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AN INVESTIGATION OF THE *COMT* GENE VAL158MET POLYMORPHISM IN PATIENTS ADMITTED TO THE EMERGENCY DEPARTMENT BECAUSE OF SYNTHETIC CANNABINOID USE

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ABSTRACT

Catechol-O-methyl transferase (COMT) enzyme has a role in the inactivation of catecholamine neurotransmitters. Functional polymorphism in the *COMT* gene has been reported to play an important role in schizophrenia, bipolar affective disorder, aggressive and antisocial behavior, suicide attempts and the pathogenesis of Parkinson's disease. In this study, we aimed to investigate the effect of the Val158Met polymorphism of the *COMT* gene on substance use, and treatment history in patients with synthetic cannabinoid (SC) intoxication. The *COMT* enzyme Val158Met polymorphisms from DNA of 49 patients who were evaluated in the Emergency Department after SC use and 50 healthy control groups aged 18-45 years, were identified by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) analyses as reported in the literature. Information regarding recurrent intake or hospitalization due to substance use was obtained from hospital records. Wild-type (WT) genotypes in 14 (28.6%) patients, heterozygous genotypes in 25 (51.0%) and homozygous genotypes in 10 (20.4%) patients were detected. Wild-type genotypes The homozygous genotype was found to be significantly higher in patients hospitalized due to drug addiction and substance use (p 0.008). The Val158 Met polymorphism of the *COMT* gene was not found to be significant in the first use after substance intake, while a significant relationship was found in terms of this polymorphism in patients with substance addiction diagnosis and treatment history.

Keywords: Addiction; Catechol-O-methyl transferase (*COMT*) gene; Synthetic cannabinoids (SC); Val-158Met polymorphism.

INTRODUCTION

The catechol-O-methyl transferase (*COMT*) gene is involved in the inactivation of catecholamine neurotransmitters. The enzyme inactivates catecholamines such as dopamine and catecholamine-containing drugs by methyl conjugation. The *COMT* gene is located on the chromosome 22q11 fragment. Deletion of this fragment leads to a complex syndrome and psychiatric symptoms including schizophrenia and other psychoses [1].

The activity of the *COMT* enzyme is regulated by a common polymorphism that causes significant variations in enzymatic activity [2].Three genotypes of alleles (Val/Val, Met/Met, Val/Met) containing valine (heat-resistant high activity) and methionine (heat-resistant low activity) were identified as a result of guanine-adenine nucleotide exchange, and valine to methionine (Val→Met) amino acid exchange at codons 108/158 in the *COMT* gene [2].

This functional polymorphism in the *COMT* gene causes the enzyme activity to change; the enzyme activity is reduced by about 4-times in the genotypes with low enzyme activity, thus the catecholamine metabolism becomes faster. The Met/Met genotype has the weakest enzymatic activity, whereas the Val/Val genotype has the highest activity. Heterozygotes were found to have moderate activity because the two alleles were codominant [3,4].

Based on this information, it is suggested that the effect of the *COMT* gene Val158Met polymorphism on behavior should be considered in the context of gene en-

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vironment interaction rather than the direct effect. Interestingly, some studies in general population samples showed that the methionine allele carriers were more susceptible to stress and environmental factors. For instance, it has been found that methionine alleles show more stress hormone response to a physical stressor in healthy individuals [3]. Methionine allele carriers show more brain activation in the limbic region to unpleasant stimuli in FMRI (functional magnetic resonance imaging) [5]. The methionine allele has been associated with excessive activity in the hippocampus and prefrontal cortex. This leads to increased sensitivity to provocative environmental stimuli [4]. The methionine allele is associated with a tendency to anxiety, a decrease in extraversion and a decrease in the search for novelty [6].

Accordingly, the hypothesis asserting that both valine and methionine alleles play a role in the development of psychopathology by interacting with specific environmental stimuli although they are different has been put forward. While the valine allele has been shown to shape the risk of psychotic disorder by interacting with cannabis, the methionine allele is associated with stress sensitivity in affective and psychotic responses to small stressors in the daily life flow [5].

Cannabinoids are a class of various chemical components that activate cannabinoid receptors within the cell, suppressing neurotransmitter release in the central nervous system (CNS). Compounds that have psychoactive properties similar totetrahydrocannabinol (THC) in cannabis (drug, stimulant, hallucinogen, *etc.*) and which are obtained by using various chemicals in the laboratory are called synthetic cannabinoids (SC). These substances are included in the hallucinogen group of the drugs [7].

