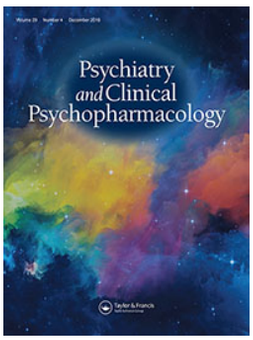

Psychiatry and Clinical Psychopharmacology

ISSN: 2475-0573 (Print) 2475-0581 (Online) Journal homepage: https://www.tandfonline.com/loi/tbcp21

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To cite this article: Selin Kurnaz, Ahmet Bulent Yazici, Ayse Feyda Nursal, Pinar Cetinay Aydin, Ayca Ongel Atar, Nazan Aydin, Zeliha Kincir & Sacide Pehlivan (2019) CNR2 rs2229579 and COMT Val158Met variants, but not CNR2 rs2501432, IL-17 rs763780 and UCP2 rs659366, contribute to susceptibility to substance use disorder in the Turkish population, Psychiatry and Clinical Psychopharmacology, 29:4, 847-853, DOI: 10.1080/24750573.2019.1688030

To link to this article: https://doi.org/10.1080/24750573.2019.1688030

Published online: 03 Dec 2019.

PSYCHIATRY AND CLINICAL PSYCHOPHARMACOLOGY
2019, VOL. 29, NO. 4, 847–853
https://doi.org/10.1080/24750573.2019.1688030

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CNR2 rs2229579 and COMT Val158Met variants, but not CNR2 rs2501432, IL-17 rs763780 and UCP2 rs659366, contribute to susceptibility to substance use disorder in the Turkish population

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ABSTRACT

OBJECTIVE: Substance use disorders (SUD) are among the most important public health problems throughout the world. We investigated whether COMT (Val108/158Met), CNR2 (rs2501432 and rs2229579), UCP2 (rs659366), and IL-17 (rs763780) gene variants were associated with SUD and its clinical parameters in a Turkish population.

METHODS: We conducted a case–control study among 136 subjects with SUD and 100 healthy controls. Six variants were analysed by the PCR-RFLP method.

RESULTS: The CNR2 rs2229579 T/T genotype and T allele increased in SUD groups than controls while the C/C genotype and C allele were more prevalent in the control group compared to the SUD group ($p = 0.000$ and $p = 0.001$, respectively). The COMT Val108/158Met Val/Val genotype and Val allele were significantly associated with polysubstance abuse ($p < 0.05$). There was no significant difference between the SUD group and control group regarding genotype and allele frequencies of COMT (Val108/158Met), CNR2 (rs2501432), UCP2 (rs659366) and IL-17 (rs763780) variants.

CONCLUSIONS: This is the first study that discussed the relation of these variants and SUD patients in the Turkish population. The results of the analysis indicated that the CNR2 rs2229579 variant has an effect on susceptibility to SUD, suggesting that this variant might play a role in the physiopathology of SUD. The COMT Val108/158Met variant might be an important factor affecting polysubstance use.

ARTICLE HISTORY
Received 9 August 2019
Accepted 8 October 2019

KEYWORDS
Substance use disorder; variant; PCR-RFLP; COMT; CNR2; UCP2; IL-17

Introduction

A "substance" refers to any psychoactive compound that can lead to health and social problems, such as addiction. These substances may be legal or illicit. Substance use disorders (SUDs) manifest a global threat to public health and bear a devastating social and economic impact on individuals as well as their families. According to DSM-V [1], SUD are classified depending on the types of pathological behaviours and four classes of criteria (impaired control, social impairment, risky use, and pharmacological criteria). The mechanism of addiction formation has distinct steps: the beginning of substance use, the transition from trying out to regular use, and the real development of addiction. Environmental factors such as friend pressure, parental monitoring, and the availability of a substance play an important role in the initial attempt to drink, smoke, or take illegal drugs. Following the first step, the transition from regular substance use to dependence varies among the individuals and is largely subject to genetic control [2]. The role of genetic factors in the development of alcohol and other drug addiction has been shown by many family, twin, and adoption studies in general population samples.

