

μ-Opioid Receptor Gene (*OPRM1*) Polymorphisms A118G and C17T in Alcohol Dependence: A Turkish Sample



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SUMMARY

Objectives: Previous investigations on opioid system genetics have identified polymorphisms of the *OPRM1* gene expressing μ-opioid receptors to be significantly associated with some features of alcohol dependence (AD). In the present study, we evaluated the relationship between single nucleotide polymorphisms (SNP) in the *OPRM1* gene, A118G (rs1799971, Asn40Asp) and C17T (rs1799972, Arg6Val), and AD diagnosis, level of alcohol consumption, and AD severity in a Turkish sample.

Methods: 121 AD patients and 117 healthy male subjects were included in the study. *OPRM1* A118G (N40D) and C17T (A6V) polymorphisms were evaluated using PCR - RFLP (polymerase chain reaction – restriction fragment length polymorphism) method. We evaluated the association between the presence of SNPs and AD diagnosis, family history of AD, AD severity evaluated via the Michigan Alcoholism Screening Test (MAST), the daily average and maximum quantity of alcohol consumed.

Results: There was no significant difference in *OPRM1* A118G genotype frequencies between the AD and control groups. T allele frequency for the *OPRM1* C17T SNP was very low (0.006) in the sample population. *OPRM1* A118G SNP G118 allele carriers showed significantly higher levels of AD severity as indicated by the MAST.

Conclusion: The *OPRM1* G118 allele was significantly associated with more severe AD in the Turkish population. Similar to other European populations, the frequency of the *OPRM1* T17 allele was very low.

Keywords: Alcoholism, *OPRM1*, Single Nucleotide Polymorphisms, Genetics

INTRODUCTION

Both genetic and environmental factors play an important role in the emergence of alcohol dependence (Thome et al. 2000). According to twin and adoption studies, the heritability of alcohol dependence (AD) may be as high as 50-60% (Kendler et al. 1997, Prescott and Kendler 1999). Studies evaluating intermediate phenotypes have also clarified the contribution of hereditary factors in AD (Ducci and Goldman 2008, Hines et al. 2005, Ray et al. 2010a).

A full understanding of the genetics of opioid system is a necessary step in delineating the genetic origins of AD. Opioid receptors are abundantly expressed in structures important for reward processing in the brain, such as the amygdala, the ventral tegmental area and the nucleus accumbens (Minami and Satoh 1995). Research on pharmacological effects of opioids has established that μ-opioid receptors (MOR) are responsible for the rewarding and addictive properties of opium (Kieffer and Gaveriaux-Ruff 2002). In later studies, the role

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of opioid receptors was found to extend beyond opium and its derivatives, but also to other addictive substances including alcohol (Shippenberg et al. 2008). A portion of the positive reinforcement associated with alcohol is considered to be attributable to the opioid system. MOR blockade results in a decline in voluntary consumption of alcohol in animal models (Gilpin and Koob 2008, Robert et al. 2000). Alcohol consumption is reinforced through an increase in activity mediated by β -endorphin, an endogenous ligand of MOR, resulting in a concurrent increase in dopamine levels in the mesolimbic pathway (Gianoulakis 2009, Koob and Kreek 2007). A relative decrease in endogenous opioidergic activity producing a negative reinforcing effect after cessation of alcohol intake following a period of heavy drinking is considered to be a major contributor to dependency syndrome (Oswald and Wand 2004).

The *OPRM1* gene single nucleotide polymorphism (SNP) A118G (Asn40Asp, N40D, rs1799971), resulting with a adenine to guanine switch at the 118th position, is a notable example of a SNPs affecting peptide sequence within the receptors of the opioidergic pathway investigated in addiction. Frequency of this polymorphism is reported to range between 10 and 50% in different ethnic populations, (Mura et al. 2013). This missense polymorphism, resulting in a switch from Asparagine to Aspartic acid on the N terminal of the extracellular portion of the receptor, leads to the dissipation of asparagine linked glycosylation sites (Singh et al. 1997). Asp40 (G118) *OPRM1* has three times greater affinity to β -endorfin relative to the Asn40 (A118) isotype (Bond et al. 1998). Additionally, expression of *OPRM1* Asn40 (A118) is up to 10 times higher relative to Asp40 (G118) (Zhang et al. 2005).

