European Review for Medical and Pharmacological Sciences 2016; 20: 1373-1378

Relationship between the DAT1 gene and the effects of methylphenidate administration in adult attention deficit hyperactivity disorder: a magnetic resonance spectroscopy study

A.N. INCI KENAR[1], G.A. ÜNAL[2], H. GÜLER[3], B. ALBUZ[1], Y. KIROĞLU[4], M.E. ERDAL[3], H. HERKEN[1]

[1]Department of Psychiatry, Faculty of Medicine, Pamukkale University, Denizli, Turkey
[2]Psychiatry Clinic, Kozan State Hospital, Adana, Turkey
[3]Department of Medical Biology and Genetics, Faculty of Medicine, Mersin University, Mersin, Turkey
[4]Department of Radiology, Faculty of Medicine, Pamukkale University, Denizli, Turkey

Abstract. – OBJECTIVE: This study investigated the relationship between DAT1 gene polymorphisms and the effects of methylphenidate (MPH) administration on N-acetyl aspartate (NAA), creatine (Cr), and choline (Cho) levels in the anterior cingulate cortex, prefrontal cortex, striatum, and cerebellum in adult patients with attention deficit hyperactivity disorder (ADHD). This was the first study to investigate the relationship between DAT gene variable number tandem repeat (VNTR) polymorphisms and the responses of brain metabolites to MPH.

PATIENTS AND METHODS: Samples in this study were collected from 60 patients aged between 18 and 60 years with ADHD according to DSM-IV criteria. Genetic analysis of DAT1 gene polymorphisms was carried out using blood samples obtained after a detailed clinical evaluation. Levels of NAA, Cr, and Cho were measured in the anterior cingulate cortex, prefrontal cortex, striatum, and cerebellum by magnetic resonance spectroscopy. After this evaluation, 10 mg of MPH was given orally to patients, and the levels of the same metabolites were measured 30 min later.

RESULTS: No marked difference in NAA, Cr, or Cho levels was detected before and after MPH administration with respect to the DAT1 gene VNTR polymorphisms. A considerable increase in Cr levels in the cerebellum was identified after MPH administration in individuals with the 10/10 repeat genotype as the DAT1 VNTR polymorphism (p=0.008).

CONCLUSIONS: An increase in the previously decreased blood flow after MPH therapy may induce an increase in creatine levels in patients with the 10/10 repeat genotype. Our results thus suggest that the 10R allele as the DAT1 gene VNTR polymorphism might be associated with MPH-related changes in brain metabolites in adults with ADHD.

Key Words:
Adult attention deficit hyperactivity disorder, DAT, Methylphenidate, Magnetic resonance spectroscopy.

Introduction

The effects of specific genetic variations on brain function in patients with attention deficit hyperactivity disorder (ADHD) have not yet been clarified. Very limited data on this topic have been reported. A multidisciplinary scientific approach, such as using genetic and brain imaging analyses, is essential to better understand the pathophysiology of complex disorders like ADHD. However, neuroimaging techniques have an important role in the understanding of structural and functional changes in ADHD-related brain areas. Recent advances in these techniques have provided an opportunity for further investigations, such as those on the relationships between brain structures and functions with response to psychostimulant drug treatments. Functional neuroimaging studies reported that glucose metabolism and regional blood flow declined in prefrontal and cerebellar areas but rose in the parieto-occipital cortex in a resting state, and that these variables returned to normal after psychostimulant drug treatment[1]. To the best of our knowledge, only two magnetic resonance spectroscopy (MRS) studies have investigated the effect of methylphenidate (MPH) on brain metabolites in adult ADHD, as reported in the English medical literature[2,3]. Kronenberg et al[2] reported that an analysis of the anterior cingulate cortex (ACC) showed a significantly decreased

Corresponding Author: Ayse Nur Inci Kenar, MD; e-mail: drinci79@hotmail.com

signal of choline (Cho)-containing compounds as well as increased N-acetyl-aspartate (NAA) levels after treatment with MPH, whereas total creatine (Cr) remained unchanged, although their study included a small number of cases (n=7). Unal et al[3] reported that the levels of Cho increased after MPH administration in the striatum in the combined type of ADHD.

