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Association of the Uncoupling Protein 2-866 G/A Polymorphism with Family History and Duration of Tobacco Use Disorder in a Turkish Population

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ABSTRACT

Background: A variety of substances cause neurotoxicity by increasing intracellular oxidative stress, followed by mitochondrial dysfunction. Uncoupling proteins (UCPs) act as membrane transport proteins and reduce reactive oxygen products and mitochondrial calcium influx. We aimed to study *UCP2*-866 G/A gene polymorphism in tobacco use disorder (TUD) by comparing genotype distributions between TUD patients and healthy controls considering clinical parameters.

Methods: One hundred eighteen patients with TUD and 96 healthy volunteers were included in the study. The diagnosis of the patients were then confirmed, based on the DSM-5 criteria. Polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) were used to determine *UCP2* gene polymorphism.

Results: Our results demonstrated that the *UCP2* genotype distribution and allele frequencies of the TUD patient group were significantly different from those of the control group. When the *UCP2* genotype and the allele frequency distributions were compared between the two groups according to the family history of TUD in the patient group, the *UCP2* genotype and allele frequency distributions were significantly different. The GG genotype or G allele percentage was significantly higher in patients with a family history of TUD, than the patients without a family history of TUD. Comparing clinical parameters based on the *UCP2* genotype, the disorder's duration was significantly different between the groups of *UCP2* genotype. The duration of TUD was significantly shorter in patients with GG genotype than other genotypes.

Conclusions: In summary, the *UCP2*-866 G/A gene polymorphism might be associated with family history and duration of TUD in Turkish patients.

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INTRODUCTION

Tobacco use disorder (TUD) is a critical public health problem, leading to high mortality and morbidity in patients and high public health costs to communities. Tobacco smoking kills more than 7 million people worldwide each year and is responsible for 70-90% of lung cancer.[1,2] TUD defines substance abuse by the patients who compulsively consume tobacco products, despite knowing their health problems. Continued tobacco use despite awareness of persistent or recurrent physical (tolerance or withdrawal) or psychological (impaired control of tobacco use) complaints is one of the diagnostic criteria underlying TUD described in the Diagnostic and Statistical Manual of Mental Disorders 5th edition (DSM-5).[3]

Uncoupling proteins (UCPs) act as membrane transport proteins and reduce reactive oxygen products and mitochondrial calcium influx, causing neuronal dysfunction and cell death by checking proton gradient and ATP synthesis in the mitochondrial membranes. UCP2, UCP4, and UCP5, called ''neuronal UCPs,'' are believed to have neuroprotective and neuromodulatory effects in the central nervous system.[4] There are three well-known *UCP2* polymorphisms: the −866G/A polymorphism (rs659366), the Ala55Val polymorphism (rs660339), and the 45 bp insertion/deletion (Ins/Del) polymorphism.[5] Evidence accumulated recently showed increased oxidative stress or calcium dysregulation by mitochondrial dysfunction

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as a likely cause of vulnerability to neurodegenerative diseases and psychiatric disorders such as schizophrenia and bipolar disorder.[4,6] It was also shown that a variety of substances such as cocaine, amphetamine, and methamphetamine cause neurotoxicity by increasing intracellular oxidative stress, followed by mitochondrial deoxyribonucleic acid (mtDNA) and mitochondrial dysfunction.[7] To our knowledge, this is the first detailed study that has evaluated the relationship between the *UCP2*-866 G/A gene (rs659366) polymorphism and TUD according to clinical characteristics. We aimed to evaluate the relationship between TUD and *UCP2* gene polymorphism by comparing genotype distributions of *UCP2* between patients and healthy controls, considering clinical parameters.

METHODS

Patient Selection

This case–control study utilized a consecutive sampling design. One hundred eighteen patients diagnosed with TUD who consecutively admitted to the Bakirkoy Mazhar Osman Mental Health and Neurology Training and Research Hospital outpatient clinic, and 96 age-, gender-, and ethnicity-matched healthy volunteers. The study was approved by the Clinical Research Ethics Committee of Istanbul University School of Medicine, under the ethical standard for human experimentation established by the Declaration of Helsinki (1945/November 30, 2015).[8] The participants were informed in detail about the study's purpose, method, and procedures, and their written informed consent was obtained. The interview was started off by filling out data forms that included sociodemographic and clinical information.

Inclusion and Exclusion Criteria

Subjects from 18 to 65 years of age, of either gender, who had been diagnosed with TUD according to the DSM-5 criteria, had no other systemic/neurological disease that could affect cognitive functions (dementia, epilepsy, Parkinson's disease, or head trauma accompanied by loss of consciousness), were literate, and willing to participate in the study, were enrolled. We had excluded subjects who refused participation or had mental retardation, neurodevelopmental disorders such as autism, or a diagnosis of substance use disorders (SUDs) other than TUD due to DSM-5 criteria.