Catechol-*O*-methyltransferase (COMT) is an enzyme found all over the mammalian central nervous system which breaks down the catecholamine neurotransmitters dopamine, epinephrine, and norepinephrine. A common G-to-A transition in exon 4 of the COMT gene, causing a valine (val)-to-methionine (met) substitution at the amino acid position 108 or 158 (depending on the splice variant), results in a four-fold reduction in enzyme activity in met homozygotes, whereas heterozygotes manifest intermediate activity [3]. The endocannabinoid system plays a role in susceptibility to substance abuse. There are two well-defined cannabinoid receptors (CNRs), CNR1/CB1 and CNR2/CB2, that mediate endocannabinoid signalling [4]. CNR2 has been classically defined as the peripheral cannabinoid receptor because CNR2 is expressed principally in some peripheral and immune cells [5]. Uncoupling protein 2 (UCP2) is a member of an anion-carrier protein family found in the mitochondrial inner membrane. In the central nervous

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system, mammalian *UCP2* mRNA and protein expression occurs at highest levels in regions that could be described as high-risk for stress [6]. The Interleukin 17 (IL-17 or IL-17A) is a fundamental pro-inflammatory cytokine that is primarily released from T cells and is now believed to be the defining cytokine of a recently discovered new subset of T-helper cells, Th17 [7]. New studies have reported that cells of the central nervous system also express IL-17.

Therefore, in this study, we investigated whether COMT (Val108/158Met rs4680), CNR2 (rs2501432, 315A/G), CNR2 (rs2229579, 1073C/T), UCP2 (rs659366, −866A/G) and IL-17 (rs763780, −7488A/G) gene variants were associated with SUD and its clinical parameters in a Turkish population.

Methods

Study population

This case–control study included 136 subjects with SUD (female/male: 4/132) and 100 age-matched healthy controls (female/male:52/48). The subjects with SUD were selected from among the individuals with a positive urine test in the Department of Psychiatry, Bakirkoy Research and Training Hospital for Psychiatry Hospital, Istanbul Turkey and Department of Psychiatry, Sakarya University Training and Research Hospital, Sakarya Turkey [8]. The healthy control group was similar in terms of age and sex distribution; with 100 healthy individuals who did not have a personal history of any psychiatric disorder and chronic use of any drugs. All members of the patient and control groups were of the same ethnic origin, declared as Turkish ethnicity. Informed written consent was obtained from all the subjects. This study protocol was approved by the Local Ethics Committees of Istanbul University, Faculty of Medicine (2015/1945), in accordance with the ethical standard for human experimentation established by the Declaration of Helsinki.

Genotyping

All study participants provided 2 ml of venous blood from the antecubital vein, which was added to 1% ethylenediaminetetra-acetic acid tubes for DNA isolation. DNA was extracted from peripheral blood samples using a salting-out procedure [9]. The genotypes of COMT (Val108/158Met), CNR2 (rs2501432 and rs2229579), UCP2 (rs659366), and IL-17 (rs763780) gene variants were determined by the PCR-RFLP method as described by previous methods [10–13]. Primer sequences used and PCR conditions in the study are shown in Table 1.

Statistical analysis

Statistical analyses were done by SPSS (Statistical Package for Social Sciences, Chicago, IL, USA) 22.0 package program. Differences between patients and controls in distribution of genotype were compared using χ^2-test or Fisher's exact test as appropriate. The relative risk associated with rare alleles was estimated as an odds ratio (OR) with a 95% confidence interval (CI). All p values ≤ 0.05 were accepted as statistically significant.

Results

A total of 136 patients with SUD and 100 controls were included in the present study. The mean age of the SUD group and control group were 29.17 ± 7.99 and 31.01 ± 10.77 years, respectively. Gender, age, marriage, education, working condition, smoking, and alcohol use of SUD patients were analysed. The baseline and demographic features of the study participants are presented in Table 2. A significant difference in the gender, education status, smoking, and alcohol use was observed between the patient and the control group. The majority of the patient group consisted of males ($p < 0.001$). Education level was lower in the SUD group than control ($p < 0.001$). Smoking and alcohol use were increased in the SUD group than control ($p < 0.001$ and $p < 0.001$, respectively).