The majority of pharmacogenetic studies on ADHD has focused on MPH responses and was performed in children. A meta-analysis conducted on children investigating the effects of DAT1 on MPH responses showed a significant association between the 10/10 genotype as the variable number tandem repeat (VNTR) polymorphism and a low response to MPH[4]. However, there are few reports of pharmacogenetic investigations in adult ADHD. To the best of our knowledge, only three pharmacogenetic studies have investigated the relationship between DAT1 gene polymorphisms and the effects of MPH administration in adult ADHD[5-7]. One such study reported an association between DAT1 gene polymorphisms and the response to MPH[6]. However, no studies have investigated the relationship between DAT1 gene polymorphisms and the response to MPH by MRS, as reported in the english literature.

In the present study, we evaluated the relationship between DAT1 gene polymorphisms and the effects of MPH administration on NAA, Cr, and Cho levels in the striatum, prefrontal cortex (PFC), cerebellum, and ACC in adult ADHD patients.

Patients and Methods

Study Design

This study was performed in accordance with the principles of the Declaration of Helsinki and was approved by the local Institutional Review Board. Written informed consent was obtained from all subjects. A total of 60 patients between the ages of 18 and 60 years, meeting the DSM-IV criteria for adult ADHD, were included in this study. All patients were from the same clinic and were of turkish origin. Patients accompanied by neurological/chronic disease, mental retardation, a psychotic disorder, a psychiatric disorder due to organic factors, and who were illiterate were excluded from the study.

Patients were evaluated using the Adult ADHD Diagnosis and Evaluation Scale and the Wender Utah Rating Scale (WURS). The validity and reliability of WURS for Turkish individuals were established by Oncu et al[8], with a threshold score of 36. The validity and reliability for Turkish individuals of the Adult Attention Deficit Disorder (ADD)/ADHD DSM-IV-based Diagnostic Screening and Rating Scale were established by Gunay et al[9]. Patients who scored 36 points or more on the WURS and gave an answer of 2 or 3 points for at least 6 of the 9 questions in the first and/or second parts of the Adult ADHD Diagnosis and Evaluation Scale were diagnosed with ADHD.

Magnetic Resonance Spectroscopy

This study was performed using a 1.5-Tesla magnetic resonance device (GE Medical Systems, Milwaukee, WI, USA) with a standard head coil. The magnetic resonance protocol was as follows: horizontal plane, 10-mm thickness; repetition time (TR)/echo time (TE), 3000/88.2; field of view, 10; matrix, 512×512; and next: 1. T2-weighted fast spin echo sequences were obtained using the aforementioned parameters. MRS was performed using a single-voxel (^{1}H-voxel) technique with voxels placed in each of the striatum, PFC, cerebellum, and ACC. The volume of interest was placed in related areas manually with visual confirmation that they contained related brain tissue and were predominantly in the chosen areas. A chemical shift selective pulse process was used to inhibit water-derived signals. Subsequently, a point-resolved spectroscopy technique was used (TR/TE, 3000-35). Consequently, short-time TE spectra were obtained from the volume of interest of ACC and dorsolateral PFC areas, and the metabolite ratios obtained by the General Electric Software Spectral Analysis Program were evaluated.

^{1}H MRS analysis was performed by an expert radiologist, and NAA, Cho, and Cr values were measured in the striatum, PFC, cerebellum, and ACC. Methylphenidate (Ritalin® 10 mg, Novartis Pharma AG, Basel, Switzerland) was given orally to patients, and NAA, Cho, and Cr values were measured again after an interval of 30 min.

DNA Isolation and Molecular Analysis

DNA was isolated from peripheral blood leukocytes by a standard phenol/chloroform method and genotyped by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. PCR was performed with an automated thermal cycler (Veriti; Applied Biosystems, Foster City, CA, USA) using

the sense primer 5′-TGTGGTGTAGGGAACGGCCTGAG-3′ and the antisense primer 5′-TTCCTGGAGGTCACGGCTCAAGG-3′.

Statistical Analysis

Data analysis was performed using IBM Statistical Package for Social Sciences v16 (SPSS Inc., Chicago, IL, USA). The Kolmogorov-Smirnov test was used to test for normality. The Kruskal-Wallis test was used to compare brain metabolite levels between the DAT1 genotypes before and after MPH administration. A p-value of <0.05 was accepted as statistically significant. To determine the effects of MPH on brain metabolite levels in each genotype, the study group was divided into three subgroups according to DAT1 gene VNTR polymorphisms. Here, a p-value of <0.017 (0.05/3) was accepted as statistically significant. For each genotype, the change in brain metabolite levels after MPH administration was analyzed by a paired t-test.

Results

A total of 60 patients met the eligibility criteria for this study. Among these 60 patients (48 males, 12 females), the mean age was 28.98±7.66 years (range, 18 to 59 years).

