

Association of the DRD2 TaqIA, 5-HT1B A-161T, and CNR1 1359 G/A Polymorphisms with Alcohol Dependence

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ÖZET:

DRD2 TaqIA, 5-HT1B A-161T VE CNR1 1359 G/A polimorfizmlerinin alkol bağımlılığı ile ilişkisi

Amaç: Alkol bağımlılığı alkolü yıkan enzimler ve dopaminerjik, gama aminobutirik asiderjik, glutamaetrik, opiyat, kolinerjik ve serotonerjik sistemle ilgili genetik değişkenlerle ilişkilidir. Endojen kannabinoid sistemle ilgili genetik değişkenlerin de alkol bağımlılığında etkili olduğu gösterilmiştir. Bu çalışmada, alkol bağımlılığı ile DRD2 TaqIA, 5-HT1B A-161T ve CNR1 1359 G/A (rs1049353) polimorfizmleri arasındaki ilişkiyi araştırmak amaçlanmıştır. **Yöntem:** Çalışmaya DSM-IV tanı kriterlerine göre alkol bağımlılığı tanısı almış 123 kişi ve 125 sağlıklı gönüllü alınmıştır. Yazılı onam formunun alınmasının ardından hastalardan venöz kan örnekleri alınmıştır. Venöz kanlar ethylenediaminetetra asetik asit (EDTA) içeren tüplere alınmıştır. Tam kandan tuzlama tekniği ile DNA izole edilmiştir. Polimerize zincir reaksiyonu kullanarak literatürde belirtildiği gibi genetik analiz yapılmıştır. İstatistiksel analiz için 17.0 programı kullanılmıştır.

Bulgular: DRD TaqIA polimorfizmi hem çalışma hem de kontrol grubunda analiz edilmiştir. Çalışma grubunda, A1/A1 genotipi 5 kişide (4.0%), A1/A2 genotipi 51 (41.5%) kişide ve A2/A2 genotipi 67 (54.5%) kişide saptanmıştır. Kontrol grubunda ise, A1/A1 genotipi 6 (4.8%) kişide, A1/A2 genotipi 40 (32.0%) kişide A2/A2 genotipi 79 (62.2%) kişide saptanmıştır. 5-HT1B reseptör A-161T gen polimorfizmi için ise, A/A genotipi 61 (49.6%) kişide, A/T genotipi 53 (43.1%) ve T/T genotipi (7.3%) kişide tespit edilmiştir. Kontrol grubunda ise, A/A genotipi 84 (67.2%), A/T genotipi 39 (31.2%) ve T/T genotipi 2 (1.6%) kişide tespit edilmiştir. CNR1 1359 polimorfizmi için ise G/G genotipi hem çalışma hem de kontrol grubunda en sık saptanan polimorfizmdir. Çalışma grubunda 75 (61.0%) kişide, kontrol grubunda ise 84 (67.2%) kişide bu polimorfizm görülmüştür. G/A genotipi ise çalışma grubunda 39 (31.7%) kişide, kontrol grubunda ise 38 (30.4%) kişide görülmüştür. A/A genotipi hem çalışma hem de kontrol grubunda en nadir görülen genotiptir ve çalışma grubunda sadece 9 (7.3%) kişide, kontrol grubunda ise 3 (2.4%) kişide gözlenmiştir. Çalışılan 3 polimerimden sadece 5-HT1B A-161T polimorfizmi alkol bağımlılığı ile ilişkili bulunmuştur.

Sonuç: 5-HT1B reseptör A-161T polimorfizmi alkol bağımlılığı için umut veren bir belirteç olabilir, ancak bu bulguları netleştirmek için ileri çalışmalara ihtiyaç duyulmaktadır.

Anahtar sözcükler: alkol bağımlılığı, DRD2 TaqIA, 5-HT1B A-161T, CNR1 1359 G/A polimorfizmi

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ABSTRACT:

Association of the DRD2 TaqIA, 5-HT1B A-161T, and CNR1 1359 G/A polymorphisms with alcohol dependence

Objective: Alcohol dependence is associated with genetic variants of alcohol-metabolizing enzymes and genes related to dopaminergic, gamma-aminobutyric acid, glutamatergic, opioid, cholinergic, and serotonergic systems. Genetic variations in the endogenous cannabinoid system are also involved in alcohol dependence. The present study aimed to evaluate the association between three polymorphisms, DRD2 TaqIA, 5-HT1B A-161T and CNR1 1359 G/A (rs1049353), and alcohol dependence.

