The Effect of Single Dose Methylphenidate on Neurometabolites according to COMT Gene Val158Met Polymorphism in the Patient with Attention Deficit Hyperactivity Disorder: A Study Using Magnetic Resonance Spectroscopy

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Objective: Attention deficit/hyperactivity disorder (ADHD) is a common neurodevelopmental disorder. Thus, the present study aimed to determine the effects of a single dose of methylphenidate (Mph) on neurometabolite levels according to polymorphisms of the catechol-O-methyltransferase (COMT) gene.

Methods: This study evaluated the neurometabolite levels including N-acetylaspartate (NAA), creatine (Cr), and choline (Cho) of ADHD patients, before and after treatment with Mph (10 mg) according to the presence of COMT polymorphisms. The spectra were obtained from the dorsolateral prefrontal cortex (DLPFC), anterior cingulate cortex (ACC), cerebellum, and striatum.

Results: The NAA levels of the val/val and val genotype carriers (val/val and val/met genotypes) increased in the DLPFC and ACC, respectively, following Mph treatment. The NAA/Cr ratio was lower in the DLPFC of val carriers than in the met/met genotype carriers prior to Mph administration. The Cho levels of the val/met genotype and val carriers increased in the striatum following Mph treatment. Following Mph treatment, the Cr levels of the met/met genotype carriers were higher than those of the val/met genotype and val carriers. Additionally, after Mph treatment, there was a significant increase in Cr levels in the DLPFC of the met/met genotype carriers but a significant decrease in such levels in the striatum of val/met genotype carriers.

Conclusion: These findings suggest that polymorphisms of the COMT gene can account for individual differences in neurochemical responses to Mph among ADHD patients. Therefore, further studies are needed to fully characterize the effects of the Val158Met polymorphism of the COMT gene on treatment outcomes in patients with ADHD.

KEY WORDS: Catechol-O-methyltransferase; Neurometabolite; Attention deficit disorder with hyperactivity; Methylphenidate.

INTRODUCTION

Attention deficit hyperactivity disorder (ADHD) is a neurodevelopmental disorder that manifests during childhood and is characterized by attentional problems and/or hyperactivity-impulsivity in varying degrees of severity; it has a worldwide pooled prevalence of 5.29%. ADHD is a chronic disorder that negatively affects several dimensions of life, particularly the academic, occupational, and social domains, during both childhood and adulthood. In terms of its etiology, there are disruptions of the dopaminergic and noradrenergic pathways, which regulate attention, and an inhibition of executive function via alterations of the cortico-striato-thalamic-cortical networks.

The genetic heritability of ADHD ranges from 70% to 90%, and a number of studies have investigated candidate genes that may contribute to the manifestation of ADHD. The catechol-O-methyltransferase (COMT) gene, which plays a role in the removal of dopamine (DA) from the synaptic space, is frequently studied as a possible candidate gene. A single nucleotide polymorphism (SNP) of the COMT gene that includes a guanine (G) to adenine (A) mutation at codon 158 results in an amino acid substitution of methionine (met) for valine (val) during enzyme synthesis. This polymorphism, which is known as either Val158Met or rs4680, results in an enzyme with two Val alleles (val/val genotype) that is more thermo-
stable⁹ and degrades DA three-to-four times more rapidly than the two met isoforms (met/met genotypes) which, in turn, results in lower levels of DA in the synaptic space.⁸

