in vivo *29*: 129-132 (2015)

The Relationship Between Catechol-O-Methyltransferase Gene Val158Met (*COMT*) Polymorphism and Premorbid Cannabis Use in Turkish Male Patients with Schizophrenia

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Abstract. *Background/Aim: One of the risk factors for increasing psychotic disorders is the use of cannabis. It has been shown that the inactivation of dopamine and other catecholamines causes a common polymorphism generating substantial variations in COMT enzyme activity. We aimed to understand the role of cannabis in the etiology of schizophrenia with and without pre-morbid usage. Patients and Methods: The study group consisted of 80 male patients and genotyping of COMT enzyme Val158Met gene polymorphisms were detected by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Results: It was found that the Val/Val genotype is significantly higher in patients with premorbid cannabis use (88.9%) compared to patients without pre-morbid cannabis use (68.4%). Also, the mean total positive and negative syndrome scale (PANSS) score seen in the Val/Val genotype group is significantly higher than the scores of the patients with the Met allele. Conclusion: The findings from this study confirm the association between COMT Val158 Met polymorphism and pre-morbid cannabis use in causing schizophrenia.*

Cannabis use, which is gradually becoming more widespread among psychotic patients, worsens the severity of psychosis and causes frequent relapses (1, 2). Studies have indicated that the use of cannabis increases the risk of developing psychotic disorders by two-fold (3) and may lead to higher risk during adolescence (4). However, psychosis is not observed in a significant number of young people using cannabis. It has been hypothesized, on the basis of these findings, that cannabis leads to psychosis in genetically susceptible individuals (5).

Catechol-O-methyltransferase (*COMT*) gene encoding the catechol-O-methyltransferase enzyme, which inactivates dopamine and other catecholamines in the post-synaptic field, is regulated by a common polymorphism causing substantial variations in COMT enzyme activity (6). A transition in *COMT* gene exchanging valine to methionine amino acid at position 108/158 results in two common enzyme variants (Val and Met). While the Met variant of COMT is associated with low enzymatic activity, the Val variant is associated with increased enzymatic activity. The Val variant is related to increased COMT activity resulting in decreased dopamine transmission in the prefrontal cortex and increased level of dopamine in the mesolimbic pathway (7).

Studies have applied experimental methods to measure the psychotic effects induced by cannabis and found that among people who are susceptible to psychosis carriers of the Val allele are most sensitive to tetrahydrocannabinol (THC)-induced psychotic experiences (8, 9). Henquet *et al*. (8) indicated that, of the patients exposed to cannabis, carriers of the Val allele are more sensitive to cannabis-induced psychotic experiences compared to carriers of the Met allele. Their further study revealed that cannabis is one of the factors affecting the development and course of psychotic disorders (9). However, cannabis shows this effect in patients with genetic susceptibility to psychosis.

Kantrowitz *et al*. (10), who conducted the study on different ethnic groups, could not find any significant interaction between the Val/Val genotype and the use of cannabis regarding the risk of psychosis development in African-American and White American patients using cannabis before the age of 18. Zammit *et al*. (11) have reported that cannabis use does not increase the likelihood of schizophrenia according to variation in *COMT*.

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Key Words: Schizophrenia, *COMT* Val158Met, cannabis.

In the study conducted by Pelayo-Teran *et al*. (12), on patients with schizophrenia spectrum disorders, it was established that there is a significant interaction between the use of cannabis and the *COMT* gene and that the Val genotype moves the onset of schizophrenia to an earlier age when it interacts with cannabis.

Earlier studies conducted mostly on patients diagnosed with psychotic spectrum disorders, such as schizophrenia, schizo-affective disorder, schizophreni form disorder and brief psychotic disorder, have yielded confusing results.

The present study aimed to compare *COMT* Val158Met polymorphisms in patients with schizophrenia, with and without pre-morbid cannabis use, and to investigate whether the use of cannabis has a role in the etiology of schizophrenia in the presence of high activity Val allele.

