Effects of Nitric Oxide Synthase-1 Exon 1f-VNTR Gene Polymorphism on the Clinical Symptoms of Alcohol Dependence, Impulsivity and Comorbid Attention Deficit Hyperactivity Disorder

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SUMMARY

Objective: We planned to compare individuals with alcohol dependence (AD) and healthy controls on the frequency of NOS1 exon 1f-VNTR gene polymorphism and to investigate the effects of this polymorphism on the clinical symptoms of alcohol dependence, impulsiveness and comorbid attention deficit hyperactivity disorder (ADHD) symptoms.

Method: A total of 282 participants consisting of 153 patients and 129 age and gender matched healthy individuals were inluded in the study. All participants were evaluated with Structured Clinical Interview for DSM-IV Axis 1 disorders (SCID-I) and Michigan Alcohol Screening Test (MAST), Barratt Impulsiveness Scale (BIS-11), UPPS Impulsive Behavior Scale, Adult Attention Deficit and Hyperactivity Diagnosis Scale (ADHDS), Family History Research Diagnostic Criteria (FHDRC). The QF-PCR fragment protocols were used for genetic analyses. Allele fragments of ≤176 bp and >176 bp sizes were separated and 3 different genotypes were determined as the SS, SL and LL. Associations of these genotypes with symptoms of AD severity, impulsiveness and comorbid ADHD were investigated.

Results: The AD and control groups did not differ significantly on the basis of NOS1 exon 1f-VNTR gene polymorphism. Also, significant correlations between this polymorphism and symptoms of AD severity, impulsiveness and ADHD were not determined.

Conclusion: Results of our study do not indicate a significant association between the NOS1 exon 1f-VNTR genotypes and AD, subgroups of AD, impulsiveness or comorbid ADHD semptoms.

Keywords: Alcohol dependence, attention deficit hyperactivity disorder, nitric oxide synthase, impulsiveness

INTRODUCTION

It has been accepted that genetic factors involved in the aetiopathogenesis of alcohol dependence (AD) explain 60% of the variance while the remaining 40% are determined by environmental factors (Thome et al. 2000, Basu et al. 2004, Reilly et al. 2017). The genes encoding neurotransmitters such as dopamine, serotonin, GABA and glutamate have been the subject of interest for the recent studies on the genetic basis of AD. Also, the genes encoding the enzymes of alcohol metabolism such as protein kinase-C, adenylcyclase, alcohol dehydrogenase and aldehyde dehydrogenase or the enzymes acting in the biochemical steps after alcohol metabolism on the genes of the opioid receptors of the reward system have

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been investigated (Foroud et al. 1999, Ayhan et al. 2014, Gürel et al. 2016).

It is known that alcohol and substance addiction disorder may be frequently seen among patients with attention deficit and hyperactivitiy disorder (ADHD) or vice versa (Biederman at al. 1995, Wilens et al. 1995, Osland et al. 2017). It has also been known that the onset of comorbid addiction syndromes including AD occurs at earlier ages and with more severity in ADHD (Wilens et al. 1997, Wilens 2004). Given the high genetic burden observed in both AD and ADHD after investigation of their common genetic features, it was proposed that comorbidity of these disorders was a phenotype for the severe dependence (Johann et al. 2003). The reports suggest that AD and ADHD may have a common genetic basis or share a similar genetic aetiology for increased incidence of comorbid disorders such as impulsiveness.

Nitric oxide (NO) is a free radical gas formed during the conversion of L-arginine to citrulline by the NO synthase (NOS), with molecular properties differing from neurotransmitters while partaking in various biological processes in the organism (Dawson and Dawson 1995, Mustafa et al. 2009). NO was reported to provide non-synaptic transmission between glutamatergic and monoaminergic systems in the brain (Kiss and Vizi 2001, Matsumoto et al. 2006) and has been thought to function as secondary messenger molecule in the central nervous system. Hence, the NOS system may be a common target for evaluating the clinical and genetic relationships of ADHD, impulsiveness and AD (Aspide et al. 1998, Uzbay and Oglesby 2001, Grammatikopoulos et al. 2002).