New psychoactive substances declared by the European Monitoring Center for Drugs and Drug Addiction (EMCDDA) are aminoindanes, arylalkylamines, benzo-diazepines, cathinones, tryptamines, phenethylamines, piperazines, and plants, *etc.* One of these substances called SC (its vernacular name is Bonzai) was first seen in Turkey in 2010 [8].

Pharmacological effects include cognitive impairment, learning and memory disorders, euphoria and hallucinations, sleep disorders and hyperphagia. It has been shown that its pharmacological effects are mediated by cannabinoid receptors in humans and mice. Two cannabinoid receptors, CB1 and CB2, have been identified [9]. While there are CB1 receptors in the CNS, there are also CB1 and CB2 receptors in peripheral tissues [9,10]. Activation of CB1 causes a decrease in cAMP and leads to psychoactive side effects such as agitation by inhibiting some neuro-transmitters [acetylcholine, dopamine, norepinephrine, glutamine and γ-aminobutyric acid (GABA)] [11]. The binding affinities of SCs to their receptors are quite strong compared to natural cannabinoids. This study aimed to determine whether there is a relationship between the *COMT* enzyme polymorphism Val158Met and SCs which have become a major public health problem.

MATERIALS AND METHODS

This study was performed prospectively in patients between 18-45 years of age admitted to the Emergency Department at the Bursa Yuksek Ihtisas Training and Research Hospital, Bursa, Turkey, between May 25 2018 and September 15 2018 with SC intake. A total of 50 patients and 50 healthy control group were included in the study. The SC use, the history, interviews with family and/or relatives, and medical records were evaluated. Patients and their relatives included in the study were informed about the research and written informed consent was obtained. Written approval was obtained from the ethics committee of the faculty (2011-KAEK-25 2018/04-05).

Genetic Examination. Genetic examination of the study was performed in the laboratory of the genetics department of our hospital. DNA was isolated from patients' blood samples using a DNA isolation kit. DNA isolation was primarily performed from blood samples of patients using the RTA brand DNA isolation kit (RTA Laboratory, Kocaeli, Turkey) following the original protocol. The Sanger sequencing method was used to detect the variation of p.Val158Met/c.472G>A (rs4680) located in the exon 4 region of the *COMT* gene (NM_000754). For this purpose, site-specific primer synthesis was performed using the Primary 3 program (https://ihg.helmholtz-muenchen.de/ihg/Exon Primer.html) (forward 5'-ATC CAA GTT CCC CTC TCT CC-3'; reverse 5'-AGG CTG GGA AGC ACT GAG-3'). The synthesized specific primers were treated with polymerase chain reaction (PCR) kit (Zeydanlı, Ankara, Turkey) in appropriate quantities and under suitable conditions as specified in the instruction manual.

All PCR procedures were performed on a thermal cycler (Applied Biosystems, Foster City, CA, USA). Second sequence PCR was performed with a BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems) using the first PCR products. The products obtained after the second PCR were subjected to purification. The Zymo Research Sequencing Clean-up purification kit (Zymo Research Corp., Irvine, CA, USA) was used for this process. After purification, all products were loaded onto ABI PRISM® 3500 Genetic Analyzer (Applied Biosystems). After execution, the codon 158 region of the *COMT* gene was analyzed using the SeqScape Software (Applied Biosystems) (Figure 1).

As shown in Figure 1, the codon 158 (c.472G>A) exchange region of the *COMT* gene is marked with a red frame. Three different genotypes are formed after execution. **a)** WT; **b)** homozygote p.Val158Met exchange; **c)** heterozygote p.Val158Met exchange. In those with WT,

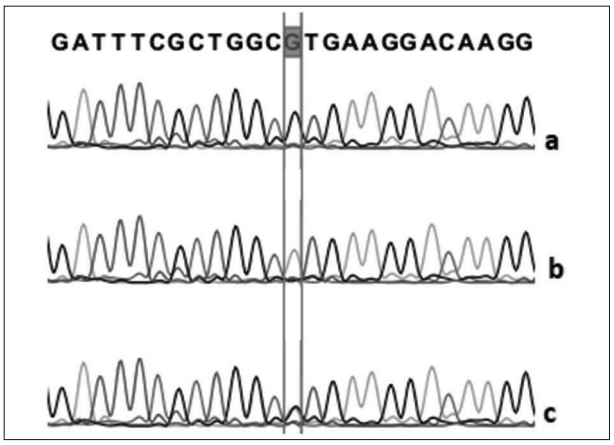

Figure 1. Sequence analysis image of the codon 158 region of the *COMT* gene. **a)** WT; **b)** homozygote p.Val 158Met exchange; **c)** heterozygote p.Val158Met exchange.

both copies are normal and unchanged (no Val158Met exchange has occurred in both copies). In heterozygous ones, one copy has the Val158Met exchange, while the other copy has not. In homozygous ones, the Val158Met changes in both copies.