MAIN POINTS

- The *UCP2*-866 G/A gene polymorphism might be associated with family history and duration of TUD.
- The GG genotype or G allele percentage was significantly higher in patients with a family history of TUD than the patients without a family history of TUD.
- The duration of TUD was significantly shorter in TUD patients with wild-type GG genotype than other genotypes.

DNA Analysis

Blood samples were obtained from participants at the Istanbul Faculty of Medicine Laboratory of Medical Biology to isolate their DNA material. *UCP2*-866 G/A gene (rs659366) polymorphism in the promoter of the human *UCP2* gene was determined by polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP).[9]

Statistical Analysis

Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) version 21.0 (IBM SPSS Corp.; Armonk, NY, USA). Descriptive statistics included mean, standard deviation, median, minimum, maximum, frequency, and percentage. The odds ratio (OR) and the 95% CI were also calculated. The Pearson chi-square test was used to compare genotype distributions of *UCP2* polymorphism in TUD patients with the control group, and to compare genotype distributions of *UCP2* polymorphism in TUD patients based on the family history. The Shapiro-Wilk test evaluated the suitability of continuous variables to normal distribution. Since our data were not normally distributed, intergroup comparisons of continuous variables (age, duration of TUD, age of onset, cigarettes smoked per day, and cumulative smoking amount (pack-year)) were performed by Kruskal–Wallis testing. Genotype distributions in both the patients and the healthy controls were analyzed according to the Hardy–Weinberg Equilibrium (HWE) (mid-*P* adjustment). Statistical significance was accepted as $P < .05$ for the results of all analyses. The power analysis was performed with the "G*power" software (Version 3.0.5, http://www.psycho.uni-duesseldorf.de/abteilungen/aap/gpower3/), post hoc goodness of fit χ^2 test, with an "-error" probability of .05.

RESULTS

Sociodemographic Characteristics and UCP2 Genotype Frequencies of Participants

The TUD patients were evaluated according to sociodemographic characteristics and clinical parameters, as shown in Table 1. According to the *UCP2* genotype distribution, 28.8% ($n = 34$) of the patients diagnosed with TUD had AA, 43.2% ($n = 51$) had AG, and 28% ($n = 33$) had GG genotypes. In the control group, 42.7% ($n = 41$) had AA, 42.7% ($n = 41$) had AG, and 14.6% ($n = 14$) had GG genotypes.

UCP2-866 G/A Genotyping

When the *UCP2* (AA, AG, GG) genotype and the allele frequency (A, G) distributions of TUD patients were

Table 1. Sociodemographic Characteristics and Clinical Parameters of Patients Diagnosed With TUD.

	Tobacco Use Disorder (*N* = 118) Mean ± SD	
Age	28.95 ± 7.47	
Smoking onset age (years)	17.23 ± 4.06	
Duration of disorder (years)	11.72 ± 7.12	
Cigarettes per day	21.72 ± 9.14	
Cumulative smoking amount (pack-year)	13.00 ± 10.75	
Sex	*N*	%
Female	5	4.2
Male	113	95.8
Education		
Literate	12	10.2
Primary School	19	16.1
Secondary School	60	50.8
High School	25	21.2
University	2	1.7
Marital status		
Single	52	44.1
Married	66	55.9
Working status		
Unemployed	65	55.1
Officer/employee/trader	53	44.9
Family history of TUD		
No	37	31.4
Yes	81	68.6
Family history of AUD		
No	83	70.3
Yes	35	29.7
Family history of SUD		
No	105	89
Yes	13	11

SD, standard deviation; resist., resistance; TUD, tobacco use disorder; AUD, alcohol use disorder; SUD, substance use disorder.

compared with the control group (Table 2), TUD patients had a higher frequency of the GG genotype than the control group (GG vs. AA+AG) (χ^2 (1, *N* = 118) = 5.5, *P* = .019). The AA genotype frequency was higher in the control group compared to TUD (AA vs. AG+GG) (χ^2 (1, *N* = 118) = 4.4, *P* = .034). The *UCP2* allele frequency distributions of TUD patients were also significantly different from those of the control group (A vs. G) (χ^2 (1, *N* = 118) = 8, *P* = .005).