NOS inhibition augments the sedatif-hypnotic effect of alcohol which is reversed by drugs that imitate NO (Adams et al. 1994). In experimental animal models, NOS inhibition decreases alcohol consumption and its effects on the locomotor system (Rezvani et al. 1995, Aspide et al. 2000); and ADHD like symptoms such as briskness, impulsivity, aggression, learning disability, anxiousness and attention deficit were observed in NOS1 knock out mice (Gao et al. 2015). The NO level in humans with ADHD was found to be higher than in healthy controls (Ceylan et al. 2010). These results support the hypothesis that NO has a mediating effect on the symptoms of phenotypes related to ADHD and AD. In one of the recent studies focused on the NOS system genetics carried out on 3200 participants, those with ADHD, B type personality disorder, history of suicidal attempt, aggressive and violent behaviour had increased incidence of expression of the SS allele of NOS1 exon 1f-VNTR polymorphism. The S allele was proposed to have a role in the development of impulsiveness or other psychopathologies by reducing the transcription of the gene and was identifed as a "risk allele" (Reif et al. 2009). Similarly, increased impulsiveness in ADHD associated with NOS1 exon 1f-VNTR gene SS allele homozygosity was reported by Hoogman et al. (2011).

In the only reported study on polymorphism related to alcohol use, the starting age of alcohol use was earlier, the quantities consumed were higher and effects were more distinct in healthy individuals carrying the SL and LL alleles of NOS1 exon 1f-VNTR as compared to the homozygotic SS carriers (Laas et al. 2011). The incidence of this polymorphism, alcohol use behaviour and its relation to related phenotypes has not been investigated.

The main purpose of this study is to compare the incidence of NOS1 exon 1f-VNTR gene polymorphism in healthy and AD diagnosed individuals, and to assess the relationship of the polymorphism with impulsiveness and ADHD in both groups. The relationship between NOS1 exon 1f-VNTR gene polymorphism and features of AD such as the starting age and family history were queried to obtain the guidelines for testing the significance of this polymorphism in determining the AD subtypes.

It was hypothesised at the outset that the incidence of carrying the NOS1 exon 1f-VNTR gene S allele would be increased, and a relationship would be demonstrated between an increased incidence of expressing the S allele and early age of starting alcohol use, severity of AD, and elevated scores on psychometric tests on ADHD and impulsiveness.

METHOD

This study was conducted in Hacettepe University Faculty of Medicine (HUTF) Department of Psychiatry and Ankara Numune Training and Research Hospital, Alcohol and Drug Research Treatment and Training Center (AMATEM). The experimental groups were selected from male inpatients who had just started treatment for AD and the control group consisted of healthy male volunteers employed in the two hospitals. The two groups were matched on gender and age basis by being males in the age range of 18-65.

The AD group did not have any substance use disorder except alcohol use and cigarette smoking; and participants meeting these criteria but having a history of substance use were also included in the study. The control group did not have any alcohol or substance use disorder and used only cigarettes. Not being literate, having lifelong schizophrenia spectrum disorders, bipolar disorder and any comorbid disorder affecting the cognitive functions comprised the exclusion criteria for both groups. Only male particpants were enrolled in the study.

Sociodemographic details were obtained from all participants during the inteviews. The Structured Clinical Interview for Diagnostic and Statistical Manual of Axis I Mental Disorders)

(DSM-IV- SCID-I) and the Michigan Alcoholism Screening Test (MAST) were used for determining the intensity of AD and for screening alcoholism level of the healthy participants. To assess family history Family History Research Diagnostic Criteria (FH-RDC), to determine the level of impulsiveness Barratt Impulsivity Scale-11 and UPPS Impulsive Behavior Scale, and to determine comorbidity with ADHD symptoms the Adult Attention Deficit and Hyperactivity Disorder Scale were used in this study.

