Original article / *Araştırma*

Investigation of Catechol-O-Methyltransferase and Cannabinoid Receptor 2 gene variants in tobacco use disorder or tobacco use disorder and schizophrenia comorbidity*

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ABSTRACT

Objective: *The purpose of this study was to investigate whether functional variants of Catechol-O-Methyltransferase (COMT) (rs4680) and Cannabinoid Receptor 2 (CNR2) (rs2501432) genes play a role in tobacco use disorder (TUD) or tobacco use disorder and schizophrenia (TUDSch) comorbidity.* ***Methods:*** *This study consisted of 163 participants with TUD, 60 participants with TUDSch, and 106 gender-, age- and ethnicity-matched non-smoker controls (HNC). While the TUD and TUDSch were diagnosed according to the DSM-5, the severity of TUD was rated according to the Fagerstrom Test for Nicotine Dependence. Genotyping of COMT and CNR2 genes was determined using the polymerase chain reaction-restriction fragment length polymorphism method (PCR-RFLP).* ***Results:*** *Distributions of genotypes and allele frequencies were compared among the groups. Patients with TUDSch had higher frequency of COMT Val/Val genotype compared to the TUD and HNC groups (p=0.001, p=0.034, respectively). Patients with TUD had higher frequency of Val/Met genotype than TUDSch and HNC groups (p=0.001, p=0.033, respectively). The frequency of the Val allele was higher in TUDSch than the HNC group, whereas the frequency of the Met allele was higher in TUD than in the TUDSch group (p=0.047, p=0.001, respectively). Additionally, patients with TUD had higher frequency of TT CNR2 genotype than the HNC group (p=0.019).* ***Conclusion:*** *While the Val/Val genotype of the COMT gene is associated with an increased risk for TUDSch, the Val/Met genotype is associated with an increased risk for TUD. Additionally, the TT CNR2 genotype was associated with increased risk for TUD in the Turkish population.* ***(Anatolian Journal of Psychiatry 2020; 21(x):xxx-xxx)***

Keywords: *COMT, CNR2, tobacco, schizophrenia, PCR-RFLP*

Tütün kullanım bozukluğu veya tütün kullanım bozukluğu ve şizofreni komorbiditesinde Katekol-O-Metiltransferaz ve Kannabinoid Reseptör 2 gen varyantlarının incelenmesi

ÖZ

Amaç: *Bu çalışmanın amacı, Katekol-O-Metiltransferaz (COMT) (rs4680) ve Kannabinoid Reseptör 2 (CNR2)*

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(rs2501432) genlerinin işlevsel varyantlarının tütün kullanım bozukluğu (TUD) veya tütün kullanım bozukluğu ve şizofreni (TUDSch) komorbiditesinde rol oynayıp oynamadığını araştırmaktır. ***Yöntem:*** *Bu çalışmanın örneklemini 163 TUD, 60 TUDSch ve 106 cinsiyet, yaş ve etnisite eşleşmeli sigara içmeyen kontrol grubu (HNC) oluşturmuştur. TUD ve TUDSch tanıları DSM-5 tanı ölçütlerine göre konulurken, TUD şiddeti Fagerstrom Nikotin Bağımlılığı Testine göre derecelendirilmiştir. COMT ve CNR2 genlerinin genotiplendirilmesi, polimeraz zincir reaksiyonu ve restriksiyon parça uzunluk polimorfizmi yöntemi kullanılarak belirlenmiştir.* ***Bulgular:*** *Gruplar arasında genotip ve allel frekans dağılımları karşılaştırılmış olup COMT val/val genotipi sıklığı TUDSch hastalarında, TUD ve HNC gruplarına göre daha fazla bulunmuştur (sırayla p=0.001, p=0.034). Val/met genotipi sıklığı, TUD hastalarında, TUDSch ve HNC gruplarına göre daha fazla bulunmuştur (sırayla p=0.001, p=0.033). Val alelinin sıklığı TUDSch hasta grubunda HNC grubuna göre daha fazla saptanmış, met alelinin sıklığı ise TUD hasta grubunda, TUDSch hasta grubuna göre daha fazla bulunmuştur (sırayla p=0.047, p=0.001). Ek olarak, TUD hastalarında TT CNR2 genotipi sıklığı HNC grubuna göre daha fazla saptanmıştır (p=0.019).* ***Sonuç:*** *COMT geninin val/val genotipi TUDSch için artmış bir riskle ilişkilendirilmiş olup, val/met genotipi ise TUD için artmış riskle ilişkilendirilmiştir. Ek olarak, Türk popülasyonunda TT CNR2 genotipi artmış TUD riski ile ilişkili bulunmuştur.* ***(Anadolu Psikiyatri Derg 2020; 21(x):xxx-xxx)***

