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Determination of the Relationship Between Craving and 4-repeat Allele of *DRD4* Gene Polymorphism in the Early Withdrawal Period of Alcohol Use Disorders

ABSTRACT

Objective: Alcohol use disorder (AUD) is a disease with chronic relapses. Risk factors of craving, which is thought to be one of the predictors of relapse, have been studied for a long time. The *DRD4* gene is located on chromosome 11p and has a 48-base pair variable number of tandem repeat (VNTR) polymorphisms in the 3rd exon. This study aimed to investigate if a relationship existed between craving and *DRD4* VNTR polymorphism and to determine the predictors of craving.

Methods: A total of 125 patients with AUD were included in the study. The sociodemographic data form, the Michigan Alcoholism Screening Test (MAST), the Obsessive Compulsive Drinking Scale (OCDS), and the Penn Alcohol Craving Scale (PACS) were applied to the patients. Polymerase chain reaction (PCR) was used to determine the *DRD4* VNTR variant of all participants in the peripheral blood sample.

Results: In the 4R/4R homozygous group, it was found that the age at first alcohol use was higher and the scores of the OCDS on the seventh day were lower, but this relationship could not be demonstrated in further statistical analyses. In the stepwise linear regression model, the age at first alcohol use, MAST score, duration of AUD, and delirium tremens history were found to be the predictors of craving.

Conclusion: *DRD4* VNTR polymorphism does not play a role as a predictor of craving. A decrease in age at first alcohol use, an increase in the MAST score, and the presence of delirium tremens were found to be the predictors of craving among the participants of this study.

Keywords: Receptors, dopamine, polymorphism, alcohol use disorder, craving

Introduction

Alcohol use disorder (AUD) is a chronic relapsing disorder. Craving is being investigated as one of the factors that may be associated with relapse. Craving is defined as an uncontrolled desire and an intense emotional-physiological need for alcohol intake in patients with AUD.[1] This experience is thought to contribute to sustaining AUD and its relapse.[2,3]

Dopamine, which plays an important role in the regulation of the brain reward system, is also closely related to craving. It has been reported, based on imaging and drug studies, that the dopaminergic system has a key role in craving.[4,5] Deterioration in striato-thalamo-orbitofrontal pathways has an impact on craving and loss of control in AUD.[6] Dopamine D4 receptors localized in the amygdala, hypothalamus, pituitary, and cerebral cortex are very important in this field.[7,8] In addition, the expression of dopamine receptors in the prefrontal cortex, which is related to attention and cognition, may be interesting for its behavioral phenotype determination.[9]

The *DRD4* gene is located on chromosome 11p and has a 48-base-pair (bp) variable number of tandem repeats (VNTRs) polymorphism in the 3rd exon, repeated between 2 and 11 times, with the most common variants as 2R, 4R, and 7R repeats (reps).[8] Studies on the functional significance of length-sequence changes of the *DRD4* receptor have been extensively investigated

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in AUD.[10] The 7R variant has been shown to have less potential than the 4R variant to inhibit the formation of cyclic adenosine monophosphate (cAMP).[7] It has been thought that individuals with the *DRD4* long allele may have high levels of cAMP, and it may result in chronic upregulation of cAMP with the chronic use of alcohol. This chronic upregulator, an alcohol-related stimulant, is believed to increase phasic D2/D4 stimulation and play a role in the occurrence of craving. The presence of this long allele (again 7 reps; DRD4L) has been shown to increase the urge to drink after alcohol intake.[11,12] However, this 7R allele is rarely found in some populations.[13] Individuals are grouped according to their *DRD4* tandem reps polymorphisms. Those with 7 or more reps (long alleles) are grouped as DRD4L carriers, and those with 6 or less reps (short alleles) are grouped as DRD4S carriers. DRD4L carriers have been shown to experience greater problems with alcohol than DRD4S homozygotes.[14,15]

This study aimed to investigate if there was a relationship between craving and *DRD4* VNTR polymorphism and identify properties that may be related to craving. Our hypothesis was that craving would be more severe in DRD4L carriers during the alcohol withdrawal period. It is very important to know the role of genetic factors in the etiology and treatment of AUD for developing individual treatments.