Methods: One hundred twenty three patients, who were diagnosed as having alcohol dependence according to the DSM-IV criteria and 125 healthy volunteers, were included in the study. With written informed consent, a blood sample was drawn from each individual. Venous blood samples were collected in ethylenediaminetetra acetic acid (EDTA) containing tubes. DNA was extracted from whole blood by the salting out procedure. Genetic analyses were performed as described in the literature by using a Polymerase Chain Reaction method. SPSS 17.0 software was used for statistical analysis.

Results: The DRD2 TaqIA polymorphism was analyzed in the study and control groups. In the study group, the A1/A1 genotype was observed in 5 (4.0%) patients, the A1/A2 genotype was observed in 51 (41.5%) patients and the A2/A2 genotype was observed in 67 (54.5%) patients. In the control group, the A1/A1 genotype was observed in 6 (4.8%) subjects, the A1/A2 genotype was observed in 40 (32.0%) subjects and the A2/A2 genotype was observed in 79 (62.2%) subjects. For the 5-HT1B receptor A-161T gene polymorphism, the A/A genotype was detected in 61 (49.6%) patients, the A/T genotype was detected in 53 (43.1%) and the T/T genotype was detected in 9 (7.3%) patients. In the control group, the A/A genotype was detected in 84 (67.2%) subjects, the A/T genotype was detected in 39 (31.2%) subjects, and the T/T genotype was detected only in 2 (1.6%) subjects. The G/G genotype was the most common genotype in both study and control groups for CNR1 1359 gene polymorphism. It was detected in 75 (61.0%) study patients and in 84 (67.2%) control subjects. The G/A genotype was observed in 39 (31.7%) patients of the study group and 38 (30.4%) subjects of the control group. The A/A genotype was the most rare genotype in both groups; it was detected only in 9 (7.3%) study patients and 3 (2.4%) control subjects. Of the three polymorphisms investigated, 5-HT1B A-161T was the only one found to be associated with alcohol dependence.

Conclusions: The 5-HT1B receptor A-161T polymorphism might be a promising marker for alcohol dependence; however, future studies are needed to clarify these findings.

Keywords: alcohol dependence, DRD2 TaqIA, 5-HT1B A-161T, CNR1 1359 G/A, polymorphism

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INTRODUCTION

Alcohol dependence, a chronic relapsing disorder, is characterized by repetitive alcohol drinking patterns causing a loss of control over alcohol consumption¹. Susceptibility to the development of alcohol dependence is associated with 50%-60% heritability among both males and females². The high heritability of alcohol dependence indicates that genetic factors are important in the disease process. Until recently, the only genes unambiguously associated with variation in the risk of developing alcoholism were those that encode alcohol-metabolizing enzymes and neurotransmitters, such as dopamine, gamma-aminobutyric acid (GABA) and serotonin³. More recently, studies on epigenetic factors and possible associations of genes involved in the cannabinoid and opiate systems are on the rise.

Polymorphisms in genes encoding the neurotransmitters and enzymes intrinsically involved in addiction have been investigated in candidate gene studies. Associations between alcohol dependence and genetic variants of alcohol-metabolizing enzymes and genes related to dopaminergic, GABAergic, glutamatergic, opioid, cholinergic, and serotonergic systems have been intensively investigated⁴. Dopamine is one of the most frequently studied neurotransmitters in addiction genetics, and the dopamine receptor D2 (DRD2) TaqI-A1 polymorphism was the first gene polymorphism associated with alcohol dependence^{5,6}. In their meta-analysis, Smith and colleagues reported that individuals with the A1A1 and A1A2 genotypes were 1.38 times more at risk for alcoholism than those with the A2A2 genotype⁷. Several major lines of evidence have supported the role of dysfunction of the human serotonin receptor (5-HT1B) in the pathophysiology of alcohol dependence⁸. It has been reported that 5-HT1B knockout mice show greater aggression, impulsivity, and alcohol and cocaine consumption^{9,10}. Many genetic variants of the 5-HT1B gene have been determined by polymerase chain reaction, and the A-161T polymorphism in the promoter region of the 5-HT1B receptor gene has been found to be

associated with alcohol dependence¹¹. Researchers have also suggested that the endocannabinoid system may play a role in many of the physiological and behavioral effects of chronic ethanol exposure, including tolerance and dependence¹². Various genetic variations in the endocannabinoid system have also been associated with different kinds of substance abuse¹³. Studies regarding the association of the cannabinoid receptor 1 (CNR1) 1359 G/A (rs1049353) polymorphism with alcoholism have reported an excess frequency of the A allele in alcoholics versus healthy controls¹⁴. These results, however, are controversial, since this observation was not replicated in other studies¹⁵.

