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Association of the Neuropeptide Y LEU7PRO rs16139 and NEUREXIN 3 rs760288 Polymorphisms with Alcohol Dependence

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ABSTRACT:

Association of the Neuropeptide Y LEU7PRO rs16139 and NEUREXIN 3 rs760288 polymorphisms with alcohol dependence

Objective: Alcoholism is associated with genetic variants of genes related to alcohol metabolizing enzymes, dopaminergic, gamma-aminobutyric acidergic, glutamatergic, opioid, cholinergic, and serotonergic systems. Neurpeptide Y system and Neurexins were shown to be associated with alcohol dependence. Recent studies identified new genetic polymorphisms related to endogenous cannabinoid system, neuropetide Y and neurexin. In the present study, we aimed to investigate the association of Neurpeptide Y LEU7PRO rs16139 and neurexin 3 gene rs760288 polymorphisms with alcohol dependence in patients with alcohol dependence and healthy control subjects.

Methods: 123 patients who were diagnosed with alcohol dependence according to the DSM-IV criteria and 159 healthy volunteers were included in the study. Blood samples were used to investigate polymorphisms. The genotyping of the neurexin 3 gene rs760288 and the neuropetide Y gene Leu7Pro rs16139 polymorphisms was performed using TaqMan SNP Genotyping Assays Real-Time PCR System.

Results: Of the 2 genetic polymorphisms investigated in the present study, we detected association between and neurexin 3 gene rs760288 polymorphisms with alcohol dependence.

Conclusions: Neurexin gene polymorphisms might be an important factor in development of alcohol addiction.

Keywords: alcohol dependence, neuropeptide Y, neurexin, polymorphism

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INTRODUCTION

Alcohol dependence is associated with high heritability; however, research on specific genetic factors has so far resulted in unreliable outcomes. Besides, genetic variability in the genes encoding alcohol metabolizing enzymes, which is the strongest finding that has been found to date, alcoholism has been associated with genetic variants of genes related to dopaminergic, gamma-aminobutyric acidergic, glutamatergic, opioid, cholinergic, and serotonergic system[1,2]. Recent studies identified new genetic polymorphisms related to endogenous cannabinoid system, Neuropeptide Y (NPY) and Neurexins (NRXNs)[3-5].

NPY is a 36 amino-acid peptide, which modulates emotional processes, stress response, and anxiety. It has been suggested that NPY system might be involved in the pathogenesis of alcoholism[6]. The association between the genetic polymorphisms of NPY and alcoholism has been studied in a few studies[4,7]. Lappalainen et al. found that NPY Pro7 allele is a risk factor for alcohol dependence and Frances et al. reported that NPY 1258G>A polymorphism was associated with greater alcohol consumption but contradictory

results have also been reported[8,9].

Neurexins are presynaptic transmembrane proteins that function as cell adhesion molecules, binding to neuroligins to stabilize the synapse[10]. In addition to modulating neurotransmitter receptors and ion channels, ethanol has been reported to inhibit cell–cell adhesion[11]. Recently, NRXN3 polymorphisms were shown to be associated with impulsivity, substance use problems and alcohol dependence[5,12]. However, only few studies are available. We decided to investigate the association of NPY and neurexin polymorphisms with alcohol addiction because of the rising evidence showing that these molecules were involved in development of alcohol addiction.

In the present study, we aimed to investigate the association of NPY-LEU7PRO (rs16139) and NRXN3 gene rs760288 polymorphisms with alcohol dependence in patients with alcohol dependence and healthy control subjects.

METHOD

Subjects

The present study was conducted in the Alcohol and Substance Abuse Center of Denizli. Written informed consents were obtained from all participants before the study and the study was approved by the Local Ethics Committee. The study included 123 randomly selected patients between 18 and 65 years of age, who were diagnosed as alcohol dependence according to DSM-IV criteria. Patient assessment was performed using the Structured Clinical Interview for DSM-IV axis I disorders (SCID-I). Patients having any axis I disorder other than smoking dependence, and those having clinically significant organic diseases, mental retardation and a history of substance abuse (except alcohol and tobacco) in the previous 12 months, were excluded from the study. A hundred and fifty-nine volunteers, whose demographic characteristics were suitable for the study with no severe chronic physical diseases and history of mental disorders, not related to the patients in the study group, and were willing to participate in the study, served as the control group. All subjects included in the study were of Turkish origin.

Subjects were assessed for eligibility on the first day of hospitalization. A semi-structured questionnaire, which was developed by the researchers, including items on socio-demographic data was administered to all subjects.