Comparison of Genotype Distributions of UCP2 Polymorphism in TUD Patients due to Family History of TUD, Alcohol Use Disorder (AUD), or SUD

Comparing the *UCP2* genotype and the allele frequency distributions between the two groups according to the presence of the family history of TUD, AUD, or SUD in the patient group (Table 2) demonstrated that the *UCP2* genotype (GG vs. AA+AG) (χ^2 (1, *N* = 81) = 5.5, *P* = .018) and allele frequency (A vs. G) (χ^2 (1, *N* = 81) = 7.3, *P* = .007) distributions of TUD patients were significantly different between the groups of patients due to the family history of TUD. The GG genotype or G allele percentage was significantly higher in patients with a family history of TUD than the patients without a family history of TUD.

Comparison of Clinical Parameters According to UCP2 Genotype Distribution in Patients with TUD

When clinical parameters (age, duration of TUD, age of onset, cigarettes per day, and cumulative smoking amount (pack-year)) were compared between the three genotype groups in reference to the *UCP2* genotype of the patients with TUD (Table 3), the duration of TUD was significantly different between the groups of the *UCP2* genotype (*H*(2) = 8.34, *P* = .015). The duration of TUD was significantly shorter in TUD patients with GG genotype than other genotypes (AA and AG).

DISCUSSION

Nicotine is the main addictive ingredient of tobacco products, and maintains TUD. Researches have reported that nicotine could be as addictive as heroin, cocaine, and methamphetamine.[10] Nicotine works as an agonist at nicotinic acetylcholine receptors (nAChRs) and broadly expanded cholinergic receptors in the central and peripheral nervous systems, and other tissues. This system includes dopaminergic neurons that originate in the ventral tegmental area and release dopamine in areas such as the prefrontal cortex, hippocampus, amygdala, and the nucleus accumbens (NAc).[11] The impact of nicotine on mitochondrial function has been shown in different experimental studies (isolated mitochondria, intact cells, and animal models). Mitochondrial nAChR and respiratory complex I are estimated targets for nicotine action in mitochondria.[12]

In the present study, we reported a statistically significant difference between *UCP2* genotype and allele frequency distributions of TUD patients and control groups. The participants carrying the GG genotype or G allele had a higher risk of developing TUD, in the Turkish population. A limited number of studies on the relationship between addiction and mitochondrial dysfunction could be seen when the literature was reviewed. Firstly, Lehrmann et al. reported that the postmortem prefrontal cortex of cocaine use disorder (CUD) patients had increased transcripts of molecules that are important for mitochondrial activity.[13] Again, Feng et al. demonstrated that transcriptional profiling of the NAc shows enriched gene ontology of mitochondrial-related transcripts after chronic usage of cocaine.[14] Recently, Chandra et al. showed a role

Table 2. Analysis of the Pearson Chi-Square Test Results of Genotype Distributions of *UCP2* Polymorphism in TUD Patients Due to the Family History

TUD	Genotype	Patients, *n* = 118 (%)	Control, *n* = 96 (%)	χ^2 Value	OR (95% CI)	*P*
UCP2						
	AA	34 (28.8)	41 (42.7)	4.489	0.54 (0.30-0.95)	.034*
	AG	51 (43.2)	41 (42.7)	0.006	1.02 (0.59-1.76)	.940*
	GG	33 (28)	14 (14.6)	5.532	2.27 (1.13-4.55)	.019*
Allele						
	A	119 (50.4)	123 (64.1)			
	G	117 (49.6)	69 (35.9)	8.015	0.57 (0.38-0.84)	.005*
Family History of TUD	Genotype	Yes, *n* = 81 (%)	No, *n* = 37 (%)	χ^2 Value	OR (95% CI)	*P*
UCP2						
	AA	19 (23.5)	15 (40.5)	3.614	0.44 (0.19-1.03)	.057*
	AG	34 (42)	17 (45.9)	0.163	0.85 (0.38-1.86)	.686*
	GG	28 (34.6)	5 (13.5)	5.589	3.38 (1.18-9.64)	.018*
Allele						
	A	72 (44.4)	47 (63.5)			
	G	90 (55.6)	27 (36.5)	7.389	0.46 (0.26-0.80)	.007*

*Pearson chi-square.
TUD, tobacco use disorder; OR, odds ratio; CI, confidence interval.

for altered mitochondrial fission in the NAc during early cocaine abstinence, and they thought that disrupting mitochondrial fission in CUD could be a potential treatment.[15] Although recent studies also indicate that smoking may act on mitochondrial activity and disrupt the respiratory chain and cause lack of energy,[16] the altered brain energy homeostasis and transcriptional mechanisms reported in the cocaine-dependent brain have not been studied in TUD. However, since we know that the nAChRs influenced by nicotine contain dopaminergic neurons originating from a brain reward nucleus similar to those in CUD, the significant results in our study did not indeed seem to surprise us.