We also evaluated characteristics of SUD. These characteristics are presented in Table 3. The allele and genotype frequencies of CNR2 rs2229579 variant in the SUD group and control group are summarized in Table 4. CNR2 rs2229579 T/T genotype increased in SUD groups than controls, while the C/C genotype

Table 1. Primer sequences and PCR conditions of the gene variants.

Variants	Primer sequences	Annealing temperature-Cycle number	Restriction enzyme	References
COMT Val108Met	F: 5′-TCG TGG ACG CCG TGA TTC AGG-3′ R: 5′-AGG TCT GAC AAC GGG TCA GGC-3′	55°C/35	*Nla III*	[9]
COMT Val158Met	F: 5′-ACT GTG GCT ACT CAG CTG TG-3′ R: 5′-CCT TTT TCC AGG TCT GAC AA-3′	58°C/35	*Nla III*	[9]
CNR2 rs2501432	F: 5′- GAA CAG GTA TGA GGGCTT TCG GCG G-3′ R: 5′-CAC CCC ATG GAG GAA TGC TGG GTG ACA G-3′	67°C/35	*MspI*	[10]
CNR2 rs2229579	F: 5′-CTC TGC CCA TCA CTG CCG G-3′ R: 5′-GGG TCC GTG TCT AGG TGT CTG G-3′	57°C/35	*BanII*	[10]
UCP2 rs659366	F: 5′-CAC GCT GCT TCT GCC AGG AC-3′ R: 5′-AGG CGT CAG GAG ATG GAC CG-3′	65°C/35	*MluI*	[12]
IL-17 rs659366	F: 5′-GTG TAG GAA CTT GGG CTG CAT CAA T-3′ R: 5′-AGC TGG GAA AAA CAA AC-3′	52.2°C/35	*Nla III*	[11]

Table 2. Baseline and demographics features of the patients and controls.

	SUD group *n* (%)	Control group *n* (%)	*p*	OR
Age	29.17 ± 7.99	31.01 ± 10.77		
Gender				
Male	132 (97.1)	48 (48.0)	**<0.001**	35.75
Female	4 (2.9)	52 (52.0)		
Marriage			0.891	0.938
Single	71 (56.3)	55 (57.9)		
Married	55 (43.7)	40 (42.1)		
Education				
İlliterate	9 (7.1)	0 (0)	**<0.001**	
Primary school	26 (20.5)	8 (8.4)		
Secondary school	64 (50.4)	4 (4.2)		
High school	26 (20.5)	8 (8.4)		
College	2 (1.6)	75 (78.9)		
Working condition			0.498	1.222
Working	66 (52.4)	45 (47.4)		
At rest	60 (47.6)	50 (52.6)		
Smoking			**<0.001**	
Yes	125 (97.7)	0 (0)		
No	3 (2.3)	100 (100)		
Alcohol use			**<0.001**	
Yes	57 (44.5)	0 (0)		
No	71 (55.5)	100 (100)		

Note: The results that are statistically significant are shown in boldface.

was higher in the control group compared to the SUD group ($p = 0.000$). Also, the CNR2 rs2229579 T allele was more prevalent in the SUD group than the patient group ($p = 0.000$).

Genotype and allele frequencies of COMT (Val108/158Met), CNR2 (rs2501432), UCP2 (rs659366), and IL-17 (rs763780) were not statistically different between patients and healthy controls (data not shown).