Heterozygotes (val/met genotype carriers) are associated with intermediate levels of COMT activity,¹⁰,¹¹ and healthy met/met genotype carriers exhibit superior performance in a number of cognitive paradigms, including the letter-number-sequencing test (⁰²) and n-back task, (⁰³) compared with individuals with the val/val genotype. The Val allele is related to decreased prefrontal cortical activation in healthy adults (⁰⁴) and impaired working memory function in healthy adults and children.¹⁴,¹⁵ Cheon et al.¹⁶ evaluated the association between the Val158Met polymorphism and treatment response and found that the response to methylphenidate (Mph) was better in val/val genotype carriers than in carriers of other genotypes. However, Bellgrove et al.¹⁷ observed that children with ADHD carrying the val/val genotype exhibited superior sustained attention than met carriers (met/met and val/met genotypes). On the other hand, studies of the relationship between the etiology of ADHD and polymorphisms of the COMT gene have reported equivocal results. For example, Keresztesy et al.¹⁸ reported that the Val allele is more common in children with ADHD than in healthy controls, whereas other studies (¹⁹-²¹) and meta-analyses (²²,²³) did not observe any type of relationship between these variables.

¹H magnetic resonance spectroscopy (¹H MRS) is a non-invasive neuroimaging technique that can be used in vivo to assess levels of neurometabolites, such as N-acetylaspartate (NAA), creatine (Cr), and choline (Cho), in a variety of brain areas. Courvoisier et al.²⁴ found higher levels of Cho in the prefrontal cortex (PFC) of children diagnosed with ADHD compared with healthy controls. Similarly, Husarova et al.²⁵ assessed children with ADHD during the second month of atomoxetine treatment and identified decreases in NAA levels and the NAA/Cr ratio in the left dorsolateral PFC (DLPFC) in conjunction with an increase in the Cho/Cr ratio in the right DLPFC. Amor²⁶ reported that drug-naïve children with a diagnosis of ADHD had lower Cho levels in the left PFC compared with healthy controls and ADHD children who had received treatment. A meta-analysis conducted by Aoki et al.²⁷ showed that, although the results of the adult ADHD patients and controls were similar, the NAA levels of ADHD-diagnosed children were higher than those of healthy controls in the medial PFC area. Thus, the present authors speculated that age-related changes in ADHD symptoms may have a neuronal basis even though no significant alterations in other metabolites have been reported.

Therefore, the present study utilized ¹H MRS to investigate alterations in the neurometabolite levels of adult ADHD patients following a single dose of Mph according to the presence of the Val158Met polymorphism of the COMT gene. To the best of our knowledge, the present study is the first to investigate pre- and post-Mph neurometabolite levels in adult ADHD patients based on this COMT polymorphism. It was hypothesized that different COMT gene polymorphism carriers would exhibit different treatment outcomes in terms of neurometabolite levels in different brain regions following a single dose of Mph.

METHODS

Subject Characteristics

The present study assessed 57 adult patients who were between 18 and 60 years of age and who met the criteria of the Diagnostic and Statistical Manual of Mental Disorders 4th edition, text revision (DSM-IV-TR) for ADHD.²⁸ All patients were recruited from the psychiatry outpatient clinic of Pamukkale University in Turkey, and the absence of other psychotropic diagnoses was confirmed by structured interviews using the Turkish version of the Structured Clinical Interview for DSM-IV Clinical Version (SCID-I CV).²⁹ Additionally, the Turkish version of an adult attention-deficit disorder (ADD)/ADHD DSM-IV-based diagnostic screening and rating scale was used to assess the patients.³⁰ Patients with a neurodegenerative disorder, such as Alzheimer’s disease or Parkinson’s disease, and/or a clinical evaluation of an intellectual disability were excluded from the present study. The local Ethics Committee of Pamukkale University approved the study protocol (approval no. 60116787/020/27537), and all patients were informed of the aim of the study and its procedures; all patients also provided written informed consent confirming their voluntary participation in this study.