Initial evaluations on the age of starting alcohol use, the amount of alcohol consumed and the patterns of usage were made with the patients group after the first week of hospitalisation and following the subsidence of withdrawal symptoms. The criterion for "the age of starting alcohol use at a problematic level" was based on the presence of a history of at least two social incidences such as alcohol related problem at work or home, absenteeism from work or school due to alcohol use, or being involved in violence/police arrest under the influence of alcohol. Reviewing the classification of starting age of alcohol use, shows that 20 and 25 years are depicted as the cut off points to differentiate the early and late starting ages (Babor et al. 1992) and these were implemented to form the "AD subgroups of early and late start" in this study. Another subgroup was determined by using the diagnostic criteria of the FH-RDC and was further divided on the bases of the queries as those with and without family history of alcoholism. Alcohol amount was calculated on the basis of the standard drink criterion and approxiamately equivalent amounts of alcohol were assumed to be included in raki, whiskey, gin, coniac and vodka, accepting a level of 30 units/70cl in high alcohol grade drinks, 1 unit was allowed for 0.33L beer, 0.15 L wine and 0.04 L of sherry/fruit brandy (Johnson and Ait-Daoud 2005). The amount of daily alcohol use within the previous 6 months and the lifelong daily alcohol use were also queried and these parametric data were used to assess the severity of alcohol use in addition to the scores on the MAST.

This study was approved by Hacettepe University Non-invasive Clinical Research Ethics Committee, and written informed consent were received from all participants. The study was supported financially by the Scientific and Technological Research Council of Turkey (TUBITAK) as the Project No: 113 S513 of TUBITAK.

Materials

The Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I)

Diagnoses of alcohol and substance dependence of the participants were based on the SCID-1, the clinical interview conducted to determine the psychiatric disorders of axis-1. It has been adapted to and structured in the Turkish language (First et al. 1996, Özkürkçügil et al. 1999). In this study the A, B and ve F modules of the SCID-1 were used to assess mood disorders, psychotic symptoms and related disorders and anxiety disorders, and the E module was used to diagnose alcohol and other substance use disorders.

Michagan Alcoholism Screening Test (MAST)

The Turkish language version of the MAST was used to determine the AD severity and to exclude individuals under the risk of developing alcohol use disorder from the control group. The MAST is a 25-question self report scale answered as ''yes" or ''no", with a score for each question, developed to assess the severity of acohol use (Selzer 1971). The Turkish language version the scale has the highest discriminatory power and a cut off point within scores of 5-9, taken a 7 for the purposes of this study. (Coşkunol et al. 1995). Despite being a screening test, high scores on MAST have been associated with high severity of alcohol dependence (Ögel et al. 2012).

Family History Research Diagnostic Criteria (FH-RDC)

FH-RDC is an assessment tool developed by Andreasan et al. (1977) to determine the psychiatric disorder history in the family. In this study, the criteria of the version adapted to the Turkish language by Ayhan et al. (2015) and Gürel et al. (2016) including a history of 'legal, social, health, work/ profession and marriage' related issues of alcohol use disorder and the treatment receieved in the first and second degree relatives of the two groups of participants.

Barratt Impulsiveness Scale-11 (BIS-11)

BIS-11 is a self report scale to assess impulsiveness originally developed by Barratt in 1959. Its 11-items have high reliability as revised by Patton et al. (1995). It has 30 questions in total and three subscales on non-planning, attention and motor. High scores on BIS-11 show high level of impulsiveness (Patton et al. 1995). BIS-11 was adapted to the Turkish language by Guleç et al. (2008).

UPPS Impulsive Behavior Scale

UPPS Impulsive Behavior Scale, developed by Lynam and Whiteside (2001), is a self report, four-point likert type scale that assesses impulsiveness. It has 45 questions in total and 4 subscales on lack of premeditation, urgency, sensation seeking and lack of perseverance. Its psychometric validity and adapation to the Turkish language were made by Yargıc et al. (2011).