Anahtar sözcükler: *COMT, CNR2, tütün, şizofreni, PCR-RFLP*

INTRODUCTION

Smoking tobacco is a multifactorial behavior with genetic and environmental determinants. Genetic factors are responsible for smoking tobacco, quitting success, risk of withdrawal symptoms, and death rates associated with tobacco use.[1] Nicotine, the major psychoactive ingredient in tobacco, regulates dopamine which contributes to the development and maintenance of reward-related behaviors in the middle brain, such as smoking. Smokers regulate their smoking activity to maintain brain nicotine levels within an optimal concentration range.[2] Polymorphisms of genes associated with both nicotine metabolism and dopamine catabolism take part in the risk specifically related to tobacco use.

Catechol-O-methyltransferase (COMT) is the primary enzyme responsible for the metabolism of dopamine in cortical regions of the brain. The COMT gene is found on chromosome 22q11.21, has eight exons and produces 271 amino acids, which metabolize catecholamines.[3] COMT gene polymorphisms are associated with the activity of the enzyme: higher activity is associated with the COMT val (valine) allele, and lower activity is linked to the COMT met allele.[4,5] In codon 158 (in the rs4680 polymorphism) of the COMT gene, low enzymatic activity of the met allele which provides metabolic inactivation of dopamine is associated with nicotine dependence.[6] Val-allele carriers, however, have been reported to show a lower likelihood of smoking cessation and a higher probability of relapse compared to met/met homozygotes, suggesting that COMT may impact cognitive function.[7] Loughead et al. has shown that individuals with tobacco use disorder who have the val/val COMT genotype are more susceptible to cognitive dysfunction during tobacco deprivation compared to met-allele carriers.[8] The presence of the Val allele has been associated with poor performance in cognitive domains such as executive functioning due to impaired dorsolateral prefrontal cortex (DLPFC) activity in both the control group and in patients with schizophrenia.[9,10]

While tobacco smoking prevalence is 20% in the general population, the prevalence ranges from 45% to 88% in patients with schizophrenia and schizoaffective disorder.[11] The high rate of smoking in patients with schizophrenia is typically considered to be an attempt to correct neuropathology of nicotinic acetylcholine receptors (nAChRs) and/or dopaminergic systems associated with schizophrenia using exogenous nicotine.[12] Nicotine directly binds to high-affinity nAChRs in cortical areas and increases dopamine release from dopaminergic projections; additionally, nicotine activates low-affinity nAChRs to increase release of dopamine in the cortex via excitatory glutamatergic projections to the PFC.[13,14] These effects are thought to underlie the cognitive-enhancing properties of smoking.[15,16]

The endocannabinoid system plays an important role in the predisposition of individuals to substance abuse. Two types of cannabinoid receptors, CB1 and CB2, have been identified.[17] Although CB2 receptors are expressed at much lower levels in the CNS compared to CB1 receptors,18 recent studies have demonstrated a potential role for CB2 receptors in the neurobiology of psychiatric disorders.[19] In recent years, there have been significant reports concerning the role of CB2 receptors in addiction. Preclinical studies have indicated that CB2 receptors regulate cocaine, alcohol and nicotine intake.[18,20,21] CB2 receptors additionally play an important role in regulating the reinforcing and rewarding effects as well as behavioral expression of nicotine withdrawal syndrome.[20] CB2 is encoded by the CNR2 gene, mapping on

1p36.11 and Okahisa et al. reported that the Q63R variant of CNR2 gene did not affect the clinical phenotypes of methamphetamine dependence with psychosis or methamphetamine psychosis in Japanese society.[22] One of our previous studies identified that the frequency of CNR2 rs2229579 T/T genotype was significantly higher in patients with substance use disorder compared to the control group, and the frequency of C/C genotype was higher in the control group.[23]

In this study, we aimed to investigate whether functional variants of COMT and CNR2 genes are involved in the etiopathogenesis of nicotine dependence among patients with a comorbid diagnosis of tobacco use disorder (TUD) and schizophrenia with tobacco use disorder (TUDSch) by comparing these individuals to healthy non-smoker controls (HNC).