Patients and Methods

Sample group. The sample group involved 80 male patients between the ages of 18 and 65 who were diagnosed with schizophrenia, according to DSM-IV-TR diagnostic criteria, in partial or full remission and received treatment in psychiatry clinics or outpatient clinics of Bakirkoy Prof. Dr. Mazhar Osman Mental Health and Neurological Diseases Education and Research Hospital. Patients that had any general medical condition, mental retardation or alcohol/substance use disorder were not included in the sample group. While 40 of the patients included in the study had premorbid cannabis use (at least 5 times or more) (13), the other 40 patients, who were appointed as the control group, did not have any history of cannabis use. Earlier history of cannabis use was analyzed and investigated based on the patients' statements on the disease history, family interviews and medical records. As we could not obtain results in the PCR phase of the study, 4 patients with premorbid cannabis use and 2 patients without premorbid cannabis use were excluded from the study. Eventually, the sample group involved 74 patients, 36 of whom had pre-morbid cannabis use while the other 38 patients had no pre-morbid cannabis use. For clinical evaluation, sociodemographic form and the positive and negative syndrome scale (PANSS) (14) were applied to all patients. This study was approved by the local ethics committee of Bakirkoy Prof. Dr. Mazhar Osman Mental Health and Neurological Diseases Education and Research Hospital.

Genotyping. Polymorphisms were genotyped using the polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) method (15). PCRs were used to amplify the region of *COMT* Val158Met polymorphism. PCR reactions were carried out in a final volume of 25 ml containing 50 ng/ml DNA, 2.5ml of each primer (*COMT* Val158Met Forward: 5'-CGAGGCTCATCACCAT CGAGATCG-3' *COMT* Val158Met Reverse: 5'-CTGACAACGGG TCAGGAATGCA-3') and 2.5 of 10X buffer, 2.5U 2.5 µl Taq polimerase, 25 mM 1.5 µl MgCl, 2.5 mM 3 µl. The PCR reaction was performed as follows: 5 min at 95˚C; then 35 cycles of 45s at 95˚C, 45 s at 62˚C and 45s at 72˚C; finally, 5 min at 72˚C. PCR-RFLP with NlaIII restriction enzyme (MBI Fermentas, address) at 65˚C, respectively, and all the final samples were evaluated by agarose gel electrophoresis (2%). After separation of products on 2% agarose gel, three possible genotypes were defined by three distinct patterns of bands seen on the gel for both polymorphisms.

Table I. *Sociodemographic and clinical characteristics of the patients*.

Cases, N=74	
Age: mean±SD	35.42±7.65
Total education years: mean±SD	8.97±3.85
Employment	
Employed	%12.2 (n=9)
Umployed	%87.8 (n=65)
Marital status	
Married	%9.5 (n=7)
Single	% 90.5(n=67)
Age of first episode : mean±SD	21.6±4.55
Number of hospitalizations: mean±SD	4.44±4.99

Statistical methods. The obtained data were expressed using the average, standard deviation (SD), frequency and percentage values in the statistical analysis of the study. Frequencies and percentages were compared between the groups using the Chi-square test and Fisher's exact test, when appropriate. Relative risk at 95% confidence intervals (CI) was calculated as the odds ratio (OR). The Student's *t*-test was used to compare averages of qualitative data between the groups. $p<0.05$ was set as the significance level. Statistical analyses were carried-out with the SPSS package program (revision 11.5, SPSS Inc., Chicago, IL, USA).

Results

Table I shows sociodemographic and clinical features of the patients included in the study. As only 2 patients had the Met/Met polymorphism, the patients were divided into two groups according to their enzymatic activities as Val/Val genotype group (the group with high enzyme activity) and Val/Met- Met/Met genotype group (the group with low enzyme activity), while they were statistically analyzed (16).

It has been identified that the rate of individuals with the Val/Val genotype is significantly higher in patients with pre-morbid cannabis use (88.9%) compared to patients without pre-morbid cannabis use (68.4%) and that the Val/Val genotype increases the risk of the disease 3.69-fold (Table II).