Although some researches have reported an association between A118G SNP and AD risk (Bart et al. 2005, Kim et al. 2004b, Miranda et al. 2010, Nishizawa et al. 2006, Rommelspacher et al. 2001), others reported conflicting findings (Bergen et al. 1997, Gscheidel et al. 2000, Kim et al. 2004a, Loh et al. 2004, Sander et al. 1998). In addition, there are also reports suggesting that the G118 allele has a protective effect against AD (Du and Wan 2009, Town et al. 1999). A recent investigation conducted on a large sample found no significant interaction between AD and *OPRM1* A118G (Rouvinen-Lagerstrom et al. 2013). In individuals carrying the G118 allele, stimulation, sedation, and positive mood levels after alcohol intake were significantly higher than controls. These individuals were three time more likely to have a family history of AD (Ray and Hutchison 2004, Ray et al. 2010b). In primates carrying an allele homologous to G118 a significant increase in alcohol-induced stimulation, alcohol intake and greater alcohol preference over vehicle was observed (Barr et al. 2007). Individuals carrying the G118 allele have been found to exhibit a significantly decreased level of cortisol response to stress, greater stress-induced craving,

and increased alcohol intake with stress (Pratt and Davidson 2009). Additionally, findings from a sample of heavy drinkers showed a significant increase in approach tendency to alcohol related cues and diminished responses to positive or negative stimuli (Wiers et al. 2009). In a study using functional magnetic resonance imaging, alcohol and alcohol related cues resulted in a significantly increased response in the orbito-frontal cortex, ventromedial prefrontal cortex and striatum in individuals carrying the G118 allele (Filbey et al. 2008). In a study conducted using positron emission tomography, G118 carriers exhibited a significantly increased striatal dopamine response with ethanol consumption compared to placebo (Ramchandani et al. 2011). All of these findings support a possible association between this polymorphism and alcohol's rewarding effects.

The C17T (Ala6Val, rs1799972) SNP, which is located on the first exon of *OPRM1*, corresponds to a substitution of alanine with valine in the extracellular N-terminal domain of the receptor. C17T has been primarily studied in other substance use disorders. The prevalence of C17T varies between populations (Knapman and Connor 2014). In Indian (Kapur et al. 2007) and African American samples (Crowley et al. 2003), the frequency of the T17 allele was 15-20%, whereas in German (Rommelspacher et al. 2001) and East Asian (Tan et al. 2003) populations the frequency of this allele was 1%. The TT genotype has been previously associated with cocaine and heroin dependence (Hoehe et al. 2000, Kapur et al. 2007). Significantly increased T17 allele frequency has been reported in AD subjects (Rommelspacher et al. 2001). In a study conducted in a female African-American sample population, individuals with the TT genotype showed significantly higher scores in measures of the amount, frequency, and duration of alcohol, tobacco, opiate, and cocaine use (Crystal et al. 2012).

Research on frequency of polymorphisms and their related phenotypes in the μ -opioid receptor gene is essential for understanding the role of hereditary factors in the development of AD. For this purpose, the association between clinically important *OPRM1* SNPs (Sherry et al. 2001) A118G and C17T and diagnosis of AD, intensity of alcohol use, family history of AD were evaluated for the first time in a Turkish population.

METHODS

Characteristics of Participants and Data Collection:

124 consecutive male patients at the Hacettepe University Faculty of Medicine (HUTF) Department of Mental Health and Diseases, and Ankara Alcohol and Substance Addiction Treatment and Training Centre (AMATEM) seeking treatment for AD (HUTF n=27; AMATEM n=97) and 118 healthy male subjects with no known psychiatric disorders were enrolled in the study. Written informed consent was obtained from all subjects prior to participation in the study. As

an indicator of the ethnicity of the participants included in the study, the native language of each participant and their parent's native languages were recorded. Only native Turkish speakers were included in the study. In practice, no participants were excluded from study owing to native language. Exclusion criteria included illiteracy or the presence of other disorders potentially affecting cognitive functions. The control group was assembled from healthcare personnel working in the HUTF via verbal announcement. Among the participants scanned for inclusion in the control group, individuals who had a Michigan Alcoholism Screening Test (MAST) score ≥ 5 ($n = 12$) were excluded from the study. Interviews were conducted with the patient group after the first week of hospitalization for alcohol detoxification following the alleviation of withdrawal symptoms. This study was approved by the Hacettepe University Non-interventional Clinical Research Ethics Board (FON 09/18).

Assessment of Participants

Diagnosis of alcohol and drug dependence according to DSM-IV was evaluated using the Structured Clinical Interview for DSM-IV Axis I disorders (SCID-I). SCID-I is a structured clinical interview developed to diagnose axis-I psychiatric disorders according to DSM-IV and has been fully adapted to the Turkish population (First et al. 1996, Özkürkçügil et al. 1999). The B, C, D modules evaluating mood disorders, psychotic symptoms and associated disorders and the E module evaluating diagnoses associated with alcohol and other substance use were applied to all study subjects. Patients diagnosed with lifelong schizophrenia spectrum disorders, bipolar mood disorder, and substance dependence other than alcohol were excluded from the study.