METHODS

Patient selection and procedures for blood samples

The participants were informed in detail about the purpose, methods and procedures of the study, and their written consent was obtained. Diagnosis of TUD and schizophrenia was performed according to the DSM-5, while the severity of TUD was rated following the guidelines of the Fagerstrom Test for Nicotine Dependence (FTND). Participants with a FTND score of seven and higher were enrolled in the study. Patients with TUD were taken consecutively from Yedikule Chest Diseases and Thoracic Surgery Training and Research Hospital smoking cessation outpatient clinic, and patients with schizophrenia comorbidity with TUD were taken from Istanbul Bakırköy Mazhar Osman Mental Health and Neurological Diseases Training and Research Hospital outpatient clinic. To obtain a healthy control group without TUD, individuals without any physical and mental health problems were included in the study. We included 60 patients with TUDSch (45 female [F], 15 male [M]), 163 individuals with TUD (74 F, 89 M) and 106 gender-, age- and ethnicity-matched non-smoker controls. While all DNA materials of 106 healthy controls were studied for the COMT 158 gene polymorphism since adverse enzyme conditions in the laboratory 100 healthy controls could be examined for the CNR2 rs2501432 gene polymorphism (HNC; 61 F, 39 M). In 88 of the 163 participants with TUD, CNR2 genes were investigated, whereas COMT genes were studied in 59 of 60 TUDSch participants. Genotyping of COMT (rs4680) and CNR2 (rs2501432) genes were determined using polymerase chain reaction-restriction fragment length polymerphism (PCR-RFLP) for both patient and control groups.[23] Concomitant neurodegenerative diseases, autism spectrum disorders, intellectual disability, chronic physical diseases, pregnancy, and psychiatric disorders secondary to the organic cause were determined as an exclusion criterion. This study protocol was approved by the Local Ethics Committee (2014/1195) and all the procedures performed in the study were in accordance with the Declaration of Helsinki.

Diagnostic tools and scales

Sociodemographic and Clinical Characteristics Data Form: A detailed interview data form prepared by the researchers was used and included questions about clinical information such as sociodemographic characteristics, nicotine dependence and history of disorder which was diagnosed according to the DSM-5 criteria.

Fagerstrom Test for Nicotine Dependence (FTND): First proposed in 1978 and developed in 1991,[24] the FTND consists of six questions. It is easily understood and rapidly applied. FTND scores are typically grouped into distinct levels, namely low nicotine dependence (0-3 points), medium nicotine dependence (4-6 points) and high nicotine dependence (≥7 points). A Turkish validation study of the test was performed by Uysal et al.[25] and found the FTND to be moderately reliable.[26]

Statistical analyses

Statistical analysis was performed using IBM SPSS version 21.0 (IBM Corp. released 2012; Armonk, NY, USA). Statistical significance of the differences between the patients and control groups was estimated using a logistic regression analysis. The results were statistically analyzed by calculating the odds ratios (OR) and 95% confidence intervals (CI) using the chi-square test. Statistical significance was accepted as $p < 0.05$ for the results of all analyses.

RESULTS

Comparing genotypes and allele frequencies among the participant groups revealed that the TUDSch group had a higher number of participants with the COMT (rs4680) Val/Val genotype compared to the TUD and HNC groups (p=0.001, p=0.034, respectively). In the TUD group, the val/met genotype frequency was higher compared to the TUDSch and HNC

groups (p=0.001, p=0033, respectively). Val allele frequency was higher in the TUDSch group compared to the HNC group, whereas met allele frequency was significantly higher in the TUD group than in the TUDSch group (p=0.047, p=0.001, respectively) (Table 1).

The TT CNR2 genotype was more common in the TUD group than in the HNC group (p=0.019). Based on these findings, we concluded that the COMT (rs4680) val/val genotype may be a risk factor for the TUDSch group, and the val/met genotype may be a risk factor for the TUD group (val/met: heterozygote disadvantage). Additionally, a significant increase in the frequency of the TT CNR2 genotype in the TUD group revealed that this genotype may play a role in predisposition to nicotine dependence (Table 2).