The distribution of patients with respect to the DAT1 VNTR polymorphism genotypes was as follows: 3 (5.0%) with the 9/9 genotype (inattentive subtype: 1, combined subtype: 2), 28 (46.7%) with the 10/9 genotype (inattentive subtype: 12, hyperactive subtype: 5, combined subtype: 11), and 29 (48.3%) with the 10/10 genotype (inattentive subtype: 8, hyperactive subtype: 6, combined subtype: 15). In this study, no significant difference was detected in the levels of Cho, Cr, or NAA among the DAT1 gene VNTR polymorphism genotypes before and after MPH administration (p>0.05).

After MPH administration, a statistically significant increase in Cr levels was found in the cerebellum compared with those before administration in patients with the 10/10 genotype (p=0.008). In other brain areas, after MPH administration, changes in Cr levels were not statistically significant for subjects with this genotype (p>0.05). There were no statistically significant differences in the Cho or NAA levels before and after MPH administration in any brain area for subjects with this genotype (p>0.05) (Table I).

Table I. Distribution of N-acetyl aspartate, creatine, and choline levels according to the DAT1 variable number tandem repeat polymorphism genotype before and after methylphenidate administration in the brain.

		9/9 Genotype			10/9 Genotype			10/10 Genotype		
Before MPH-After MPH		Mean	SD	p	Mean	SD	p	Mean	SD	p
Prefrontal cortex	NAA levels	-9.00000	11.53256	.309	.71429	15.36195	.808	-1.37931	11.64172	.529
	Cre levels	-2.66667	4.04145	.371	-.35714	6.76163	.782	-1.10345	9.40116	.532
	Cho levels	3.00000	7.00000	.535	1.64286	7.85618	.278	-.58621	8.99014	.728
Anterior cingulate cortex	NAA levels	-1.00000	10.39230	.883	-2.25000	14.13591	.407	-2.65517	9.38188	.139
	Cre levels	.33333	3.05505	.868	-.50000	6.94155	.706	-1.82759	7.46931	.198
	Cho levels	.00000	2.64575	1.000	.21429	8.83326	.899	.79310	8.65357	.625
Cerebellum	NAA levels	-2.33333	2.30940	.222	-1.14286	8.62260	.489	-3.37931	11.54004	.126
	Cr levels	2.00000	7.81025	.701	-1.21429	8.01553	.430	-4.27586	8.08834	.008*
	Cho levels	-5.33333	5.50757	.235	.89286	6.95669	.503	-2.93103	6.37479	.020
Striatum	NAA levels	-2.66667	10.96966	.715	-1.42857	10.72010	.487	2.10345	8.89761	.213
	Cr levels	-1.66667	8.14453	.757	.25000	9.10281	.886	1.03448	6.49441	.398
	Cho levels	-5.66667	4.04145	.136	-3.25000	7.15244	.023	-.44828	9.18301	.795

*Paired t-test was performed. A p-value of <0.017 was accepted statistically significant.

In patients with the 9/9 and 10/9 genotypes, after MPH administration, NAA, Cr, and Cho levels did not show statistically significant changes compared with the levels before MPH administration in any brain area ($p>0.05$) (Table I).

Discussion

In this study, we attempted to demonstrate a relationship between DAT1 gene polymorphisms and the effects of MPH use on NAA, Cr, and Cho levels in adult ADHD. The results of our study show that the 10/10 genotype as a DAT1 gene VNTR polymorphism may be related to MPH-associated changes in brain metabolites in adult ADHD patients. This is the first study to investigate the relationship between DAT1 gene VNTR polymorphisms and the responses of brain metabolites to MPH. Additionally, the present report is the second to demonstrate an association between the 10/10 genotype and response to MPH in adult ADHD[6].

Two studies have reported a lack of an association between the DAT1 gene and the effects of MPH administration in adult ADHD[5-7]. Contini et al[7] studied −839 C>T, Int8 VNTR, and 30-VNTR polymorphisms of the DAT1 gene. They failed to demonstrate that any of the DAT1 polymorphisms or haplotypes had an effect on the response to MPH. They supposed that, although the DAT protein is important, the DAT1 gene does not have clinical significance in adult ADHD. Mick et al[5] reported no association between DAT1 VNTRs and the response to MPH. However, Kooij et al[6] found that the 10/10 genotype as a DAT1 gene VNTR polymorphism was associated with a lower MPH response when compared with a single 10-repeat allele. They suggested that a high dose of MPH is needed for individuals with the 10/10 genotype to achieve appropriate blockade of DAT because the 10/10 genotype is associated with higher DAT protein availability[10]. However, this disagrees with Shumay et al[11], who reported that lower DAT levels associated with the 10/10 genotype and the 9R allele have an additive effect on DAT density[11]. Additionally, it was shown that adult patients with ADHD having low DAT availability did not seem to respond to MPH therapy[12]. Thus, low DAT activity is related to a poor response to MPH therapy[13]. Furthermore, in a meta-analysis, a significant association between a low response to MPH and the 10R/10R genotype was reported[4].