All patients were examined with a single-voxel ¹H MRS, and the spectra were obtained from the DLPFC, anterior cingulate cortex (ACC), striatum, and cerebellum via a clinical 1.5-T magnetic resonance scanner (GE Medical System, Milwaukee, WI, USA). For this procedure, the following parameters were employed: a point-resolved spectroscopy sequence with water suppression, a chemical shift selective imaging sequence, an echo time (TE)/repetition time (TR) ratio of 35/3,000 ms, and 128 averages. Additionally, the present study utilized a conventional spin-echo sequence with T2-weighted fast spin-echo parameters as follows: horizontal slices (10-mm
Table 1. Sociodemographic characteristics

| Characteristic     | Data |
|-------------------|------|
| Sex               |      |
| Female            | 12 (21.1) |
| Male              | 45 (78.9) |
| Marital status    |      |
| Single            | 35 (61.4) |
| Married           | 18 (31.6) |
| Divorced          | 4 (7.0) |
| Education         |      |
| Primary education | 5 (8.8) |
| High school       | 12 (21.1) |
| University        | 40 (70.1) |
| Methylphenidate use |      |
| Yes               | 33 (57.9) |
| No                | 24 (42.1) |

Values are presented as number (%).
types according to COMT polymorphism ($\chi^2=4.29$, $p=0.29$; Table 2). In total, 37 of the males (82.2%) and nine of the females (75.0%) were val carriers, but there was no significant difference in the gender distribution according to COMT polymorphism ($\chi^2=0.31$, $p=0.57$).

For the metabolic assessments, the patients were categorized according to the COMT polymorphisms in two different ways, and all statistical analyses were performed accordingly. For the first categorization, the patients were divided into three groups (val/val, val/met, and met/met genotype carriers) and their neurometabolic variables were compared. For the second categorization, the patients were divided into two groups: val genotype carriers (val/val and val/met; i.e., rapid metabolizers) and met/met genotype carriers (slow metabolizers). After a single 10-mg dose of Mph, there was a significant increase in NAA levels in the DLPFC of the val genotype carriers ($p=0.04$) and the ACC of the val carriers ($p=0.03$; Table 3 and Fig. 1). The pre-Mph NAA/Cr ratio was significantly lower in the DLPFC of val carriers than that in the met/met genotype carriers ($p=0.02$), but there were no significant post-Mph alterations in NAA levels or NAA/Cr ratios in the other brain regions that were investigated. Cho levels in the striatum of the val/met and val genotype carriers significantly increased after a single dose of Mph compared with pre-medication levels ($p=0.01$ and $p=0.007$, respectively). However, there were no significant medication-related changes in Cho levels in the other brain regions based on polymorphism (Table 3 and Fig. 1). Although the pre-medication Cr levels in the DLPFC were similar in all groups, after Mph treatment there were higher Cr levels in the met/met genotype carriers than in the val/met and val genotype carriers.

Table 2. ADHD subtypes and the COMT gene polymorphism

| COMT gene polymorphism | Val/val | Val/met | Met/met |
|------------------------|---------|---------|---------|
| Attention deficit type  | 9 (45.0)| 8 (40.0)| 3 (15.0)|
| Hyperactivity impulsivity type | 5 (55.6)| 3 (33.3)| 1 (11.1)|
| Combined type          | 6 (21.4)| 15 (53.6)| 7 (25.0)|

Values are presented as number (%).

ADHD, attention deficit hyperactivity disorder; COMT, catechol-O-methyltransferase.

$p=0.29$, $X^2=4.29$; by chi-square test.

Table 3. Comparison of the neurometabolites between the COMT gene Val158Met polymorphism groups

| COMT gene polymorphism | Val/val | Val/met | Met/met | p value* | p value† |
|------------------------|---------|---------|---------|----------|----------|
| Comparison of NAA levels before and after Mph |
| DLPFC Before Mph 68 (50-117)† | 66 (45-98) | 74 (53-107) | 0.486 | 0.04† |
| After Mph 71 (51-115)† | 59.5 (39-92) | 70 (60-113) | 0.097 | 0.174 |
| ACC Before Mph 62.5 (51-126) | 58 (39-87) | 66 (51-83) | 0.225 |
| After Mph 68.5 (54-113) | 63 (47-94) | 62 (56-79) |
| Striatum Before Mph 63.5 (50-97) | 62 (45-82) | 61 (52-76) | 0.684 |
| After Mph 67 (45-94) | 59 (45-85) | 68 (55-105) | 0.146 |
| Cerebellum Before Mph 61 (38-96) | 60.5 (31-78) | 57 (37-89) | 0.724 |
| After Mph 66 (38-85) | 60.5 (38-84) | 61 (52-88) | 0.521 |