Table 1. Comparison of the COMT 158 polymorphism among participants with TUDSch, TUD, and HNC

COMT 158 Genotypes	TUDSch (n=59) Sayı	%	TUD (n=163) Sayı	%	HNC (n=106) Sayı	%	OR*	%95 CI*	p
val/val	32	54.2	35	21.5	39	36.8	0.491[a]	0.257-0.938[a]	**0.034**[a]
							1.375[b]	0.794-2.381[b]	0.261[b]
							0.231[c]	0.122-0.435[c]	**0.001**[c]
val/met	15	25.4	84	51.5	40	37.7	1.778[a]	0.878-3.600[a]	0.123[a]
							0.570[b]	0.346-0.938[b]	**0.033**[b]
							3.119[c]	1.609-6.044[c]	**0.001**[c]
met/met	12	20.5	44	27.0	27	25.5	1.339[a]	0.620-2.891[a]	0.567[a]
							0.869[b]	0.449-1.513[b]	0.675[b]
							1.448[c]	0.703-2.981[c]	0.383[c]
Allele									
val	79	66.9	154	47.2	118	55.7	0.620[a]	0.387-0.991[a]	**0.047**[a]
met	39	33.1	172	52.8	94	44.3	1.402[b]	0.991-1.984[b.]	0.064[b]
							2.262[c]	1.456-3.516[c]	**0.001**[c]

** Fisher's Exact Test; [a]: comparison of genotype frequencies between TUDSch and HNC groups; [b]: comparison of genotype frequencies between TUD and HNC groups; [c]: comparison of genotype frequencies between TUDSch and TUD. TUDSch: Tobacco Use Disorder+Schizophrenia; TUD: Tobacco Use Disorder; HNC: Healthy Non-smoker Controls*

Table 2. Comparison of the CNR2 rs2501432 polymorphism among participants with TUDSch, TUD, and HNC.

CNR2 genotypes	TUDSch (n=60) Sayı	%	TUD (n=88) Sayı	%	HNC (n=100) Sayı	%	OR*	%95 CI*	p
T/T	6	10.0	21	23.8	11	11.0	1.112[a]	0.389-3.181[a]	1.000[a]
							0.383[b]	0.172-0.848[b]	**0.019**[b]
							2.821[c]	1.063-7.483[c]	0.050[c]
T/C	30	50.0	32	36.4	43	43.0	0.754[a]	0.397-1.434[a]	0.416[a]
							1.273[b]	0.706-2.296[b]	0.456[b]
							0.836[c]	0.433-1.613[c]	0.619[c]
C/C	24	40.0	35	39.8	46	46.0	1.278[a]	0.668-2.446[a]	0.512[a]
							1.241[b]	0.693-2.223[b]	0.553[b]
							0.782[c]	0.403-1.517[c]	0.504[c]
Allele									
T	42	35.0	74	42.0	65	32.5	0.894[a]	0.555-1.442[a]	0.714[a]
C	78	65.0	102	58.0	135	67.5	0.664[b]	0.436-1.011[b]	0.069[b]
							1.347[c]	0.834-2.177[c]	0.228[c]

** Fisher's Exact Test; [a]: comparison of genotype frequencies between TUDSch and HNC groups; [b]: comparison of genotype frequencies between TUD and HNC groups; [c]: comparison of genotype frequencies between TUDSch and TUD. TUDSch: Tobacco Use Disorder+Schizophrenia; TUD: Tobacco Use Disorder; HNC: Healthy Non-smoker Controls*

DISCUSSION

The val158met polymorphism is located in exon three of the COMT gene (rs4680).[27] The met (A) allele promotes reduced activity of the gene, which results in three- to four-fold decrease in COMT enzyme activity, suggesting that lower enzyme activity produces a relative increase in dopamine activity as a result of decreased inactivation.[28] In our study, a significantly higher frequency of the val/met genotype was identified in the TUD group compared to the TUDSch and HNC groups. Furthermore, higher frequency of the COMT (rs4680) val/val genotype was found in the TUDSch group compared to the TUD and HNC groups. When the studies investigating the relationship between COMT and nicotine dependence are reviewed in the literature, it is seen that several studies have pointed out the potential mechanism by which COMT val158met polymorphism can modulate the risk of nicotine dependence and treatment response.[29]