In our study, while no remarkable differences in NAA, Cr, or Cho levels were determined to be associated with DAT1 genotypes after MPH administration, a considerable increase in Cr levels after MPH administration was found in the cerebellum of patients with the 10/10 repeat genotype. Cr is a relatively constant component of cellular energy metabolism and is a reservoir of high-energy phosphate. While the level of Cr is increased in a hypermetabolic state, it decreases in a hypometabolic one[14]. In ADHD, it is known that cerebellar blood flow and glucose metabolism are low, but these variables normalize after psychostimulant therapy[1]. In our study, an increase in previously decreased blood flow after MPH therapy may have induced an increase in Cr levels because the hypometabolic state did not normalize in the cerebellum in patients with the 10/10 genotype. Thus, no or a poor response to MPH can be considered to be consistent with the findings of Kooij et al[6].

Previous studies have reported that the 10/10 genotype as a DAT1 VNTR polymorphism was much more strongly related to the hyperactivity symptoms of ADHD, among others, in the combined ADHD subtype than in the inattentive ADHD subtype or cases with inattentive symptoms[15]. It has been reported that cerebellar dysfunction could be responsible for the motor signs in ADHD[16]. In the present study, it was determined that 53.8% of the patients with hyperactivity and the combined subtype had the 10/10 genotype, while 72.4% of patients with the 10/10 genotype had hyperactivity and the combined subtype. As such, it is suggested that cerebellar activation is lower in patients with the 10/10 genotype than in those with other genotypes. Since the hypometabolic state did not normalize in the cerebellum after MPH administration, it can cause an increase in Cr levels. However, it makes us think non-responsiveness and/or poor response to MPH.

On the other hand, it has been reported that abnormal cerebral findings in children with ADHD normalize with the onset of adolescence, but the decrease in cerebellar volume continues into adulthood[17]. The present study was performed only in adults. After MPH therapy, only the cerebellar levels of Cr changed. After MPH administration, no changes in PFC or ACC levels or in cerebral metabolites in the striatum were observed. These findings may be related to volumetric normalization of these cerebral regions with the onset of adulthood, as well as the persistent decrease in cerebellar volume.

The limitations of this study include the potential impact of long-term drug treatment, as received by the majority of patients, on MRS metabolite levels, the inability to exclude the effects of smoking on the treatment response to MPH, the absence of a control group, the use of low Tesla units in cranial magnetic resonance imaging, and the evaluation of a unilateral field.

Clarification of the neuropathology of ADHD will require further studies with control groups, in which the effects of age and gender are minimized; neuroimaging techniques with a higher resolution should also be used. In addition, few pharmacogenetic studies have been performed on individuals with ADHD. This work was the first in which cerebral metabolites were investigated from a pharmacokinetic perspective.

Conclusions

Our findings suggest that the 10R allele as a DAT1 gene VNTR polymorphism might be related to MPH-associated changes in brain metabolites in ADHD patients. ADHD is a neuropsychiatric disorder caused by dysfunctional frontostriatal pathways. Genetic factors play a role in its etiopathogenesis. Demonstration of a correlation between the response to MPH and some variants of the DAT1 gene can lead to the ability to predict the response to MPH. More researches on the DAT1 gene and genes related to a predisposition to ADHD, as well as the response to stimulating treatment, may open up new horizons in the diagnosis and treatment of ADHD. This work provides a foundation for further studies.

Conflicts of interest

Funding was provided by the Commission of Scientific Research Projects, Pamukkale University, Denizli, Turkey.