Comparison of Cho levels before and after Mph

| COMT gene polymorphism | Val/val | Val/met | Met/met | p value* | p value† |
|------------------------|---------|---------|---------|----------|----------|
| DLPFC Before Mph 41 (25-66) | 38 (29-62) | 37 (26-52) | 0.196 |
| After Mph 42 (29-70) | 39.5 (26-62) | 40 (36-51) | 0.170 |
| ACC Before Mph 38.5 (24-65) | 37 (25-50) | 38 (22-54) | 0.945 |
| After Mph 41 (24-44) | 37.5 (26-52) | 35 (32-48) | 0.405 |
| Striatum Before Mph 36 (24-53) | 33 (22-45)† | 36 (28-55) | 0.239 |
| After Mph 36.5 (27-57) | 35.5 (27-56)† | 36 (26-47) | 0.929 |
| Cerebellum Before Mph 46.5 (31-61) | 40.5 (25-61) | 45 (25-59) | 0.196 |
| After Mph 41 (27-64) | 41.5 (29-56) | 44 (31-64) | 0.492 |

Comparison of Cr levels before and after Mph

| COMT gene polymorphism | Val/val | Val/met | Met/met | p value* | p value† |
|------------------------|---------|---------|---------|----------|----------|
| DLPFC Before Mph 40 (29-70) | 40.5 (29-53) | 39 (28-55)† | 0.804 |
| After Mph 43 (33-69) | 39 (23-60)* | 42 (38-66)* | 0.016* |
| ACC Before Mph 41.5 (29-73) | 40.5 (30-57) | 43 (36-49) | 0.357 |
| After Mph 44 (31-69) | 42.5 (33-62) | 43 (34-55) | 0.591 |
| Striatum Before Mph 47.5 (36-69)† | 42 (29-51) | 46 (38-59) | 0.054 |
| After Mph 43 (34-66)† | 41.5 (34-58) | 48 (36-61) | 0.463 |
| Cerebellum Before Mph 53 (24-70) | 49 (34-66) | 54 (29-65) | 0.396 |
| After Mph 52 (27-74) | 52.5 (27-65) | 55 (41-74) | 0.542 |

Values are presented as median (range).

COMT, catechol-O-methyltransferase; DLPFC, dorsolateral prefrontal cortex; ACC, anterior cingulate cortex; Mph, methylphenidate; NAA, N-acetylaspartate; Cho, choline; Cr, creatine.

By *Kruskal Wallis test and †two related sample test (Wilcoxon).
val/met and val carriers (\(p = 0.01\) and \(p = 0.03\), respectively; Table 3). Consistent with these findings, the Cr levels in the DLPFC of the met/met genotype carriers significantly increased after a single dose of Mph compared with their pre-medication levels (\(p = 0.01\); Table 3 and Fig. 2). In the val/val genotype carriers, Cr levels in the striatum significantly decreased after Mph treatment (\(p = 0.01\)), but the Cr levels in the other brain regions were not affected (Table 3).

**DISCUSSION**

Of the 57 adult ADHD patients assessed in the present study, the number of rapid metabolizers (val carriers; \(n = 46\)) was approximately fourfold higher than that of slow metabolizers (met/met genotype; \(n = 11\)). The Val allele enhances the hypo-dopaminergic state in the synaptic space to a greater degree than the Met allele due to its thermostability,\(^9\) and healthy met/met genotype carriers exhibit superior cognitive performance compared with val/val genotype carriers.\(^{12,13}\) Similarly, the Val allele has a stronger association with impaired working memory function than does the Met allele in healthy adults and children,\(^{14,15}\) and it is also associated with decreased prefrontal cortical activation in healthy adults.\(^{14}\) In contrast to these findings, other studies\(^{24,32}\) and meta-analyses\(^{22,23}\) have reported no relationship between ADHD and COMT gene polymorphisms.