Table 3. Characteristics of substance use.

		n (%)
Prevalent Substance	Synthetic cannaboid	80 (58.8)
	Cannaboid	27 (19.9)
	Heroin	21 (15.4)
	Ecstasy	8 (5.9)
Usage	Inhalation	110 (88.0)
	Eat/drink	9 (7.2)
	Injection	6 (4.8)
Frequency of usage	Everyday	66 (52.0)
	Once a week	61 (48.0)
Polysubstance usage	Yes	66(52.0)
	No	61(48.0)
Secondary substance	Ecstasy	22 (34.9)
	Cannaboid	21 (33.3)
	Synthetic cannaboid	9 (14.3)
	Cocaine	6 (9.5)
	Heroin	2 (3.2)
	Volatile substance	2 (3.2)
	Methamphetamine	1 (1.6)
Psychosis findings	Yes	65 (49.2)
	No	67 (50.8)
Psychosis	Substance-related psychosis	43 (78.2)
	Other	18 (21.8)
Suicide attempt	Yes	32 (25.0)
	No	96 (75.0)
Self-mutilation	Yes	32 (25.0)
	No	96 (75.0)
Family history of psychosis	Yes	25 (20.5)
	No	97 (79.5)
Family history of smoking	Yes	102 (80.3)
	No	25 (19.7)
Alcohol usage in family	Yes	38 (30.9)
	No	85 (69.1)
Substance usage in family	Yes	13 (10.4)
	No	112 (9.6)

Table 4 . Genotype and allele frequencies of CNR2 rs2229579 variant.

CNR2 rs2229579	SUD group n:136 (%)	Control group n:100 (%)	*p*	OR
Genotypes				
T/T*	38 (27.9)	2 (2.0)	**0.000**	OR1 = 15.69
T/C	46 (33.8)	38 (38.0)		OR2:21.92
C/C	52 (38.2)	60 (60.0)		
Alleles				
T	122 (44.9)	42 (21)	**0.000**	3.059
C	150 (55.1)	158 (79)		

Note: Data were analysed by χ^2 test. The results that are statistically significant are shown in boldface.
*Reference category for odds calculation.

Additionally, we compared whether polysubstance usage was associated with frequencies of genotype and allele of these variants. There was a statistically significant difference between patients and controls regarding genotype and allele frequencies of the COMT Val108/158Met variant (Table 5). COMT Val108Met and Val158Met Val/Val genotypes were more prevalent in the polysubstance user group than the non-polysubstance user group, while Met/Met genotype was higher in the non-polysubstance group user compared to the polysubstance user group (respectively, $p = 0.033$, $p = 0.013$). Also, COMT Val108Met and Val158Met Val alleles were associated with polysubstance use ($p = 0.013$ and $p = 0.001$, respectively).

Additionally, we investigated the relationship between age at onset for SUD and SUD-related psychosis. SUD-related psychosis was significantly associated with age of onset for SUD. SUD-related psychosis was more common in the subjects with age of onset ≤18 ($p = 0.001$, OR: 12.95) (Table 6).

Discussion

SUDs are chronic relapsing psychiatric disorders manifested by the compulsive and dyscontrolled use of a

Table 5. Genotype and allele frequencies of COMT gene variants according to polysubstance usage.

COMT Val 108Met	Polysubstance usage		*p*	OR
Genotypes	Yes *n* (%)	No *n* (%)		
Val/Val*	25 (38.5)	15 (24.6)	**0.033**	OR1:1.464
Val/Met	33 (50.8)	29 (47.5)		OR2:4.047
Met/Met	7 (10.8)	17 (27.9)		
Alleles				
Val	83 (63.8)	59 (48.4)	**0.013**	1.885
Met	47 (36.2)	63 (51.6)		
COMT Val158Met	Polysubstance Usage			
Genotypes	Yes *n* (%)	No *n* (%)		
Val/Val*	35 (53.0)	17 (28.3)	**0.013**	OR1:2.433
Val/Met	22 (33.3)	26(43.3)		OR2:3.888
Met/Met	9 (13.6)	17(28.3)		
Alleles				
Val	92 (69.7)	60 (50)	**0.001**	OR:2.300
Met	40 (30.3)	60 (50)		

Note: Data were analysed by χ^2 test. The results that are statistically significant are shown in boldface.
*Reference category for odds calculation.

Table 6. The relationship between onset age of SUD and SUD-related psychosis.