Methods

Sample Selection

Patients who were hospitalized in the Alcohol and Drug Addiction Treatment Center of the Ankara Numune Training and Research Hospital owing to alcohol dependence were included in the study. Interviews were carried out with 156 patients. Moreover, 13 patients with comorbid anxiety disorder, 5 patients with attention-deficit hyperactivity disorder, 9 patients who used mixed substances other than alcohol, and 4 patients who did not agree to participate in the study were excluded from the study. A total of 125 male patients between the ages of 18 and 65 years, who were diagnosed with alcohol dependence according to the Diagnostic and Statistical Manual of Mental disorders-4th Edition (DSM-IV)-TR criteria and who had the cognitive capacity to understand the scales to be used in the study and follow the instructions were included in the study.

Written and verbal informed consent was obtained from all the participants. The study was approved by the Ethics Committee of Ankara Numune Training and Research Hospital (Approval Date: May 25, 2009; Approval Number: 62).

Measurement Tools

Structured Clinical Interview for DSM-IV-TR Axis I Disorders (SCID-I): SCID-I is considered to be the gold standard, semi-structured assessment method for clinical disorders in DSM-IV axis I. It was developed by First et al.[16] Adaptation and reliability studies for the Turkish population were conducted by Corapcioglu et al.[17]

MAIN POINTS

- *The most common allele in the participants was 4R/4R followed by the 2R/2R and the 2R/4R allele.*
- *There is no difference in craving between those with and without the 4R / 4R genotype in PACS score.*
- *The age at first alcohol use, MAST score, duration of alcohol use, and history of delirium tremens predicted craving.*

Obsessive Compulsive Drinking Scale (OCDS): OCDS is a scale developed by Anton et al.[18] to measure craving in patients with AUD. It is a sensitive tool for measuring the obsessive and compulsive features of alcohol-related thoughts, drinking urges, and the ability to resist these thoughts and urges in patients with AUD. The Turkish reliability and validity studies of this scale were performed by Evren et al.[19]

Penn Alcohol Craving Scale (PACS): PACS is a 5-item self-rating scale that evaluates craving in terms of frequency, severity, and duration of thoughts on drinking, as well as the ability to resist drinking.[20] The Turkish reliability and validity studies of this scale were performed by Evren et al.[21]

Michigan Alcoholism Screening Test (MAST): MAST is an assessment tool developed by Gibbs that contains 25 questions based on a self-report, which questions whether the person faces problems related to alcohol use and severity of the problem.[22] The Turkish version of MAST is valid and reliable for screening severity of dependence in alcohol-dependent patients.[23]

Procedure

The patients were evaluated on the 0th and seventh day of hospitalization using PACS and OCDS. Depending on the severity of alcohol withdrawal symptoms, patients received 0 to 40 mg/day diazepam orally. Diazepam doses were reduced and discontinued within 1 week. Blood samples were taken on the seventh day of hospitalization for genotyping.

Genotype

DNA was isolated from peripheral blood leukocytes by the standard phenol/chloroform method, and the extracted DNA was stored at -20°C until analysis. The *DRD4* 48-bp VNTR variant in exon III with a variable length was genotyped as described previously.[13] The following primers were used: forward 5′-CGCGACTACGTGGTCTACTCG-3′ and reverse 5′-AGGACCCTCATGGCCTTG-3′. The polymerase chain reaction (PCR) conditions were as follows: 10 minutes at 95°C followed by 30 cycles of 20 seconds at 95°C, 20 seconds at 55°C, 40 seconds at 72°C, and a final extension of 5 minutes at 72°C. PCR products were electrophoresed on 3.5% agarose gel and visualized by ethidium bromide staining under ultraviolet illumination.

Statistical Analysis

Data were coded and analyzed using the Statistical Package for the Social Sciences, version 22.0, (IBM Corp.; Armonk, NY, USA). The normality of the distribution of the total scores was tested using the Shapiro-Wilk test and skewness and kurtosis values. Descriptive statistics are given as percentages or mean (standard deviation). The chi-square test was used to compare categorical variables, and the Student *t*-test was used to compare continuous variables. Patients were grouped according to 4R/4R homozygous and nonhomozygous genotypes as previously grouped according to allele frequency.[24] PACS and OCDS scores on days 0 and 7 were compared using the Student *t*-test. Internal consistency of PACS and OCDS was assessed using the Cronbach alpha. The general linear model was applied as an advanced statistical test to confirm significance. Spearman correlation analysis was used to show the correlation between total PACS and total OCDS values on days 0 and 7. The stepwise linear regression model was used to evaluate variables that predict craving (PACS and OCDS). The statistical significance level was accepted as $P < .05$.