The primary goal of the present study was to investigate the underlying genetic mechanisms of neurotransmitters that were related to the neurobiology of alcohol addiction (dopaminergic, serotonergic and endocannabinoid). Association between DRD2 TaqIA, 5-HT1B A-161T and CNR1 1359 G/A (rs1049353) polymorphisms and alcohol dependence was investigated in patients with alcohol dependence and healthy control subjects.

MATERIALS AND METHODS

Subjects

The present study was conducted in the Alcohol and Substance Abuse Center of Denizli State Hospital. The study was approved by the Local Ethics Committee, and all participants provided written informed consent. Randomly selected patients between 18 and 65 years of age with a diagnosis of alcohol dependence according to DSM-IV criteria were included in the present study. The Structured Clinical Interview for the DSM-IV (SCID-I) was used to evaluate the patients, and patients with any axis I disorder other than smoking dependence were excluded from the study. Patients were also excluded if they had clinically significant organic disorders, mental retardation or a history of substance abuse (except alcohol and tobacco) in the previous 12 months. The control group consisted of 125 volunteers, whose demographic characteristics were suitable for the study, who had no severe chronic physical diseases and no history of

mental disorders, who were not related to the patients in the study group, and who were willing to participate in the study. Our study and control group all were of Turkish origin.

Patients were assessed for inclusion criteria on their first day of hospitalization. A semi-structured questionnaire, which was developed by the researchers, consisting of items covering sociodemographic data and detailed history of alcohol use was administered to all patients.

Molecular Analysis

With written informed consent, a venous blood sample was drawn from each individual into tubes containing ethylenediaminetetraacetic acid (EDTA). DNA was extracted from whole blood by a previously described salting-out procedure¹⁶.

A Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) assay was used to determine the Taq1A (rs17115461) polymorphism within the DRD2 gene. The oligonucleotide primers, used to amplify the region containing this polymorphism, have been described previously¹⁷. The genotyping of the CNR1 1359 G/A (rs1049353) and 5-HT1B A-161T (rs130058) polymorphisms was performed using a pre-designed TaqMan Single Nucleotide Polymorphism (SNP) Genotyping Assay (Applied Biosystems, Foster City, CA, USA). The Assays-on-Demand SNP genotyping kit (Applied Biosystems) was used for the real-time polymerase chain reaction. All SNP amplification assays were performed in accordance with the manufacturer's instructions. All procedures were performed in a blinded manner to the status of the patients and other characteristics of the participants. Two independent investigators scored the gel images and entered the data.

Statistical Analysis

The Predictive Analysis Software (PASW) Statistics 17.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Descriptive analysis was used for sociodemographic data. The chi-square test was used for categorical and the t test was used for continuous variables. A p value of <0.05 was considered statistically significant.

RESULTS

The study group consisted of 123 patients with alcohol dependence [111 (90.2%) males; 12 (9.8%) females] and the control group comprised 125 healthy volunteers [111 (88.7%) males; 14 (11.2%) females]. The mean age of the study group was 35.19±10.18 years, and the mean age of the control group was 34.53±14.22 years. There were no statistically significant differences between the study and control groups in terms of age and gender ($p>0.05$). The social demographic factors of the study and control groups are shown in Table 1.

The DRD2 Taq1A polymorphism was analyzed in the study and control groups. In the study group, the A1/A1 genotype was observed in 5 (4.0%) patients, the A1/A2 genotype was observed in 51 (41.5%) patients, and the A2/A2 genotype was observed in 67 (54.5%) patients. In the control group, the A1/A1 genotype was observed in 6 (4.8%) subjects, the A1/A2 genotype was observed in 40 (32.0%) subjects, and the A2/A2 genotype was observed in 79 (62.2%) subjects. The A1 allele was found in 56 (32.2%) study subjects and 46 (26.3%) controls. The A2 allele was found 118 (67.8%) study subjects and 119 (73.7%) of the control group. There was no statistically significant difference between the study and control groups in terms of the