	Onset age of SUD		
	≤18 *n*(%)	>18 *n*(%)	*p*
SUD-related psychosis	34 (91.9)	7 (46.7)	**0.001**
Other	3 (8.1)	8 (53.3)	

Note: The results that are statistically significant are shown in boldface.

drug or activity, resulting in maladaptive and destructive outcomes. When analysing the risk factors for addictions, it is crucial to underline the biological events that are involved in these activities and to develop drugs that can hinder the molecular mechanisms to prevent and to treat the addictions. Studies have reported the important role of heritable impacts on individual differences in addiction. Twin studies suggested that genetic factors account for more than half of the cases. The genetic impact for addictions is not related to a contribution of a single gene; however, it is the result of the interaction of different genes that along with environment factors stimulate a condition of "susceptibility" to the disorder. The substance use and behaviours will be represented in the brain but, more significantly, drug use modifies the brain chemistry in ways that keep and potentially enhance consumption [14]. Addictive drugs cause neuronal changes in cortical and basal ganglia structures, as well as in the mesocorticolimbic dopamine system, resulting in alterations of synaptic reorganization and function [15]. Dopaminergic brain systems have been implicated in drug reward [16], hence the genes that have a role in these circuits are likely candidates for susceptibility to SUDs.

COMT is an enzyme that plays a role in metabolism of various catecholamine neurotransmitters, such as dopamine and epinephrine. The *COMT* gene is 27.22 kb in length, and is found on chromosome 22q11.2 [17]. A common non-synonymous single-nucleotide polymorphism (rs4680) changes the 158th amino acid residue of the membrane-bound isoform (or 108th amino acid of the soluble form) from valine (Val) to methionine (Met). Chen et al. demonstrated that Val/Val homozygotes have higher stability than the Met/Met homozygotes, and COMT activity in Val/Val homozygotes is approximately 40% higher than in Met/Met homozygotes, while Val/Met heterozygotes have moderate enzyme activity [18]. Some studies reported that the Val158-Met variant plays a role in psychiatric phenotypes, such as schizophrenia and bipolar disorder [19,20], and reported that individuals with the high-activity COMT variant may have greater genetic vulnerability to drug abuse [21]. Serý et al. found a relationship between the Val158Met variant of the COMT gene and alcoholism in male subjects [22]. In different studies, it was found that COMT Val158Met variant was associated with heroin [23], cocaine [24], methamphetamine [25], inhalant use [26]. In this study, although there was no association between patients and controls regarding genotype and allele frequencies of COMT (Val108/158Met), we found a significant association between COMT Val108/158Met variant and polysubstance use, with the Val allele (high activity) being over-represented in patients versus controls (Table 6).

The endocannabinoid system plays a role in the modulation of numerous physiological processes including neurotransmission, pain perception, appetite, and immune response. The discovery of an endocannabinoid physiological control system (EPCS) has resulted in the investigation of this system in the central nervous system and its possible involvement in mental disorders. It was shown that the variants of *CNR1* were linked with higher vulnerability to cannabis, alcohol, and drug addiction [27–29]. While *CNR1* has been widely investigated, only a few number of authors studied the role of *CNR2* in psychiatric disorders. CNR2 receptors are chiefly found in the immune cells, however they have also been identified in some regions of the rat brain, such as cerebral cortex, striatum, amygdala, thalamus, cerebellum, spinal nucleus, olfactory nucleus, and hippocampus [30]. Further investigation searched whether cannabinoid CB2 receptor knockout (CB2KO) mice similarly showed a higher aggression compared with wild-type during the social interaction test and resident–intruder paradigm [31]. There are several association studies between CNR2 variants gene and schizophrenia [11], eating disorders [32], depression [33], and alcoholicm [34]. Okahisa et al. reported that the CNR2 Q63R variant did not affect the risk of methamphetamine dependence and psychosis or the clinical phenotypes of methamphetamine psychosis in a Japanese population [4]. In this study, we found a significant difference between SUD patients and the control group regarding genotype and allele frequencies of the CNR2 rs2229579 variant. CNR2 rs2229579 TT genotype and T allele were associated with SUD compared to other genotypes and alleles, while CNR2 rs2229579 CC genotype and C allele were higher in healthy control group than patients (Table 5).