DNA Extraction and Analysis

Venous blood samples were collected in ethylenediaminetetraacetic acid (EDTA) containing tubes. Salting out procedure was used for DNA extraction from whole blood[13].

Genotypic Analysis of the Neurexin 3 (NRXN3) Gene rs760288 and the Neuropeptide Y (NPY) Gene Leu7Pro (rs16139) Polymorphisms

The genotyping of the NRXN3 Gene rs760288 and the NPY Leu7Pro (rs16139) polymorphisms was performed using predesigned TaqMan Single Nucleotide Polymorphism (SNP) Genotyping Assays (Applied Biosystems, Foster City, CA). SNP amplification assays were performed according to the manufacturer's instructions. In brief, 25µl of reaction solution containing 30 ng of DNA was mixed with 12.5µl of 2X TaqMan Universal PCR Master Mix (Applied Biosystems) and 1.25 µl of pre-developed assay reagent from the SNP genotyping product C_26929863_10 for the NRXN3 Gene rs760288 and C_11164473_20 for the NPY Leu7Pro rs16139 (Applied Biosystems) containing two primers and two MGB TaqMan probes. Reaction conditions consisted of preincubation at 60ºC for 1 minute and at 95ºC for 10 minutes, followed by 40 cycles at 95ºC for 15 seconds and at 60ºC for 1 minute. Amplifications and analysis were performed in an ABI Prism 7500 Real-Time PCR System (Applied Biosystems), using the SDS 2.0.3 software for allelic discrimination (Applied Biosystems).

All procedures were conducted in a manner blind to the case status and other characteristics of the participants. Scoring of gels and data entry was conducted independently by two persons.

Table 1: Distribution of the genotypes and alleles of the NPY LEU7PRO rs16139 and NRXN3 gene rs760288 polymorphisms in the study and control groups

	Study Group (n=123)			Control Group (n=125)			p
NPY LEU7PRO (RS16139) polymorphism	T/T 118 (95.9)	T/C 5 (4.1)	C/C	T/T 150 (94.3)	T/C 9 (5.7)	C/C	0.342
NPY LEU7PRO (RS16139) allele	T allele 241 (98.0)	C allele 5 (2.0)		T allele 309 (97.2)	C allele 9 (2.8)		0.371
Neurexin 3 (NRXN3) gene (RS760288) polymorphism	C/C 4 (3.3)	C/T 40 (32.5)	T/T 79 (64.2)	C/C 10 (6.3)	C/T 86 (54.1)	T/T 63 (39.6)	0.001
Neurexin 3 (NRXN3) gene (RS760288) allele	C allele 48 (19.6)	T allele 198 (80.4)		C allele 106 (33.3)	T allele 212 (66.7)		0.001

Data are presented as n (%)

Statistical Analysis

Statistical analysis of data was performed using the Predictive Analytics Software (PASW) Statistics 17.0 (SPSS Inc., Chicago, IL, USA). Descriptive analysis was used to present sociodemographic data. Comparison of the categorical variables was performed using the chi-square test and t test was used to compare continuous variables. A Hardy–Weinberg equilibrium test was performed for each sample. A p value of <0.05 was considered statistically significant.

RESULTS

The study group included 123 patients with alcohol dependence [111 (90.2%) males; 12 (9.8%) females] with a mean age of 35.19±10.18 years and the control group included 159 healthy volunteers [142 (89.3%) males; 17 (10.7%) females] with a mean age of 34.92±12.36 years. There were no statistically significant differences between the study and control groups in terms of age and gender (p=0.743).

The observed genotype frequencies did not deviate from those expected according to Hardy–Weinberg equilibrium for NPY LEU7PRO rs16139 polymorphism (p=0.695). As for the NPY LEU7PRO rs16139 polymorphism, the T/T genotype was observed in 118 (95.9%) patients and the T/C genotype was observed in 5 (4.1%) patients; C/C genotype was not present in any of the patients in the study group. In the control group, the C/C genotype was observed in 150 (94.3%) subjects, and the C/T genotype was observed in 9 (5.7%) subjects, however none of the subjects had C/C genotype, T allele and C allele was present in 241 (98.0%) and 5 (2.0%) patients in the study group, and in 309 (97.2%) and 9 (2.8%) subjects in the control group, respectively. The distribution of genotypes and alleles of the NPY LEU7PRO rs16139 polymorphism was similar in the study and control groups (p=0.342).