Adult Attention Deficit and Hyperactivity Disorder Scale (AADHDS)

The adult ADHDS has three subscales on Attention Deficit, Hyperactivity/ Impulsivity and ADHD Related Symptoms. First and second subscales are generated by the DSM-IV attention deficit and hyperactivity sections while the third

subscale is based on clinical practice. Total scores from first and second subscales sum up to the ADHD scores and total scores from third subscale sum up to the ADHD related symptoms scores. (Turgay 1998, Günay et al. 2006). ADHD level is assessed on ADHD total scores and ADHD Related Symptoms. This scale was used to assess the ADHD level of all participants.

Genetic Analyses

All genetic studies were carried out in the laboratory of Hacettepe University, Faculty of Medicine, Department of Medical Genetics, starting with 5-6 peripheral blood samples collected in EDTA (ethylenediaminetetraacetic acid) tubes stored at -20 °C. before isolating the DNA by using the Qiagen DNA Blood Mini Kit protocol. Genotyping of the NOS1 Exon 1f-VNTR zone was done by the QF-PCR (Quantitative Fluorescence – Polymerase Chain Reaction). The primer pair used in the study were determined as the NOS1F: FAM-5'-CCCTGCGTGGCTACTACATT-3' and the NOS1R:5'-GTTTCTTCTGGGCTCCAAAGCATACAT-3' containing the the underlined bases of the ''PIG Tail". The fragment size of the genes after PCR were determined by the ABI 3130 DNA sequencer analyzer instrument and the Gene Mapper program. According to the analysis results, the allele sizes were separated as the short (≤176 bp) and the long (>176 bp) into two groups, and three different genotypes as the SS, SL and LL were identified in the patient and control samples (Hoogman ve ark. 2011).

Statistical Analyses

The statistical analyses of this study were made on the Statistical Package for the Social Sciences (SPSS 21 Version). Numeric variables were presented with standard deviation, mean and median (max. and min.) values, while categorical variables were presented with numbers and percentages. The Chi-Square Test or Fisher's Exact Test was used to determine any difference between the independent groups of categorical variables. Normal distribution of the numerical variables was tested by the Shapiro Wilk Test and the homogeneity of the variances by the Levene test. The difference between two independent groups of numerical variables was analysed using the T-Test for independent groups when criteria for parametric tests were met, and by the Mann Whitney U test when these criteria were not met. The Kruskal Wallis test was used for comparison of multiple independent groups of numerical variables. Correlations between numerical variables were determined by the Spearman Correlation test. The corrections in the differences between the patient and control groups on the basis of the numerical variables of age and education were assessed using the multiple variance analysis (MANOVA). Statistical significance was accepted within the limit of $p<0.05$.

In this study, the analyses related to the NOS1exon 1f-VNTR gene were based on the assumption of equi-dominance of the S and L alleles in the individual analysis of the conditions SS, SL and LL and by accepting the S or the L allele as dominant in analysing the conditions SS, SL+LL; LL, SS+SL individually.

RESULTS

Sociodemographic Features

Initially a total 282 participants comprising 153 AD patients and 129 healthy controls were included in this study, but 3 AD patients without DNA data had to be excluded. A further 29 partipants were excluded on the basis of the MAST, with 14 having ≥ 7 scores and 15 having no results. In the final results included the data of 150 patients and 100 control individuals.

In controlling the AD and the control groups on age and years of education, the mean age of the AD group (44.68 ± 9.63) was significantly higher than that of the control groups (35.99 ± 7.98) ($p<0.01$) and the year of education of the AD group (11.67 ± 3.50) exceeded that of the control group (10.02 ± 3.52) ($p<0.01$).

The Relationship Between NOS1 Exon 1f-VNTR Gene Polymorphism and Alcohol Dependence

AD and control groups were both shown to be compatible with Hardy-Weinberg equilibrium. The NOS1 exon 1f-VNTR gene polymorphism did not differ significantly between the AD and control groups on the basis of the equi-dominance model. The differences between the two groups on the bases of the genotypic models SS and SL+LL with L allele dominance and the LL and SS+SL with S allele dominance were also not statistically significant (Table 1).