References

1) Guney E, Senol S, Sener S. Neuroimaging methods in Attention Deficit Hyperactivity Disorder. [Article in Turkish] Klinik Psikiyatri 2008; 11: 84-94.
2) Kronenberg G, Ende G, Alm B, Deuschle M, Heuser I, Colla M. Increased NAA and reduced choline levels in the anterior cingulum following chronic methylphenidate. A spectroscopic test-retest study in adult ADHD. Eur Arch Psychiatry Clin Neurosci 2008; 258: 446-450.
3) Unal GA, Kenar AN, Herken H, Kiroglu Y. Association of adult attention deficit hyperactivity disorder subtypes and response to methylphenidate HCL treatment: a magnetic resonance spectroscopy study. Neurosci Lett 2015; 604:188-192.
4) Purper Ouakil D, Wohl M, Orejarena S, Cortese S, Boni C, Asch M, Mouren MC, Gorwood P. Pharmacogenetics of methylphenidate response inattention deficit/hyperactivity disorder: association with the dopamine transporter gene (SLC6A3). Am J Med Genet B Neuropsychiatr Genet 2008; 147: 1425-1430.
5) Mick E, Biederman J, Spencer T, Faraone SV, Sklar P. Absence of association with DAT1 polymorphism and response to methylphenidate in a sample of adults with ADHD. Am J Med Genet B Neuropsychiatr Genet 2006; 141: 890-894.
6) Kooij SJ, Boonstra AM, Vermeulen SH, Heister AG, Burger H, Buitelaar JK, Franke B. Response to methylphenidate in adults with ADHD is associated with a polymorphism in SLC6A3 (DAT1). Am J Med Genet B Neuropsychiatr Genet 2008; 147: 201-208.
7) Contini V, Victor MM, Marques FZ, Bertuzzi GP, Salgado CA, Silva KL, Sousa NO, Grevet EH, Belmonte-de-Abreu P, Bau CH. Response to methylphenidate is not influenced by DAT1 polymorphisms in a sample of Brazilian adult patients with ADHD. J Neural Transm (Vienna) 2010; 117: 269-276.
8) Oncu B, Olmez S, Sentürk V. Validity and reliability of the Turkish version of the Wender Utah Rating Scale for attention-deficit/hyperactivity disorders in adults. [Article in Turkish] Türk Psikiyatr Derg 2005; 16: 252-259.
9) Gunay S, Savran C, Aksoy UM, Maner F, Turgay A, Yargic I. The Norm Study, Transliteral Equivalence, Validity, Reliability of Adult Hyperactivity Scale in Turkish Adult Population. [Article in Turkish] Türkiye'de Psikiyatri 2006; 8: 98-107.
10) Heinz A, Goldman D, Jones DW, Palmour R, Hommer D, Gorey JG, Lee KS, Linnoila M, Weinberger DR. Genotype influences in vivo dopamine transporter availability in human striatum. Neuropsychopharmacology 2000; 22: 133-139.
11) Shumay E, Chen J, Fowler JS, Volkow ND. Genotype and ancestry modulate brain's DAT availability in healthy humans. PLoS One 2011; 6: e22754.
12) Krause J, la Fougere C, Krause KH, Ackenheil M, Dresel SH. Influence of striatal dopamine transporter availability on the response to methylphenidate in adult patients with ADHD. Eur Arch Psychiatry Clin Neurosci 2005; 255: 428-431.
13) la Fougère C, Krause J, Krause KH, Josef Gildehaus F, Hacker M, Koch W, Hahn K, Tatsch K, Dresel S. Value of 99mTc-TRODAT-1 SPECT to predict clinical response to methylphenidate treatment in adults with attention deficit hyperactivity disorder. Nucl Med Commun 2006; 27: 733-737.
14) Kuruoglu AC. Neuroimaging techniques in alcohol dependence. [Article in Turkish] Dahili Tıp Bilimleri Psikiyatri 2005; 1: 28-34.

15) Mill J, Xu X, Ronald A, Curran S, Price T, Knight J, Craig I, Sham P, Plomin R, Asherson P. Quantitative trait locus analysis of candidate gene alleles associated with attention deWcit hyperactivity disorder (ADHD) in Wve genes: DRD4, DAT1, DRD5, SNAP-25, and 5HT1B. Am J Med Genet B Neuropsychiatr Genet 2005; 133B: 68-73.

16) Silk TJ, Vance A, Rinehart N, Bradshaw JL, Cunnington R. White matter abnormalities in attention deficit hyperactivity disorder: a diffusion tensor imaging study. Hum Brain Mapp 2009; 30: 2757-2765.

17) Castellanos FX, Lee PP, Sharp W, Jeffries NO, Greenstein DK, Clasen LS, Blumenthal JD, James RS, Ebens CL, Walter JM, Zijdenbos A, Evans AC, Giedd JN, Rapoport JL. Developmental trajectories of brain volume abnormalities in children and adolescents with attention deficit/hyperactivity disorder. JAMA 2002; 288: 1740-1748.