The val allele is also associated with a three- to four-fold increase in enzymatic activity and a reduction in prefrontal dopamine levels.[5,27] Previous studies have suggested that the COMT val allele is associated with increased susceptibility to nicotine use disorder as well as high relapse risk.[7,30] A 2010 meta-analysis concluded that the val/val genotype may be a risk factor for the development of nicotine use disorder.[31] In addition, COMT val carriers exhibit poorer performance compared to met allele carriers in both working memory and continuous attention.[32,33] Furthermore, it has been shown that val/val patients with smoking tobacco may be more sensitive to changes in executive functioning of prefrontal brain in abstinence compared to met allele carriers.[8] Smoking-induced dopamine is increased in individuals with TUDs homozygous for the COMT val allele, and this in turn may contribute to the rewarding effects of nicotine.[34] In both the control group and patients with schizophrenia, the presence of the val allele has been associated with poor performance in cognitive domains due to impaired DLPFC activity, such as executive function and working memory.[9,10] Since patients diagnosed with schizophrenia exhibit poor cognitive performance, and nicotine-like agonists have restorative effects on sensory gating deficiencies,[12] the higher rate of the COMT (rs4680) val/val genotype in the TUDSch group compared to the TUD and HNC groups (p=0.001 and 0.034, respectively) may be interpreted as a compensatory genetic background. Nicotine administration and smoking have been found to be effective in improving neuropsychological deficits associated with Sch. In support of this, the sensory information processing deficits of schizophrenia appear to be sensitive to the nicotinic system.[35] Individuals with TUDs diagnosed with schizophrenia suffer from more severe cognitive dysfunction during deprivation than individuals without schizophrenia, and cognitive deficits are associated with failure in smoking cessation.[36,37] The increase in dopamine release in the PFC is responsible for the pro-cognitive effects of nicotine.

Individuals diagnosed with schizophrenia have greater difficulty quitting smoking; the rate of smoking cessation among people without any mental disorders or addiction is 42.5%, while smoking cessation among individuals with psychotic disorders ranges between 10% and 27.2%.[38,39] Several studies have reported a relationship between the val/val genotype and a poor response to smoking cessation therapies; [7,30,40] however, other studies that have reported opposite results.[41,42] Low rates of smoking cessation in patients with schizophrenia may be explained by the fact that the val/val genotype dominated the patient group in our study. Taken together, these findings suggest that smoking cessation may be particularly challenging for smokers with the val/val genotype. Understanding the genetic basis of withdrawal-related cognitive dysfunction in schizophrenia may assist in developing smoking cessation therapies and cognitive enhancers for this population that is challenging to treat. Therefore, the role of the COMT genotype should be investigated in larger samples. Accordingly, COMT genotyping may help detect the TUD patients with the highest risk of cognitive impairment on those who quit smoking and guide the development of more effective treatments, such as specific treatment involving neurocognitive treatment therapies.[43]

Several neuropsychiatric disorders are associated with polymorphisms of the CNR2 gene. A low level of CNR2 function has been associated with increased risk of schizophrenia, depression, drug abuse, eating disorders and autism spectrum disorders.[44] While no previous studies have investigated CNR2 gene polymorphisms in nicotine dependence, we found that the frequency of TT genotype was significantly higher in the TUD group compared to the other two groups, suggesting that this gene variant may confer risk for nicotine use disorder. Compared to the TUDSch group, the frequency of TT genotype in the TUD group was at the limit of significance (p=0.05). A

previous study from our group found that, compared to the control group, the frequency of the CNR2 rs2229579 T/T genotype was significantly higher in patients diagnosed with substance use disorder, of whom 98% were cigarette smokers and 66% were polysubstance users; in contrast, the C/C genotype was found at a higher rate in the control group.[23] The results of both of our studies indicate that the T/T genotype is associated with predisposition to substance use disorders.

If the limitations of the study should be considered, results must be evaluated with attention as a relatively small sample size can limit the statistical power. Again this study must be studied in different ethnic groups with a larger sample size in terms of validating results. In conclusion, the results of our study suggest that the val/val genotype of the COMT gene (rs4680) may be associated with increased risk for TUDSch, while the val/met genotype is associated with increased risk for TUD. Furthermore, the TT CNR2 (rs2501432) genotype was associated with increased risk for TUD in the Turkish population. Further studies with larger groups and different ethnicities are needed to determine the impact of these gene variants on the risk of developing TUD and TUDSch.

Authors' contributions: *S.P.: planning, design, materials and laboratory samples, revised the manuscript, supervision of the study; P.Ç.A.: planning, design, revised the manuscript, supervision of the study; H.M.A.: design, drafted the manuscript, revised the manuscript; M.A.U.: design, materials and laboratory samples, revised the manuscript; Ü.S.: materials and laboratory samples, revised the manuscript; M.P.: acquired, analyzed, and interpreted of all data; revised the manuscript, supervision of the study.*

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