The predominance of val carriers in the present patient group appears to support the Val allele-ADHD relationship in terms of disease etiology. The conflicting reports from several candidate gene studies may be due to the multigenic and multifactorial etiology of ADHD because, although the COMT polymorphism may affect the development of ADHD, this does not occur in isolation. To better understand this relationship, ADHD-related networks and other factors (particularly catecholamine-related polymorphisms) that may have an impact on the development of ADHD should be researched on a larger scale. Consistent with the results of Yatsuga et al.\(^{32}\), the present study did not find a relationship between ADHD subtype and COMT polymorphism. It is possible that the contribution of the COMT polymorphism to ADHD is not subtype-specific or it could be that the small sample size used in the present study may have influenced the present results.

**NAA**

Following treatment with Mph, there was a significant increase in the amount of NAA in the DLPFC of the val/val genotype carriers and in the ACC of the val carriers. In contrast, Carrey et al.\(^{33}\) did not find any significant alterations in the neurometabolite levels in the right frontal region of ADHD patients after 13 weeks of treatment with atomoxetine, Mph, or Dexedrine. Husarova et al.\(^{25}\) reported that the NAA levels and NAA/Cr ratio decreased in the left DLPFC of ADHD patients after 2 months of atomoxetine treatment, but that there were no significant alterations after treatment with Mph. A meta-analysis revealed that the amount of NAA in the medial...
PFC of children with ADHD was significantly higher than that of the controls, but this difference declined with age and disappeared in adults, which suggests that age-related alterations in ADHD symptoms may have a neuronal basis.\(^{34}\)

In this respect, the present findings disagree with those of previous studies. NAA is considered to be a marker of regional neuronal activation and vitality,\(^{35}\) and neuroimaging studies conducted among ADHD patients have observed numerous alterations of this metabolite in various brain regions, principally the PFC, ACC, and striatum.\(^{36}\) Compared with healthy controls, ADHD patients show reduced perfusion, especially in the PFC,\(^ {37},^{38}\) and a study investigating the effects of treatment on hypoperfusion found that stimulant medication increases blood flow in the bilateral prefrontal, caudate, and thalamic areas.\(^ {38}\) Similarly, the chronic administration of Mph increases neuronal mitochondrial activity in rats,\(^ {39}\) and a single dose of Mph can lead to increases in neuronal activation in the frontal lobes of patients with ADHD.\(^ {40}\)

Taken together, these data suggest that Mph has a positive impact on impaired cerebral perfusion and neuronal function. Therefore, post-Mph increases in NAA levels may indicate improved perfusion and increased neuronal activation, as in the present data. As suggested by Angeline et al.,\(^ {41}\) the contrasting results among studies may be due to the presence or absence of a cognitive performance measure during the assessment process and, more importantly, they may be related to age, sex, the investigated regions, or related variations in NAA levels.

### NAA/Cr Ratio

In the present study, the pre-Mph NAA/Cr ratio in the DLPFC of val carriers was significantly lower than that of the met/met genotype carriers. During the literature search for this study, it became clear that no previous studies had investigated the relationship between the NAA/Cr ratio and COMT polymorphisms. Jin et al.\(^ {42}\) reported a lower NAA/Cr ratio in the striatum of children with ADHD relative to that of healthy controls, and this did not change after a single 10-mg dose of Mph. In contrast, Fayed et al.\(^ {43}\) found that the prefrontal cortico-subcortical NAA/Cr ratio is higher in ADHD children than in healthy controls, and Wiguna et al.\(^ {44}\) reported a significantly increase in the NAA/Cr ratio in the bilateral PFC of ADHD patients after 12 weeks of Mph treatment. Contradictory results in the literature may be due to changes in NAA levels according to age, sex, and the brain area investigated.