The mean total PANSS score and the mean PANSS aggression subscale score of the Val/Val genotype group is significantly higher than the scores of the patients with Met allele (Table III).

Discussion

The most important finding of our study is that the rate of individuals with the Val/Val genotype is significantly higher in patients with pre-morbid cannabis use (88.9%), compared to patients without pre-morbid cannabis use (68.4%) and that the Val/Val genotype increases the risk of the disease by 3.69-fold. As it was reported in similar recent studies, we found the use of

Table II. *Comparison of the Val/Val genotype and Val/Met+Met/Met genotypes by premorbid cannabis users and non-users.*

Genotypes of *COMT* Val 158Met	All groups (N=74)	Cannabis (+) schizophrenia (N=36)	Cannabis (–) schizophrenia (N=38)			
	N (%)	N (%)	N (%)	p	OR	%95CI
Val/Val	58 (%78.4)	32 (%88.9)	26 (%68.4)	0.030*	3.69	1.06-12.81
Val/Met+Met/Met	16 (%21.6)	4 (%11.1)	12 (%31.6)			

Fisher's exact test; *$p<0.05$; OR: Odds ratio; CI: Confidence intervals.

Table III. *Comparison of the clinical features according to the COMT Val/Val genotype.*

N=74	Val/Val N=58	Val/Met+Met/Met N:16	t	*p*
+ Age of first episode : mean ±SD	21.20±0.6	23.06±0.92	–1.55	n.s.
+ Number of hospitalizations: mean±SD	4.50±0.6	4.25±0.99	0.01	n.s.
PANSS total score: mean±SD	58.18±1.9	50.06±2.85	0.20	0.04*
PANSS positive subscale: mean±SD	15.24±0.7	12.56±1.4	0.68	n.s.
PANSS negative subscale: mean±SD	16.8±5.9	14.75±1.5	–0.96	n.s.
PANSS general psychopathology subscale: mean±SD	22.15±5.19	19.56±0.88	0.71	n.s.
PANSS aggression subscale: mean±SD	3.89±0.19	3.18±0.75	0.82	0.01**

Student *t*-test, *$p<0.05$ **$p<0.01$; n.s.: non-significant; PANSS: positive and negative syndrome scale; SD: Standard deviation.

cannabis to be associated with the Val158Met polymorphism of the *COMT* gene in the context of gene × environment interaction in the etiology of schizophrenia (4, 8, 12, 17). Caspi *et al*. (4) reported that the risk of developing schizophreniform disorder at the age of 26 or older after adolescent cannabis exposure is 10.9-times higher for individuals homozygous for *COMT* Val158Met Val allele.

It has been suggested that cannabis use in adolescence, which is a critically important period for brain development (18), may make a causal contribution to the development of psychosis (2, 4). As the present work is a retrospective study, the failure to exactly determine the onset of cannabis use constitutes a limitation for the study.

The studies supporting the correlation between *COMT* Val158Met polymorphism and cannabis use (8, 9) have revealed the importance of gene × environment interactions. The effect of cannabis use on the increase in hallucination levels in Val/Val patients is consistent with the tonic-phasic dopamine hypothesis and the results of the studies on abnormal reactivity of the dopamine system to environmental stimuli. It has been deducted that as the *COMT* Val158Met Val allele is associated with low prefrontal dopamine activity, mesolimbic phasic dopamine exhibits less tonic inhibition. The mesolimbic dopamine system has an important role in the development of psychotic symptoms. Although neither cannabis nor *COMT* Val158Met polymorphism is sufficient alone to affect that phenomenon, the combination of these two may create a significant effect (9).

There are also studies not supporting the correlation between *COMT* Val158Met polymorphism and premorbid cannabis use (10, 19). Zammit *et al*. (19) suggested that there is a relationship between cannabis use and schizophrenia; however, that relationship is not associated with *COMT* Val158Met polymorphism. Kantrowitz *et al*. (10) investigated the correlation between cannabis use and development of schizophrenia spectrum disorders in African-American and White American patients diagnosed with schizophrenia, schizoaffective disorders and psychotic disorder not otherwise specified and found no significant correlation in either group. Costas *et al*. (17) used a method similar to this study and analyzed cannabis use retrospectively in a sample group consisting of Spanish subjects. In that study it was indicated that the interaction between cannabis and *COMT* gene polymorphism is associated with the development of schizophrenia.