Statistical analysis of the study was performed using IBM SPSS Statistics for Windows version 21.0 (IBM Corp., Armonk, NY, USA). The criteria discussed in the analysis were described as mean, standard deviation, frequency and percentage. The χ^2 test and Fisher's exact test were used to compare frequencies and percentages between groups. A p value of <0.05 was considered to be significant.

RESULTS

The study group consisted of 49 patients and 50 healthy individuals. One patient was excluded from the study because the patient's blood sample could not be studied. The *COMT* Val158Met gene polymorphism was studied in both groups. Ninety-eight percent of the patient group were males and 2.0% were females. Ninety-six percent of the control group were males and 4.0% were females. The mean age of the patient group was 26.7 ± 6.92, while the mean age of the control group was 32.4 ± 7.17. The distribution of age was normal for both groups. In these 49 patients admitted to the Emergency Department with SC intake, the Val158Met mutation in the *COMT* gene was performed by Sanger sequencing. It was used for WT Val/Val, heterozygous Val/Met and homozygous Met/ Met. Wild-type genotypes were detected in 14 (28.6%) patients, heterozygous genotype in 25 (51.0%) and homozygous genotype in 10 (20.4%) patients. Demographic data and genotype distribution of the patient and control groups are summarized in Table.1.

Table 1. Demographic and genotypic distribution of patient and control groups.

		Patients (n=49)	Controls (n=50)
Gender	**Males**	**48 (98.0%)**	**48 (96.0%)**
	Females	1 (2.0%)	2 (4.0%)
Age		26.7±6.9	32.4±7.2
Genotype	Wild-type	14	20
	Heterozygotes	25	22
	Homozygotes	10	8

Heterozygotes: p.Val158Med exchange;
Homozygotes: p.Val158Met exchange.

Of the patients included in the study, history of chronic illness, family history, substance use, Glasgow Coma Scale (GCS), and history of admission to the Bursa Treatment and Research Center for Alcohol and Drug Addiction (BTRCADA), Bursa, Turkey, were investigated. Forty-two patients (86.8%) had no history of chronic disease; one patient (2.04%) had anxiety disorder, one patient (2.04%) had bipolar disorder, one patient (2.04%) had depression, two patients (4.08%) had epilepsy and two patients (4.8%) had psychosis. This information was obtained from the patients and their relatives by scanning the files retrospectively. Participants stated that their family members had no history of substance use. When the medical history of the patients was examined, it was seen that 12 (24.5%) were hospitalized at the BTRCADA, while 37 (75.5%) were not hospitalized at the BTRCADA. No statistically significant difference was found between the study and control groups in terms of *COMT* gene Val158Met polymorphism (p 0.481).

According to the medical records and the information obtained from relatives of 49 patients admitted to the Emergency Department due to SC use; 12 (24.5%) patients were found to have hospitalization. Genetic mutation differences of these subjects compared to other substance users were examined.

Table 2 shows the genotypic distribution of patients with hospitalization at the BTRCADA. As WT and heterozygous genotypes were not different between the two

Table 2. Genotypic characteristics of patients with and without hospitalization at the Bursa Treatment and Research Center for Alcohol and Drug Addiction, Bursa, Turkey.

			Genotypic Characteristics		Total (n)
			Wild-Type	**Homozygotes**	
Hospitalization	yes	n (%)	33 (82.2)	4 (10.8)	37 (100.0)
	no	n (%)	6 (50.0)	6 (50.0)	12 (100.0)
Total (n)		n (%)	39 (79.6)	10 (20.4%)	49 (100.0)

Homozygotes: p.Val158Met exchange.

groups, they were defined as the same parameter (WT). Homozygous genotypes were found to be significantly higher in subjects with hospitalization and substance abuse than those without hospitalization (p 0.008).

DISCUSSION

Synthetic cannabinoids are synthetic derivatives of cannabinoids produced in the laboratory. These substances are included in the hallucinogen group of drugs [12]. Synthetic cannabinoids, which are rapidly spreading among adolescents and young people, are now on the list of prohibited substances in many countries, although they are still one of the most easily accessible and sold drugs on the market today [13]. Drug abuse continues to be a major health problem among adolescents and young adults. Most of the users of SC are young adults. The low cost of these substances is another reason why they are popular in the young age group [14]. In a study conducted by Barratt *et al.* [15], it was found that the mean age for SC users in Australia was 27 and 77.0% of the users were male. In another study conducted by Vandrey *et al.* [12], it was shown that the mean age for first use was 20 and 11.0% of high school students in the USA used SC. Hoyte *et al.* [16] found that the mean age for SC use was 22.5 and 74.3% of the users were male. In a similar study conducted in our country, the mean age was shown to be 30.23 [17]. In our study, we found that the mean age of the patients was 26.7 years and SC users were mostly male, which is consistent with the literature.