Results

The mean age of 125 men with alcohol dependence who participated in the study was 44.2 (SD = 10.2) years. Of these, 83 (66.4%) were married, 14 (11.2%) were single, and 28 (22.4%) were widowed. The mean duration of education was 8.9 (SD = 3.6) years. It was determined that 69 (55.2%) of the participants had regular jobs; 56 (44.8%) were not working or were retired. The duration of chronic alcohol use was 19.6 (SD = 10.9) years, and the age at first alcohol use was 20.1 (SD = 6.3) years. Moreover, 62 (49.6%) participants were found to have received inpatient treatment previously, and 105 (84%) participants had a family member with AUD. Furthermore, 24 (19.2%) participants had a history of epileptic seizures during alcohol withdrawal periods. A history of delirium was present in 27 (21.6%) patients. In correlation analysis, a positive, significant correlation was found between total OCDS and PACS scores on days 0 (r = 0.697, $P < .001$) and 7 (r = 0.515, $P < .001$).

Table 1. Genotype and Allele Distribution of the Participants According to Dopamine D4 Receptor Gene Polymorphism

Genotype	n = 125	Distribution of alleles	n = 250
2/2	10	2	31
2/3	1	3	11
2/4	10	4	197
3/3	1	5	3
3/4	8	6	2
4/4	86	8	6
4/5	3	-	-
4/8	4	-	-
6/6	1	-	-
8/8	1	-	-

Abbreviations: n, number of people.

It was found that the most common allele in the participants was 4R/4R (n = 86), followed by the 2R/2R allele (n = 10) and the 2R/4R allele. Genotype and allele distribution of the participants according to *DRD4* gene polymorphism is given in Table 1.

The mean MAST score of the participants was 28.9 (SD = 8.7). Total PACS scores on days 0 and 7 were 18.4 (SD = 7.9) and 8.5 (SD = 7.3), respectively, and total OCDS scores on days 0 and 7 were 25.9 (SD = 8.5) and 18.4 (SD = 9.8), respectively. Comparison of alcohol use characteristics, sociodemographic variables, and PACS and OCDS scores between those with and without 4R/4R genotype are given in Table 2. There was no statistically significant difference between the 2 groups in terms of PACS and OCDS scores on day 0 (PACS total on day 0: difference [df] = 23, $t = -1.19$, $P = .235$; OCDS total on day 0: df = 123, $t = -0.73$, $P = .469$; OCDS obsessive on day 0: df = 123, t = -0.90, $P = .371$; and OCDS compulsive on day 0: df = 123, t = -0.46, $P = .647$).

On the seventh day, PACS and OCDS were reapplied, and there was no significant difference between the 2 groups in terms of PACS scores. (PACS total on day 0: df = 123, t = -0.62, $P = .535$). However, in those with the 4R/4R variant, obsessive subscale (df = 123, t = -2.42, $P = .017$), compulsive subscale (df = 123, t = -2.04, $P = .044$), and to-

Table 2. Comparison of Alcohol Use Characteristics, Sociodemographic Variables, PACS[a] and OCDS[b] Scores According to 4-repeat Allele of the Dopamine D4 Receptor Gene Polymorphism

	4R / 4R Genotype (n = 86)	Other genotypes (n = 39)	df	t/χ^2	*P*
Age, mean (SD), years	45.2 (9.6)	41.9 (11.0)	123	1.70	.091
Age, mean (SD), years	8.9 (3.5)	9.20 (3.8)	123	-0.50	.620
Marital status, n (%)					
Married	56 (65.1)	27 (69.2)	1	0.20	.652
Single-widow	30 (34.9)	12 (30.8)			
Employement status, n (%)					
Employement	43 (50)	26 (66.7)	1	3.01	.083
Unemployement/retired	43 (50)	13 (33.3)			
Age of first alcohol use, mean (SD)	21.0 (6.3)	18.6 (5.1)	123	2.10	.035
Duration of alcohol use, mean (SD), years	18.6 (10.9)	20.6 (10.6)	123	-0.97	.336
MAST, mean (SD)	28.8 (8.7)	29.3 (7.4)	123	-0.33	.743
History of delirium, n (%)	15 (17.4)	12 (30.8)	1	2.81	.093
History of seizure, n (%)	18 (20.9)	6 (15.4)		0.53	.466
OCDS-total on day zero, mean (SD)	25.6 (8.9)	26.7 (7.7)	123	-0.73	.469
OCDS-obsessive on day zero, mean (SD)	11.5 (5.0)	12.3 (3.8)	123	-0.90	.371
OCDS-compulsive on day zero, mean (SD)	14.0 (4.4)	14.4 (4.3)	123	-0.46	.647
OCDS-total on day seven, mean (SD)	17.1 (9.1)	21.4 (10.6)	123	-2.34	.021
OCDS-obsessive on day seven, mean (SD)	7.1 (4.4)	9.3 (5.1)	123	-2.42	.017
OCDS-compulsive on day seven, mean (SD)	9.9 (5.4)	12.1 (6.2)	123	-2.04	.044
PACS-total on day zero, mean (SD)	17.6 (7.9)	19.4 (7.8)	123	-1.19	.235
PACS-total on day seven, mean (SD)	8.2 (7.5)	9.1 (6.8)	123	-0.62	.535