For the NRXN3 gene rs760288 polymorphism, the C/C genotype, C/T genotype and T/T genotype was present in 4 (3.3%), 40 (32.5%) and 79 (64.2%) patients in the study group, and in 10 (6.3%), 86 (54.1%) and 63 (39.6%) subjects in the control group, respectively. In the study group, C allele was observed in 48 (19.6%) patients and T allele was observed in 198 (80.4%) patients, whereas C allele was observed in 106 (33.3%) subjects and T allele was observed in 212 (66.7%) subjects in the control group. There was statistically significant difference between the study and control groups in terms of the distribution of genotypes and alleles of the NRXN3 gene rs760288 polymorphism (p=0.001). Also the observed genotype frequencies did not deviate from those expected according to Hardy–Weinberg equilibrium for NRXN3 gene rs760288 polymorphism (p=0.695).

Table 1 summarizes the distribution of the genotypes and alleles of the NPY LEU7PRO rs16139 and NRXN3 gene rs760288 polymorphism in the study and control groups.

DISCUSSION

Although SNP studies still constitute the majority of studies in the literature, advances in genetic technologies, such as the research on genome-wide association and copy number variants have provided important new insights into new treatments for alcohol dependence and other psychiatric disorders[14]. The number of genetic studies on alcohol dependence in Turkish population is quite limited. In a study, where Kayaaltı and Soylemezoglu aimed to determine the polymorphisms of ADH1B, ALDH2, CYP2E1*6 and CYP2E1*7B in unrelated healthy Turkish population and compare the results with other populations, they found that genotype distributions of these genes in the Turkish population were similar to those in Caucasian and some European populations, whereas they differed significantly from those in East Asian populations[15]. Cinnioglu et al. reported that Turkish subjects were genetically similar to those from European, Caucasian, and Middle Eastern populations[16].

NPY LEU7PRO rs16139 polymorphism was rarely studied in alcohol dependence. In this present study, we investigated whether there was an association between NPY LEU7PRO rs16139 polymorphism and alcohol dependence, and failed to detect any association. Likewise, Zhu et al. found no association between Pro7 allele and alcoholism in Caucasian populations[9]. Zill and colleagues reported that four polymorphisms, including Pro7 allele were not associated with alcohol dependence[17]. However, contradictory to the results of the present study and the studies mentioned above, there are studies reporting an association between NPY LEU7PRO rs16139 polymorphism and alcoholism. Lappalainen and colleagues reported that the NPY Pro7 allele was associated with alcohol dependence heritability[8]. Koehnke and colleagues found that Pro7 polymorphism was associated with the severity of alcohol withdrawal symptoms[18]. Other polymorphisms on NPY gene were also investigated. Wetherill et al. examined 39 single nucleotide polymorphisms in NPY and its receptors, and although they found no association between NPY gene polymorphisms and alcoholism, they found an association between NPY 2 receptor genes and alcoholism[4]. Mottagui-Tabar reported a novel polymorphism at position -602 in the 5' region of the NPY gene, which was significantly associated with alcohol dependence[19].

Neurexins are neuronal proteins that function as cell adhesion molecules; they bind to their extracellular binding partners, neuroligins, dystroglycan, and neurexophilins, at presynaptic sites[20]. Neurexin 3 polymorphisms were found to be associated with alcohol dependence, antipsychotic induced weight gain and borderline personality disorder phenotypes[5,21,22]. Hishimoto and colleagues reported an association between NRXN3 isoforms, rs8019381 and rs760288, and alcohol dependence[5]. Stoltenberg et al. found associations between rs11624704 and attentional impulsivity and between rs1004212 and alcohol problems in men. In the same study, weak associations between rs10146997 and TIME estimation and rs1004212 and drug problems in women were also reported[12]. Neurexin-3 polymorphisms have also been associated with smoking behavior, which suggests that this gene contributes to genetic vulnerability to addictive behaviors[23,24]. We also found that C allele was less frequent in the study group and the difference between the two groups was statistically significant. Recent genetic studies on neurexins and neuroligins indicate that these genes may play a role in vulnerability to many psychiatric disorders and especially to addiction[5]. Our study also supported these results, suggesting that neurexin 3 polymorphisms might be an important factor in the development of alcohol addiction.

This present study has some limitations like the relatively small sample size. Moreover, it is difficult to consider that single nucleotide polymorphisms affect alcohol addiction as alcohol addiction is a complex polygenic disease.

Of the two genetic polymorphisms investigated in the present study, we detected an association between NRXN3 gene rs760288 polymorphism and alcohol dependence. Neurexins are promising molecules in the pathophysiology of alcohol addiction. Further genetic analysis of polymorphisms in neurexins with more subjects might give us a better understanding of this disorder.

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