Research has been conducted between COMT polymorphisms and psychiatric disorders. The relationship between low-activity *COMT* allele and obsessive-compulsive disorder (OCD) was investigated by Karayiorgi *et al.* [18]. The COMT Val158Met polymorphism is one of the most studied polymorphisms in OCD patients. They concluded that Val158Met polymorphism caused a 40.0% reduction in COMT enzymatic activity in the brain and lymphocytes. As a result, it was found that prefrontal extracellular dopamine rates increased and a positive relationship was found with OCD [19]. It has been suggested that the COMT valine variant may increase prefrontal dopamine catabolism, is associated with a decrease in frontal lobe executive functions, and COMT is a locus that predisposes to schizophrenia based on this mechanism [2,20].

Allele variation in the *COMT* gene is thought to affect dopamine regulation in the prefrontal regions of the brain. Lachman *et al.* [21] first reported a common biallelic single nucleotide polymorphism (SNP) encoding valine for methionine at codon 158. This codon 158 SNP in the *COMT* gene was found to affect thermostability [21].Thus, a 2- to 4-times reduction in COMT enzyme activity was found to be associated with the methionine alleles of this polymorphism [22]. Catechol-O-methyl transferase enzyme activity is thought to play a role in the induction of dopamine in the prefrontal cortex in humans [23]. In our study, the relationship between the Val158Met polymorphism in the *COMT* gene and SC intoxication was found to be insignificant, which is contrary to that reported in the literature (p >0.05). This result supports that there is no Val158Met polymorphism in the *COMT* gene in one-time intake. The majority of our patients were not chronic substance users. Probably, the Val158Met polymorphism in the *COMT* gene may not be seen in one-time use. Another finding of this study was that the homozygous type (Met/Met) ratios which were significant for us in the study and control groups were close to each other. It was found as 20.4% [5] in the study group and 16.0% [22] in the control group. This information suggests that gene polymorphisms may have a low effect on substance intoxication due to one-time intake.

The relationship between behavioral phenotypes and psychiatric disorders in the Val158Met polymorphism has been investigated in many studies. In a study conducted by Craddock *et al.* [24], the Val158Met polymorphism in the *COMT* gene was found to be associated with schizophrenia, bipolar disorder, aggressive behavior and OCD. The most important finding of our study was that the homozygous genotype of hospitalized patients in the study group at the BTRCADA, was found to be statistically significant (p 0.008). This makes us think that there may be a genetic effect in drug addiction. The absence of the Val158Met polymorphism in one-time use, but the presence of this polymorphism in hospitalized patients who had repeated use, suggests that there may be a genetic predisposition in the patient group. When evaluating these patients, genetic effects should also be considered.

Conclusions. The use of SC among adolescents is increasing day by day. It poses serious problems in diagnosis, treatment and management in the emergency departments. Other factors making management, especially in agitated and confused patients, difficult, are the changes in the effects on a patient, difficulty in taking anamnesis, and confusion with other diseases.

In our study, the Val158Met polymorphism of the *COMT* gene was not found to be significant in patients admitted to the Emergency Department after substance intake, while a significant relationship was found in terms of this polymorphism in patients with substance addiction admitted to the BTRCADA. This makes us think that there may be a genetic disposition toward drug addiction and family members should be informed and guided in this respect. As there is no genetic disposition in patients with a one-time substance intake, families should be informed

about the recovery of these patients from drug addiction. The family should be advised to take measures such as preventing access to the substance and changing their social circle.

Limitations. In cases where anamnesis could not be taken from the patient, taking the history from the files and the relatives, was one of the limitations of our study. Another limitation was that the age range was determined. The age range is insufficient for the general population. However, this limitation can be overcome as the patient group using the substance was similar to the patient group we included. Furthermore, as genetic disposition does not change with age, this may not be considered as a limitation. Another limitation was the low number of patients and the fact that the study was performed in a single department. In addition, the lack of substance diversity and the inability to study the substance taken from the blood were among the limitations of the study. However, we think that these limitations can be overcome as there may be a relationship between the substance intake and the genetic disposition instead of the type and amount of the substance. We believe that this issue can be better elucidated by other multicenter studies to be conducted with a higher number of patients.

Declaration of Interest. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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