UCPs are integral proteins found in the mitochondrial inner membrane and modulate the mitochondrial membrane potential by discharge of the proton gradient produced during oxidative phosphorylation and negatively regulate mitochondrial ATP synthesis. Their capacity to regulate the passage of protons and consequent proton gradient make uncoupling proteins crucial in controlling neuromodulation and neuroprotection. UCP2 and UCP3 can diminish the generation of superoxide radicals at complex I of the mitochondrial respiratory chain by decreasing the electrical potential across the inner mitochondrial membrane

[35]. The neuroprotective effect of UCP2 has been shown by many studies in animals and cell cultures. Sullivan et al. reported that neurones from immature rat brains were more resistant to seizures compared to those from mature rat brain and that this was due to higher levels of mitochondrial uncoupling in those cells, associated with a higher expression of UCP2 [36]. Due to this significant functions, UCP variants were assessed often in some diseases including body composition and resting energy expenditure [37], energy metabolism [38], obesity [39], multiple sclerosis [40], diabetic neuropathy [41], and schizophrenia [42]. There is no study examining the relationship between UCP2 variants and dependence. In the present study, there was no significant association between UCP2 rs659366 variant and SUD.

To facilitate the therapeutic process in addiction, one of the difficulties is to define biological markers that could help in objectively determining the level of consumption, severity of addiction, degree of toxicity, and response to treatment in patients. Several studies have shown that abused drugs interact with the immune system and modify signalling and gene expression that occur in the immune response, and these effects contribute to different aspects of addiction [43]. Because the immune system modifies brain functions related to addiction and the concurrent participation of reward modulatory systems in psychiatric disorders, there is a possibility of establishing a link between inflammation, neuropsychiatric diseases, and addictive disorders. Moreira et al. demonstrated that there was a statistically significant increase in IL-6 and decrease in IL-10 serum levels between cocaine users and the control group [44]. Also, it was reported that IL-10-592C/A and IL-1α-889 C/T variants were associated with alcoholism [45,46]. These results pointed to an inflammatory status associated with dependence.

The IL-17 family members are pro-inflammatory cytokines mainly released by T-helper 17 (Th17) cells. IL-17 gene variants have been found to be linked with numerous autoimmune diseases, such as asthma [47], celiac disease [48], and inflammatory bowel disease [49]. Reduced synthesis of IL-6, IL-12, IL-17A, IFN-gamma, and high levels of IL-13 cytokines due to ovalbumin stimulation in alcohol-consuming mice have also been reported [50]. In the present study, no statistically significant difference between patients and controls was found in the frequency of the evaluated genotype and allele frequencies of the IL-17 rs763780 variant.

In this study, we found that SUD-related psychosis was significantly associated with onset age of SUD. SUD-related psychosis was more prevalent in the subjects with onset of age ≤18 ($p = 0.001$) (Table 6).

This study has several limitations. The first limitation of this study was the small sample size. Therefore, the prevalence of some homozygous variants was low in groups and thus reduced the statistical power. Further studies are needed to replicate this. Second, although our patients were recruited from two centres, genotyping was not divided according to the centre. Another limitation of the study involves the fact that gene expressions have not been evaluated.

Conclusion

Studies have been carried out over the last decades to find out the neurobiological mechanisms underlying drug addiction. To the best of our knowledge, this is the first study investigating the association between these variants and SUD in a Turkish population. This study provides insights into the CNR2 rs2229579 variant that is thought to be risk factor of substance use. Also, COMT Val108/158Met variants might be important factors affecting the polysubstance usage. As a conclusion, we can suggest that DNA research in humans can contribute to help define the substances and systems in the brain which are associated with addiction and to determine whether or not certain individuals are susceptible to substance use. Further studies are needed to understand clearly how genetic variants influence the development of vulnerability to addiction.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This study was supported by Istanbul University BAP thesis project (No: TYL-2016-20431) support programme.

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