Table 1. The Genetic Distribution of NOS-1 Exon 1f-VNTR Between AD and Control Groups

NOS-1 exon1f-VNTR	AD Group (n=150) (%)	Control Group (n=100) (%)	P
SS	21.3	17.0	0.452*
SL	53.3	51.0	
LL	25.3	32.0	
LL+SL (L dominant model)	78.7	83.0	0.398**
SS+SL (S dominant model)	74.7	68.0	0.250***

*For Co-dominant model Chi-square=1.737, df=2; **For Continuity Correction=0.563, df=1; ***For S dominant model Chi-square=1.421, df=1

Table 2. The Relationship Between ADHD and NOS1 Exon 1f-VNTR Genotype Distribution

	Having ADHD Diagnosis			ADHD Total Scores M±SD		ADHD Related Symptoms Scores M±SD	
	Yes (%)	No (%)	P		P		P
SS (n=24)	28.0	19.3	0.546[1]	16.7±11.9	0.490[4]	35.2± 24.8	0.846[7]
SL (n=59)	52.0	52.3		18.0±8.8		30.0± 17.4	
LL (n=30)	20.0	28.4		16.6±10.8		31.4±18.5	
LL+SL (n=89)	72.0	80.7	0.510[*/2]	17.5±9.5	0.520[*/5]	30.4±17.7	0.612[*/8]
SS+SL (n=83)	80.0	71.6	0.559[**/3]	31.43±18.5	0.450[**/6]	31.5±19.8	0.919[**/9]

M: Mean, SD: Standard Deviation, *P value was used to compare SS and LL+SL values. **P value was used to compare LL and SS+SL values. df=2[1-4-7]; df=1[2-3]; Kruskal-Wallis Test Chi-square=1.425[4]; =0.333[7]; Mann-Whitney U Test Z=0.643[5]; =0.755[6]; =0.507[8]; 0.102[9].

Relationship Between NOS1 Exon 1f-VNTR Gene Polymorphism and Severity of Alcohol Dependence and the Alcohol Dependence Subtypes

In AD group, significant relationships between NOS1 exon 1f-VNTR gene polymorphism and 'average amount of alcohol consumption', 'maximum amount of alcohol consumption' and the MAST scores were not demonstrable.

Similarly, in analyses made by inclusion of all the patient sampling, a relationship could not be detected between these parameters related to alcohol dependence and the indicated genotype distributions. Only in the analyses made with the data on the healthy controls, carriers of the SS allele had elevated MAST scores.

Taking the ages of 20 and 25 years individaully as the cut off points for, respectively, the 'early' and 'late' ages of starting problematic alcohol use, subgroups were formed within AD group. Also, subgroups were designated as those 'having' and 'not having' a family history of alcohol use. Comparison of these groups with each other on the bases of the NOS1 exon 1f-VNTR polymorphism did not yield statistically significant correlations.

The Relationship Between NOS-1 Exon 1f-VNTR Gene Polymorphism with Attention Deficit Hyperactivity Disorder (ADHD) and Impulsiveness

When the AD and control group scores on the Adult Attention Deficit and Hyperactivity Disorder Scale were evaluated together, significant relationships were not found between the NOS1 Exon 1f-VNTR gene polymorphism and on having ADHD, the ADHD total score, the ADHD related symptoms scores (Table 2).

Similarly, when all participants were evaluated together, the Barratt Impulsiveness Scale-11 and the UPPS Impulsive Behavior Scale total scores and the level of impulsiveness based on the the scores on the subscales did not correlate with the NOS1 exon 1f-VNTR genotype polymorphism (Table 3).