Abbreviations: df, difference; SD, standard deviation; MAST, Michigan Alcoholism Screening Test; OCDS, Obsessive Compulsive Drinking Scale; PACS, Penn Alcohol Craving Scale.
[a]Penn Alcohol Craving Scale.
[b]Obsessive Compulsive Drinking Scale.

Table 3. Stepwise Linear Regression model when craving score (PACS[a] and OCDS[b] on the days zero and seven) was taken as a dependent variable

Dependent variable	Predictor variable	B	SE	Beta	t	*P*
OCDS-total on day zero	(constant)	30.945	3.292		9.40	< .001
	Age of first alcohol use	-0.612	0.107	-0.433	5.70	< .001
	MAST score	0.227	0.077	0.222	2.949	.004
	History of delirium tremens	3.720	1.596	0.181	2.33	.021
OCDS-total on day seven	(constant)	7.905	3.167		2.50	.014
	History of delirium tremens	7.483	1.996	0.316	3.75	< .001
	MAST score	0.208	0.098	0.177	2.12	.036
	Duration of alcohol use (years)	0.148	0.074	0.164	1.99	.049
PACS-total on day zero	(constant)	24.289	2.519		9.64	< .001
	First alcohol use age	-0.329	0.114	-0.252	-2.88	.005
	History of delirium tremens	3.677	1.665	0.193	2.21	.029
PACS-total on day seven	(constant)	13.044	2.282		5.72	< .001
	Age of first alcohol use	-0.226	0.108	-0.185		
	-2.09	0.038				

Abbreviations: SE, standard error; MAST, Michigan Alcoholism Screening Test.
For OCDS on day 0, F = 21.48, *P* < .001, adjusted R^2 = 0.331; for OCDS on day 7, F = 10.24, P < 0.001, adjusted R^2 = 0.183; for PACS on day 0, F = 8.70, *P* < 0.001, adjusted R^2 = 0.110; and for PACS on day 7, F = 4.38, *P* = .038, adjusted R^2 = 0.027.
[a]Penn Alcohol Craving Scale.
[b]Obsessive Compulsive Drinking Scale.

tal scores (df = 123, *t* = -2.34, *P* = .021) were statistically significantly lower. However, when general linear models were used (dependent variable: obsessive or compulsive subscales on seventh day; covariates: age, years of alcohol use, and MAST score; and fixed factor including *DRD4* VNTR polymorphism), the relationship between *DRD4* polymorphism and obsessive (F = 2.26, *P* = .135) and compulsive (F = 1.59, *P* = .209) subscales could not be confirmed.

The age at first alcohol use, MAST score, duration of alcohol use (years), and history of delirium tremens (DT) predicted craving in the stepwise linear regression model. Age, history of seizure, and *DRD4* polymorphism were taken as independent variables in this model (Table 3).

Discussion

This is the first study to examine the relationship between *DRD4* VNTR polymorphism and alcohol craving in patients with AUD by repeated measurements. In our study, although obsessive, compulsive, and total scores in OCDS were lower in the group with 4R/4R homozygous genotype, the effect was no longer significant after multiple tests.

DRD4 VNTR polymorphism may have a potential contribution to the etiology of substance use disorders. In particular, *DRD4* variants have been shown to have a direct effect on problematic alcohol use as well as indirect effects through novelty seeking.[15,25] These direct and indirect effects may be the reason for the later age at first alcohol use detected in the 4R/4R group in our study.