Table 1: Sociodemographic characteristic of the participants

	Alcohol Group (n=123)	Control Group (n=125)	P value
Age	35.19±10.18	34.53±14.22	0.67
Education (years)	7.9±3.2	8.4±3.2	0.49
Employment (years)	7.2±3.4	8.1±3.9	0.21
Smoking (n)	86	76	0.43
Cigarette per day	18.2±7.8	15.6±6.1	0.37

Table 2: Distribution of the genotypes and alleles of the DRD2 Taq1A, 5-HT1B A-161T, and CNR1 1359 G/A polymorphisms in the study and control groups

	Study Group (n=123)			Control Group (n=125)			p
DRD2 Taq1A polymorphism	A1/A1 5 (4.0)	A1/A2 51 (41.5)	A2/A2 67 (54.5)	A1/A1 6 (4.8)	A1/A2 40 (32.0)	A2/A2 79 (62.2)	0.30
DRD2 Taq1A allele	A1 allele 56 (32.2)		A2 allele 118 (67.8)	A1 allele 46 (26.3)		A2 allele 119 (73.7)	0.27
5-HT1B A-161T polymorphism	A/A 61 (49.6)	A/T 53 (43.1)	T/T 9 (7.3)	A/A 84 (67.2)	A/T 39 (31.2)	T/T 2 (1.6)	0.001
5-HT1B A-161T allele	A allele 114 (64.8)		T allele 62 (35.2)	A allele 123 (74.5)		T allele 41 (25.5)	0.002
CNR1 1359 G/A polymorphism	G/G 75 (61.0)	G/A 39 (31.7)	A/A 9 (7.3)	G/G 84 (67.2)	G/A 38 (30.4)	A/A 3 (2.4)	0.17
CNR1 1359 allele	G allele 114 (70.4)		A allele 48 (29.6)	G allele 122 (74.8)		A allele 41 (25.2)	0.13

Data are presented as n (%).

distribution of genotypes and alleles of the DRD2 Taq1A polymorphism ($p>0.05$).

The A-161T polymorphism of the 5-HT1B receptor gene was also studied in both groups. In the study group, the A/A genotype was detected in 61 (49.6%) patients, the A/T genotype was detected in 53 (43.1%) patients, and the T/T genotype was detected in 9 (7.3%) patients. In the control group, the A/A genotype was detected in 84 (67.2%) subjects, the A/T genotype was detected in 39 (31.2%) subjects, and the T/T genotype was detected only in 2 (1.6%) subjects. The A allele was found in 114 (64.8%) of the study and 123 (74.5%) of the control group. The T allele was found in 62 (35.2.8%) of study and 41 (25.5%) of the control group. There was a significant difference between the study and control groups in terms of the distribution of genotypes and alleles of the A-161T polymorphism of the 5-HT1B receptor gene ($p<0.05$).

The CNR1 1359 G/A polymorphism was not found to be associated with alcohol dependence. The G/G genotype was the most common genotype in both study and control groups; it was detected in 75 (61.0%) study patients and in 84 (67.2%) control subjects. The G/A genotype was observed in 39 (31.7%) patients of the study group and 38 (30.4%) subjects of the control group. The A/A genotype was the most rare genotype in both groups; it was detected only in 9 (7.3%) study patients and 3 (2.4%)

control subjects. The G allele was found in 114 (70.4%) of the study and 122 (74.8%) of the control group. The A allele was found in 48 (29.68%) of study and 41 (25.2%) of control group. No statistically significant difference was found between the study and control groups in terms of the distribution of genotypes and alleles of the CNR1 1359 G/A polymorphism ($p>0.05$). The distribution of the genotypes of the DRD2 Taq1A polymorphism, 5-HT1B A-161T polymorphism, and CNR1 1359 G/A polymorphism in the study and control groups are presented in Table 2.

DISCUSSION

Genetics is one of the most rapid developing fields of medicine. New technologies such as the genome-wide association and copy number variants studies are promising to provide novel insights that might lead to new treatments for alcohol dependence¹⁸. However, studies investigating SNPs still constitute the majority of studies in the literature. Few genetic studies on alcohol dependence have been carried out in the Turkish population. Cinnioglu and colleagues reported that Turkish subjects were genetically similar to those from European, Caucasian, and Middle Eastern populations¹⁹. Kayaalti and Soylemezoglu found that genotype distributions of ADH1B, ALDH2,

CYP2E1*6, and CYP2E1*7B in the Turkish population were similar to those in Caucasian and some European populations, whereas they differed significantly from those in East Asian populations²⁰. Kortunay et al. also reported that the frequencies of variant ADH3 alleles and genotypes were similar to those in other Caucasian populations²¹.