Table 3. The Relationship Between NOS1 Exon 1f-VNTR Gene Polymorphism and Impulsivity (According to Total Scores from Barratt Impulsiveness Scale-11 and UPPS Impulsive Behavior Scale)

	SS (s=24) M±SD	SL (s=59) M±SD	LL (s=31) M±SD	P	LL+SL (s=90) M±SD	P*	SS+SL (s=83) M±SD	P**
BIS-11 Total Score	65.9±14.0	64.2 ±11.4	64.1 ±11.4	0.929[1]	64.2±11.3	0.710[2]	64.7±12.2	0.838[6]
Attention	16.9±4.4	15.5±3.5	15.9± 3.2	0.531[1]	15.6±3.4	0.289[3]	15.9±3.8	0.985[7]
Motor	21.6±5.7	20.9 ±4.7	21.8±6.0	0.817[1]	21.2±5.1	0.933[4]	21.1±4.9	0.568[8]
Non-planning	27.3±5.7	27.7±5.2	26.3±4.3	0.534[1]	27.2±4.9	0.783[5]	27.6±5.3	0.264[9]
UPPS Impulsive Behavior Scale Total Score	101.3±22.7	104.9±15.4	100.1±16.5	0.411[10]	103.2±15.9	0.915[11]	103.9±17.7	0.447[16]
Lack of Premeditation	19.9±5.8	22.3±5.9	20.9±6.0	0.247[10]	21.8±6.0	0.238[12]	21.7±6.0	0.738[17]
Urgency	30.9±10.1	32.2±7.4	31.0±8.9	0.820[10]	31.8±7.9	0.687[13]	31.8±8.2	0.267[18]
Sensation seeking	29.6±9.1	29.3±5.9	27.7±5.5	0.540[10]	28.8±5.8	0.700[14]	29.4±6.9	0.548[19]
Lack of Perseverance	20.9±4.3	20.9±4.3	20.4±3.4	0.807[10]	20.7±4.0	0.952[15]	20.9±4.3	0.219[20]

M: Mean, SD: Standard Deviation, BIS-11: Barratt Impulsiveness Scale-11, UPPS IBS: UPPS Impulsive Behavior Scale, *P value was used to compare SS and LL+SL values. ** P value was used to compare LL and SS+SL values. df=2[1], df=2[10], Mann-Whitney U Test Z=0.372[2], =1.061[3], =0.084[4], =0.275[5], =0.204[6], =0.019[7], =0.571[8], =1.117[9], =0.107[11], =1.179[12], =0.403[13], =0.386[14], =0.061[15], =1.230[16], =0.761[17], =0.335[18], =1.109[19], =0.600[20]

DISCUSSION

This study was carried out with the aim of investigating the existence of relationships between NOS1 exon 1f-VNTR genotype polymorphism and the different parameters related to AD, symptoms of ADHD comorbid with the dependence syndrome and the level of impulsiveness. Results have shown that the NOS1 exon 1f-VNTR genotype distribution showed similarity to a European sample (Reif et al. 2009) without any differences between the AD and the control groups. NOS1 eon1f-VNTR gene polimorphism was not related to AD severity of the variations of AD subgroups. The only study investigating the relationship between alcohol consumption and NOS1 exon 1f-VNTR genotype polymorphism among 593 healthy participants was reported in the literature by Laas et al. (2011). In this study, it was found that carriers of the L allele as compared to the homozygote S allele (SS) carriers had started alcohol intake at an earlier age, consumed more alcohol and reported more effects of alcohol. The authors reported that it had been shown in previous studies that the S allele of the NOS1 exon 1f-VNTR polymorphism represented a lower transcriptional activity and was S allele associated with impulsiveness and that in their study the L allele was found to be associated with AD suggesting that the described polymorphism could be associated with AD independently of a relationship with impulsiveness.

However, in our study, the results of the analyses with the AD and control groups and the AD subgroups did not show significant differences in genotypic distribution. In our analysis of the control group data, observation of elevated MAST scores of the SS genotype do not agree with the results of Laas et al. (2011).

The difference between our study and that of Laas et al. (2011) could be attributed to different reasons. Firstly, there are differences of participants. Laas et al. (2011) have worked with youthful male and female participants not evaluated diagnostically and with ages in the 15-25 year range. On the other hand, we have worked only with male participants in the age range of 18-65 years and classified on the basis of the DSM-IV criteria.