McGeary et al[26] examined the urge and *DRD4* VNTR polymorphism caused by cue reactivity in 93 untreated heavy drinkers. In this study, although the genotype with at least one long allele has been shown to have a greater subjective effect on impulse owing to alcohol cue reactivity than those with short homozygous alleles, no significant interaction on the impulse reactivity of *DRD4* VNTR polymorphism and alcohol dependence has been shown.

In another study in which cue reactivity and craving were examined in 88 heavy alcohol drinkers, DRD4L carriers showed significantly higher levels of subjective arousal and lower subjective craving levels compared with DRD4S carriers. Although the authors stated that these results did not comply with their hypotheses, no explanation could be found.[27] In addition, in another study conducted on 35 heavy alcohol drinkers, subjective craving levels were higher in patients with the DRD4L allele.[28]

There are a limited number of studies examining the *DRD4* VNTR genotype and alcohol drinking behaviors. In the functional magnetic resonance imaging paradigm within taste-cue, the *DRD4* VNTR genotype was shown to have no effect on drinking impulse, but in the pre-alcohol evaluation, it was shown that the response of the DRD4L group to the alcohol cues in the orbitofrontal cortex, anterior cingulate gyrus, and striatum was significant.[29] In a study conducted by Hutchison et al[11] in 87 heavy alcohol consumers, unlike DRD4S carriers, DRD4L carriers were aroused less and had a subjectively higher urge to drink alcohol. It has been suggested that DRD4L carriers may be more sensitive to self-medication to increase dopamine levels. Finally, in a naturalistic study of 112 heavy alcohol drinkers, DRD4L carriers had more drinking impulse after consuming alcohol compared with DRD4S homozygotes.[30]

In our study, the cravings were evaluated on the day of hospitalization and during the period of alcohol withdrawal. We found that craving, which is a dynamic element, is determined by variables such as duration of AUD, severity of addiction, age at first alcohol use, and DT history.

Healthy brain development should be promoted throughout childhood and adolescence. Exposure to neurotoxins, especially alcohol, at an early age may affect brain development, leading to the emergence of cognitive problems as well as functional consequences throughout life.[31,32] The age at first alcohol use (early exposure to neurotoxin) appears to affect craving measurements.

The severity of alcoholism was statistically significant in predicting craving (OCDS on days 0 and 7). In addition, duration of alcohol use and age at first alcohol use, which may be associated with alcoholism severity, appear to be associated with craving.[33,34] Finally, the history of DT was statistically significant in predicting craving. To explain the possible relationship between the history of DT and craving measurements, there may be a structural, biological factor that affects DT susceptibility and craving in common. In addition, DT may have long-term effects on the brain. Neuroadaptive changes may occur with glutamate-mediated stimulation of the central nervous system with withdrawal in long-term alcohol use. Neuropsychiatric complications such as autonomic hyperactivity and DT may occur.[35] The increase in excitatory neurotransmitters may be a cause of the possible brain damage mechanism.[36]

There are few studies examining the effect of DT occurring with alcohol withdrawal. In a study comparing alcoholics with and without DT, cognitive deficiency was found to be more common in the follow-up of patients with DT.[37] It is known that the frequency of the *DRD4* allele varies according to the population.[38] Our patient group had a similar distribution, and we classified them as with or without 4R/4R homozygous.

Our study has some limitations. Although our study had a large number of patients compared with studies in the literature that examined craving and *DRD4* VNTR polymorphism, the sample size was still small and included only men. It would be an advantage to have a higher number of participants for community representation in further genetic studies. Craving was evaluated during hospitalization in our study. The patients could not be followed-up after discharge, and how their craving levels changed in the long term could not be observed. *DRD4* VNTR polymorphism may be associated with the emergence of craving and relapse in long-term follow-up.

Our hypothesis that craving would be more severe in DRD4L carriers during alcohol withdrawal period could not be confirmed and no significant relationship was found between *DRD4* VNTR polymorphism and craving. As the age at first alcohol use decreases, the MAST score increases and the presence of DT seems to be a predictor for craving. It is very important to know the role of these genetic factors in the etiology of and in developing personalized treatments for AUD.

Ethics Committee Approval: *Ethics committee approval was received for this study from the Ethics Committee of Ankara Numune Training and Research Hospital (Approval Date: May 25, 2009; Approval Number: 62).*

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