The NAA/Cr ratio is a marker of brain maturation.\(^ {45}\) Childhood ADHD is related to delayed cortical maturation, especially in the PFC.\(^ {5}\) Furthermore, adult studies have demonstrated the presence of decreased gray matter and reduced cortical thickness in ADHD patients compared with controls.\(^ {46},^{47}\) It is known that cortical thickness is related to disease severity.\(^ {47}\) The number of Val alleles in ADHD patients is associated with the rate at which DA is metabolized\(^ {6},^{11}\) and, therefore, it is also related to disease severity. This indicates that the present finding that val carriers have a lower NAA/Cr ratio (a maturation marker) than the met/met genotype carriers is intuitive.

### Cho

Cho levels significantly increased in the striatum of the val and val/met genotype carriers following treatment with Mph compared with pre-medication levels. To the best of our knowledge, no studies have investigated the relationship between Cho levels in ADHD patients and COMT polymorphisms. A number of studies have assessed Cho metabolites in ADHD patients and produced variable results. Similar to the present findings, Carrey et al.\(^ {33}\) did not find any treatment-related changes in Cho levels in the PFC of ADHD children. However, this negative result in the striatal area contrasts with the findings of the present study. Kronenberg et al.\(^ {49}\) found a significant decrease in the Cho levels in the ACC of adult ADHD patients after 5-6 weeks of Mph treatment. In a study conducted by Amor,\(^ {26}\) lower levels of Cho were reported in the left prefrontal area of treatment-naïve ADHD patients compared with both treatment-receiving ADHD children and healthy controls. Jin et al.\(^ {42}\) reported a marginal increase in the Cho/Cr ratio in the striatum of children with ADHD, but a single 10-mg dose of oral Mph had no effect on these ratios. The authors speculated that this could account for approximately 20-25% of the neural loss and/or dysfunction observed in ADHD patients.

Cho is found in the cellular membrane and is an indicator of lipid metabolism and membrane integrity.\(^ {49}\) It should also be noted that Cho is an acetylcholine precursor that influences neural communication and is mediated by various neurotransmitters, including norepinephrine and DA. It has been suggested that impairments in dopaminergic pathways, as well as the imbalances in the dopaminergic-cholinergic system that are seen in ADHD, may affect Cho levels.\(^ {42}\) With respect to previous studies, these differences may be related to medication type, treatment duration and dose, and variations in the genetic structure of the recruited individuals. However, 57.9% of the present patients were medicated with Mph and, there-
There is strong evidence to support the hypothesis that alterations in striatal Cr and phospho-Cr are related to the treatment and management of ADHD, providing insights into the neurobiological mechanisms underlying the condition. Moreover, the study suggests that these alterations could be used as biomarkers to monitor treatment response and predict outcome, offering a more personalized approach to the care of ADHD patients. Further research is needed to validate these findings and develop effective treatment strategies to address the complex and dynamic nature of ADHD.
following the administration of the drug.

The present study assessed the effects of single-dose Mph on three different neurometabolites in adult ADHD patients who were categorized according to COMT polymorphism. As expected, there was a predominance of val carriers (rapid metabolizers) among the patients. Pre- and post-medication changes in neurometabolite levels were detected in certain brain areas in accordance with COMT polymorphisms, which suggests that COMT gene polymorphisms can account for individual differences in the neurochemical response to Mph in ADHD patients. The present study provides important contributions to the literature, because it is the first to investigate the effects of single-dose Mph on neurometabolite levels according to COMT polymorphisms.

To better understand the neuropathology of ADHD, further research using drug-naïve patients, larger sample sizes, and higher-resolution neuroimaging methods is required. Additionally, the effects of age and sex should be minimized, control groups should be included, and the specific effects of the COMT Val158Met polymorphism on treatment outcomes in ADHD patients should be analyzed.

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