Also, evaluation criteria of the two studies were different. Laas et al. (2011) based evaluations on the age of first alcohol intake in relation to polymorphism, whereas our study based the comparison on polymorphism between AD groups on the "problematic starting age of alcohol usage". Recruitment of only male participants in our study could be taken as a limitation. However, the reported results on the relationship between alcohol consumption and polymorphism were obtained in two of the evaluations, at ages of 18 and 25, out of the three, and was affected by the male gender in the case with 18 years of age. These results reduce the significance of working with only males in our study.

Studies on the NO system with animal models resulted in preventing the development of rapid tolerance of alcohol (Khanna et al. 1993), decreasing the effects of alcohol (Adams et al. 1994), alcohol withdrawal symptoms (Adams et al. 1995), alcohol preference and consumption (Lallemand and De Vitte 1997, Rezvani et al. 1995); whereas results were shown with the opposite effects of NO precursores and donors in repeated studies (Uzbay and Oglesby 2001). However, Ikeda et al. (1999) reported that NOS1 activity in many regions of the brain after acute and chronic ethanol administration was similar to the controls and that ethanol influenced NO system in the brain through NO pathways rather than the NOS1. Because of no significant results in terms of NOS1 exon 1f-VNTR genotype polymorphism was found in the analysis between the AD group and the control group or within the AD subgroups in our study, it could be said that these findings were compatible with the results of Ikeda et al. (1999). In the control group, the MAST score which was indicating the intensity of alcohol using was found to be low in SS allele carriers and low in L-dominant (LL + SL) allele carriers showed compatible results with the previous studies on the NOS1 exon 1f-VNTR polymorphism suggested that the S allele was associated with impulsivity, and that the S allele had a risk for impulsivity-related conditions such as alcohol use, and that L allele was a protective allele. However, similar results could not reached when comparing AD group and the control group or in the other evaluations of the control subgroups (Reif et al. 2009, Hoogman et al. 2011).

In this study, the relationship between NOS1 exon 1f-VNTR genotype polymorphism and the parameters obtained by using the Adult Attention Deficit Hyperactivity Scale in the AD and control group were investigated but statistically significant relationships were not found. In the literature on the subject, relationships were found between NOS1 exon 1f-VNTR polymorphism and impulsiveness, hyperactivity and aggression (Reif et al. 2009); and although impulsiveness was significantly increased in the SS carirers of the ADHD group, a significant difference with respect to polymorphism between the clinical samples of ADHD and control group was not demonstrated (Hoogman et al. 2011) .

According to these results, it could be suggested that NOS1 exon 1f-VNTR polymorphism may have a relationship with ADHD and impulsivity and some other phenotypic features. In our study, there was no difference found between ADHD and control group in the case of NOS1 exon 1f-VNTR genotype polymorphism. As a result, there was no correlation between the genes stated in this study, impulsivity and ADHD symptoms.

Preclinical studies support the association of NOS1 activity with impulsive-aggressive behaviors (Nelson et al. 1995, Chiavegatto and Nelson 2003). Clinical studies have found that NOS1 exon 1f-VNTR S allele is related to impulsiveness, and that environmental factors affect impulsiveness phenotype (Reif et al. 2011). Especially, positive impulsiveness was increasingly observed in SS carriers (Laas et al. 2010). In contrast to all of these studies, a relationship between NOS1 exon 1f-VNTR polymorphism and impulsiveness was not oberved in our study. This may be due to the low numbers of participants in the impulsiveness subgroups of our study, and the majority of the group investigated for impulsiveness being the participants with AD diagnosis.

CONCLUSION

According to the results of this study, NOS1 exon 1f-VNTR polymorphism was not associated with addiction related clinical features, level of impulsiveness and comorbid ADHD symptoms of the alcohol dependent participants. The results of this study differed from those reported in the literature suggesting that the S allele was related with impulsiveness, hyperactivity and aggression and that the L allele was related with alcohol intake characteristics. In order to understand which phenotypic features of ADHD and impulsiveness are related to NOS1 exon 1f-VNTR polimorphism and whether ADHD has a direct genetic relationship, further studies are needed with an ADHD patient group well diagnosed with respect to phenotypic characteristics.

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