Earlier studies (12, 20) have identified that the *COMT* gene polymorphism and cannabis use predispose the individual to psychosis and lead to earlier onset of the disease. According to those studies, while the age of onset is earliest in the Val/Val homozygous patients, it is the latest age in the Met/Met patients and in-between in the Val/Met patients. In this study, although the age of onset is earlier in patients with the Val/Val genotype compared to the patients with the Met allele, the difference is not significant. This may be due to the low number of patients included in the sample group.

The mean total PANSS score of the Val/Val genotype group is significantly higher compared to patients with the Met allele. Pelayo-Teran *et al*. (12) found in their study that the severity of negative symptoms is significantly higher in patients with Val-homozygous genotype and considered their finding to be consistent with the assumption that negative symptoms become more severe when the dopamine activity is low in the dorsolateral prefrontal cortex. In this study, the total PANSS scores are significantly higher in the Val/Val genotype group; however, there is no significant difference between the groups regarding the positive and negative sub-scale scores. Although the scores of both subscales are higher in patients with the Val/Val genotype, this difference has not reached the significance level. This may be because the total number of the subjects is low and the rate of Met allele patients is relatively lower in the sample group.

In conclusion, we found a significant correlation between pre-morbid cannabis use and *COMT* Val/Val genotype in patients diagnosed with schizophrenia. Also, the results of this study support the findings of gene × environment interaction in the cannabis–psychosis relationship. The genetic predisposition caused by the *COMT* Val158Met polymorphism may have an important role in the development of schizophrenia and other psychotic disorders in patients using cannabis, even years after their exposure to cannabis.

References

1 De Sousa KR, Tiwari AK, Giuffra DE, Mackenzie B, Zai CC and Kennedy JL: Age at onset of schizophrenia: cannabis, COMT gene, and their interactions. Schizophr Res *151(1-3)*: 289-290, 2013.

2 Radhakrishnan R, Wilkinson ST and D'Souza DC: Gone to Pot - A Review of the Association between Cannabis and Psychosis. Front Psychiatry 22(5): 54, 2014.

3 Arseneault L, Cannon M, Witton J and Murray RM: Causal association between cannabis and psychosis examination of the evidence. Br J Psychiatry *184*: 110-117, 2004.

4 Caspi A, Moffitt TE, Cannon M, McClay J, Murray R, Harrington H, Taylor A, Arseneault L, Williams B, Braithwaite A, Poulton R and Craig IW: Moderation of the effect of adolescent-onset cannabis use on adult psychosis by a functional polymorphism in the catechol-O-methyltransferase gene: longitudinal evidence of a gene X environment interaction. Biol Psychiatry *57(10)*: 1117-1127, 2005.

5 Henquet C, Di Forti M, Morrison P, Kuepper R and Murray RM: Gene-environment interplay between cannabis and psychosis. Schizophr Bull *34(6)*: 1111-1121, 2008.

6 Chen J, Lipska BK, Halim N, Ma QD, Matsumoto M, Melhem S, Kolachana BS, Hyde TM, Herman MM, Apud J, Egan MF, Kleinman JE and Weinberger DR: Functional analysis of genetic variation in catechol-O-methyltransferase (COMT): effects on mRNA, protein, and enzyme activity in postmortem human brain. Am J Hum Genet *75(5)*: 807-821, 2004.

7 Meyer-Lindenberg A, Kohn PD, Kolachana B, Kippenhan S, McInerney-Leo A, Nussbaum R, Weinberger DR and Berman KF: Midbrain dopamine and prefrontal function in humans: interaction and modulation by COMT genotype. Nat Neurosci *8(5)*: 594-596, 2005.