In the present study, we investigated the association of DRD2 TaqIA, 5-HT1B A-161T, and CNR1 1359 G/A (rs1049353) polymorphisms with alcohol dependence. The DRD2 TaqIA polymorphism is one of the most widely studied gene polymorphisms in alcohol dependence. We failed to detect any association between alcohol-dependent Turkish patients and the DRD2 TaqIA polymorphism. Association of DRD2 TaqIA polymorphism with alcoholism was first reported by Blum et al. in 1990⁵. Since then, numerous studies have been published, several of which have shown an increase in the DRD2 A1 allele frequency associated with alcohol dependence²². Recently, Lee and colleagues found an association between DRD2 TaqIA polymorphism and alcohol dependence in the Korean population²³. There are several studies, however, in which no evidence has been demonstrated for association of the DRD2 A1 allele with alcoholism²¹. In a meta-analysis on this topic, authors have reported that association of the A1 allele with alcoholism may be relatively small because a complex disorder like alcohol dependence has a multigene causality^{7,22}. Our results were in agreement with the negative results. Causes of these findings might be sample size of the present study rather than a special genetic feature of the Turkish population.

Research performed during the past 30 years has indicated that serotonin neurotransmission is associated with alcohol dependence²⁴. The serotonergic system has been shown to be important in mediating alcohol reward, preference, dependence, and craving²⁵. Studies concerning the association of alcohol dependence with serotonin polymorphisms generally have focused on the serotonin transporter gene. The short, low-activity allele of a polymorphism in the serotonin transporter gene (SLC6A4) has been linked to

alcohol dependence²⁶. In a recent meta-analysis, McHugh et al. also concluded that the short allele of the 5-HTTLPR SLC6A4 polymorphism was associated with alcohol dependence²⁷. Genetic polymorphisms in serotonin receptors have been another important topic in alcohol dependence²⁸. It has been suggested that alcohol dependence is associated with elevations in the number of serotonin 1B receptors in animal models, and that alcohol dependence is associated with elevated levels of ventral striatal 5-HT1B receptors in humans, like in rodent models²⁹. Several polymorphisms in the 5-HT1B receptor gene have been detected, and of these, T371G, A-161T, and G861C have been found to be associated with different psychiatric disorders³⁰. The A-161T polymorphism is located in the promoter region of the 5HT1B receptor gene, and this polymorphism might alter transcriptional expression of the receptor³¹. Sun et al. reported the variant A-161T to be associated with alcohol dependence at both the allelic and genotypic level in a Taiwanese Han population³¹. Cao et al. also found an association between this polymorphism and alcohol dependence in 135 male Han Chinese patients³². They suggested that the A-161T polymorphism might be a valuable genetic marker for detecting alcohol dependence. In agreement with this finding, we also found an association between alcohol dependence and the A-161T polymorphism in the present study. In contrast to these results, two papers from the literature have reported no association between alcohol dependence and the A-161T polymorphism^{33,34}.

We investigated the possibility of an association between the rs1049353 polymorphism of CNR1 and alcohol dependence, but failed to detect any difference between the alcohol-dependent and control groups in the present study. There is increasing evidence from pharmacologic and neurochemical studies that the endogenous cannabinoid system is involved in addiction to substance abuse. Of the two known cannabinoid receptors, the receptor which is mainly responsible for neurophysiological and behavioral responses related to addictive behavior is CNR1³⁵. The

association of three polymorphisms in CNR1 (rs1049353, rs806379, and AAT repeat) with substance dependence has been reported in the literature. Despite 2 reports in which, an association was found in alcoholics, a recent meta-analysis consisting of five studies did not show a significant association, in agreement with the results of the present study^{36,37}.

The current study has limitations such as small sample size and lack of examination of personality traits. Moreover, the consideration of how single nucleotide polymorphisms effect alcohol addiction

is difficult because alcohol addiction is a complex polygenetic process.

CONCLUSION

Of the three genetic polymorphisms investigated in the present study, only the A-161T polymorphism of the 5-HT1B receptor gene resulted in detection of a significant association. Although there are a limited number of studies and contradictory results, the 5-HT1B receptor A-161T polymorphism may be a promising marker for alcohol dependence.

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