The genes related to drug metabolism, cholinergic, dopaminergic, glutaminergic systems, reuptake, and vesicular packaging of neurotransmitters about learning and memory were associated with TUD.[17,18] Numerous genome-wide association studies reported polymorphisms in the gene encoding the α3, α5, and β4 nAChR subunits that increase the risk for TUD.[19,20] It was reported that the cholinergic receptor nicotinic alpha 5 subunit (*CHRNA5*) variant rs16969968 predicted delayed smoking cessation and an earlier age of lung cancer diagnosis in a recent meta-analysis study.[21] Forget et al. published a frequent non-synonymous single nucleotide polymorphism in the gene coding for the α5 subunit of nAChR, which is statistically related to the increased risk for TUD and delays smoking cessation.[22] In the study on the association of SUD with catechol-O-methyltransferase (*COMT*), cannabinoid receptor 2 (*CNR2*), interleukin-17 (*IL-17*), and *UCP2* gene polymorphisms, Kurnaz et al. showed that there was no significant difference between the SUD group and control group regarding genotype and allele frequencies of the *UCP2* (rs659366) variant.[23] In this study, collecting all the SUD patients (synthetic cannabinoid, cannabinoid, heroin, and ecstasy) in the same group and comparing genotype distributions with the control group may have prevented finding significant associations in contrast to our study.

Table 3. Analysis of the Kruskal-Wallis *H* Test Results of Clinical Parameters According to *UCP2* Genotype Distribution in Patients With TUD

	AA (*N* = 34)	AG (*N* = 51)	GG (*N* = 33)				Comparison of Dura. of Dis.
	Mean of Ranks	Mean of Ranks	Mean of Ranks	*df*	χ^2	*P**	
Age (years)	62.93	62.99	50.58	2	3.130	.209	
Age of onset	51.91	60.67	65.52	2	2.785	.248	
Dura. of dis.	66.65	64.10	45.03	2	8.342	.015	GG < AA or AG
Cig. per day	58.03	56.72	65.32	2	2.268	.322	
Pack-year	64.59	61.51	51.15	2	2.901	.234	

*Kruskal-Wallis test.
SD, standard deviation; TUD, tobacco use disorder; dura., duration; dis., disorder; cig., cigarettes.

Current studies have reported that TUD is common among members of tobacco-user families compared to those of tobacco non-user families. Strong evidence shows that children with TUD parents and siblings are at a higher risk of becoming addicted due to genetic and environmental factors.[24] Heritability estimates from twin studies of TUD range from moderate to high (31-60%).[25] In our study, the *UCP2* genotype and allele frequency distributions of TUD patients were significantly different between the groups of patients due to the family history of TUD. We also showed that the patients carrying the wild-type GG genotype or G allele had a higher risk of a family history of TUD in the Turkish population. The patients who began daily smoking at an early age are at higher risk of long-term TUD. The early onset of smoking is related to greater cigarette consumption in the future, a relative failure to quit smoking, and a more severe form of TUD.[26] Weiss et al. published that common haplotypes in the cholinergic receptor nicotinic alpha 5-alpha 3-beta 3 subunit (*CHRNA5-A3-B4*) gene cluster are related to adult TUD, especially among those who began daily smoking before age 17.[27] In our study, the duration of TUD was significantly different between the three groups of the *UCP2* genotype. The duration of TUD was significantly shorter in TUD patients with wild-type GG genotype than other genotypes, and the age of onset was nonsignificantly higher in the wild-type GG genotype group.

While this research study's strength is that this is the first study about the relationship between TUD and *UCP2* gene polymorphism according to clinical characteristics in the literature, our study also has several limitations. In our study, *UCP2-866* G/A gene (rs659366) polymorphism was examined, but it was not possible to know whether other polymorphisms may contribute to the TUD (the Ala55Val polymorphism (rs660339) in exon 4, and the 45 bp insertion/deletion (Ins/Del) polymorphism in the 3' untranslated region (3'UTR)). Secondly, this study has a small sample size, which can limit the statistical power.

In conclusion, the results of our study suggest that having the wild-type GG genotype or G allele is a disadvantage in terms of the occurrence of TUD and the presence of family history, but associated with a short duration of TUD in the Turkish population. Confirmation of the present findings with other *UCP2* polymorphisms, larger sample sizes, and studies in different ethnic populations will contribute to a better understanding of the relationship between TUD and *UCP2* polymorphisms.

Ethics Committee Approval: Ethics Committe approval was received from the Clinical Research Ethics Committee of Istanbul University School of Medicine (1945/30.11.2015).

Informed Consent: Written informed consent was obtained from all participants who participated in this study.

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