8 Henquet C, Rosa A, Krabbendam L, Papiol S, Fananás L, Drukker M, Ramaekers JG and van Os J: An experimental study of catechol-o-methyltransferase Val158Met moderation of delta-9-tetrahydrocannabinol-induced effects on psychosis and cognition. Neuropsychopharmacology *31(12)*: 2748-2757, 2006.

9 Henquet C, Rosa A, Delespaul P, Papiol S, Fananás L, van Os J and Myin-Germeys I: COMT ValMet moderation of cannabis-induced psychosis: a momentary assessment study of 'switching on' hallucinations in the flow of daily life. Acta Psychiatr Scand *119(2)*: 156-160, 2009.

10 Kantrowitz JT, Nolan KA, Sen S, Simen AA, Lachman HM and Bowers MB Jr.: Adolescent cannabis use, psychosis and catechol-O-methyltransferase genotype in African Americans and Caucasians. Psychiatr Q *80(4)*: 213-218, 2009.

11 Zammit S, Spurlock G, Williams H, Norton N, Williams N, O'Donovan MC and Owen MJ: Genotype effects of CHRNA7, CNR1 and COMT in schizophrenia: interactions with tobacco and cannabis use. Br J Psychiatry *191*: 402-407, 2007.

12 Pelayo-Terán JM, Pérez-Iglesias R, Mata I, Carrasco-Marín E, Vázquez-Barquero JL and Crespo-Facorro B: Catechol-O-Methyltransferase (COMT) Val158Met variations and cannabis use in first-episode non-affective psychosis: clinical-onset implications. Psychiatry Res *179(3)*: 291-296, 2010.

13 Veling W, Mackenbach JP, van Os J and Hoek HW: Cannabis use and genetic predisposition for schizophrenia: a case-control study. Psychol Med *38(9)*: 1251-1256, 2008.

14 Kay SR, Fiszbein A and Opler LA: The positive and negative syndrome scale (PANSS) for schizophrenia. Schizophr Bull *13(2)*: 261-276, 1987.

15 Lajin B, Alachkar A, Hamzeh AR, Michati R and Alhaj H: No association between Val158Met of the COMT gene and susceptibility to schizophrenia in the Syrian population. N Am J Med Sci *3(4)*: 176-178, 2011.

16 Bertolino A, Caforio G, Blasi G, De Candia M, Latorre V, Petruzzella V, Altamura M, Nappi G, Papa S, Callicott JH, Mattay VS, Bellomo A, Scarabino T, Weinberger DR and Nardini M: Interaction of COMT (Val(108/158)Met) genotype and olanzapine treatment on prefrontal cortical function in patients with schizophrenia. Am J Psychiatry *161(10)*: 1798-1805, 2004.

17 Costas J, Sanjuán J, Ramos-Ríos R, Paz E, Agra S, Tolosa A, Páramo M, Brenlla J and Arrojo M: Interaction between COMT haplotypes and cannabis in schizophrenia: a case-only study in two samples from Spain. Schizophr Res *127(1-3)*: 22-27, 2011.

18 Van der Knaap LJ, Schaefer JM, Franken IH, Verhulst FC, van Oort FV and Riese H: Catechol-O-methyltransferase gene methylation and substance use in adolescents: the TRAILS study. Genes Brain Behav *13(7)*: 618-25, 2014.

19 Zammit S, Owen MJ, Evans J, Heron J and Lewis G: Cannabis, COMT and psychotic experiences. Br J Psychiatry *199(5)*: 380-385, 2011.

20 Estrada G, Fatjó-Vilas M, Muñoz MJ, Pulido G, Miñano MJ, Toledo E, Illa JM, Martín M, Miralles ML, Miret S, Campanera S, Bernabeu C, Navarro ME and Fañanás L: Cannabis use and age at onset of psychosis: further evidence of interaction with COMT Val158Met polymorphism. Acta Psychiatr Scand *123(6)*: 485-492, 2011.

Received August 29, 2014
Revised September 30, 2014
Accepted October 7, 2014