The Turkish version of MAST was used to exclude individuals who exhibited a high risk for alcohol use disorder and to determine the severity of AD (Selzer 1971, Coşkunol et al. 1995). MAST is a self-assessment questionnaire consisting of 25 questions. Questions are scored as yes or no. Although designed as a screening test, higher scores have been associated with higher severity of dependence (Ögel et al. 2012).

The presence and timing of at least two social complications (work-family-social problems related to alcohol, repeated absences from work or school related to alcohol, violent behavior / arrest under the influence of alcohol) was adopted as a measure to determine the age of onset of alcohol use problems. In previous studies classifying alcohol dependence according to age of onset, age cut-off points of 20 and 25 years were used to discriminate early/late onset (Babor et al. 1992). Both of these age cut-off points were applied to establish the age of onset.

The average amount of alcohol consumed per day for the last six months, the lifetime maximum alcohol consumption in one day, and the presence of a disorder related to alcohol use among first and second degree relatives were recorded during interviews. The Family History-Research Diagnostic Criteria

developed by Andreasen and colleagues (1977) was employed to determine family history. According to these criteria, possible features related to alcohol use disorders were evaluated in the first and second degree relatives of all participants. Features recorded included legal, social, physical health, occupational, and marital problems related to alcohol use or a history of treatment for alcohol dependence. When at least one of the criteria was fulfilled for at least one of the first or second-degree relatives of the subject, family history was recorded as positive. The amount of alcohol consumed was calculated in terms of standard units. Accordingly, raki, whiskey, gin, brandy and vodka were considered to contain approximately equal amounts of alcohol, and 70 cl was recorded as 30 units. 0.33 L of beer and 0.15 L of wine were recorded as 1 unit after self-report.

Genotyping

DNA was obtained from peripheral blood leukocytes collected from all patients and controls and isolated using the QIamp DNA isolation kit (Qiagen GmbH, Hilden, Germany). Partial mismatch primers were used as the region of *OPRM1* C17T and A118G polymorphisms do not have natural recognition sites for restriction endonuclease enzymes (Gelernter et al. 1999). A 300 bp amplicon produced by using *OPRM1* SF 5' CTG ACG CTC CTC TCT GTC TCA 3' and *OPRM1* Dpn II 5' GTT CGG ACC GCA TGG GTC GGA CAG AT 3' primers for A118G was digested using 10 U Dpn II restriction enzyme. In the presence of the G allele, 25 and 275 base pair fragments were produced in the samples. A 246 bp amplicon was produced using *OPRM1*-Ban I -5' CCG TCA GTA CCA TGG ACA GCA GCG GTG 3' and *OPRM1* SR- 5' TGG AGT AGA GGG CCA TGA TCG 3' primers for C17T and was digested using 10 U Ban I restriction enzyme. 27 and 219 base pair fragments were produced in the presence of the C allele. Products were run on 3% agarose gel. The accuracy of PCR-RFLP results was controlled by sequencing of 25 randomly selected samples from all groups.

Statistical Analysis

Statistical analysis of the study was performed using IBM SPSS version 21 and G*Power version 3.1.9.2. Allele and genotype frequency in the patient and control groups was compared to the null hypothesis of Hardy-Weinberg equilibrium using a chi-square test. Compliance with a normal distribution was evaluated using the Kolmogorov-Smirnov test, Shapiro-Wilk test, and histogram plotting. T tests were implemented for independent variables in independent samples during statistical calculations. The Mann-Whitney U test was applied to data that did not conform to the normal distribution. For comparisons between three groups, the Kruskal-Wallis test was used when data was not normally distributed. Pearson's Chi-square test and Fisher's Chi-square tests were used to compare categorical variables between groups.

RESULTS

Socio-demographic features

Three individuals in the patient group and one individual in the control group were excluded due to inadequate DNA extraction. A total of 121 individuals diagnosed with AD and 117 healthy individuals were included in the study. The average age in the AD group was 44.3 ± 9.6 years and average age for the control group was 33.8 ± 7.7 years. There was a significant difference in age between the two groups ($U=2.55$ $p<0.01$). The average duration of education for the AD and control groups were 8.9 ± 3.8 and 10.6 ± 3.9 years, respectively. The average duration of education between the two groups was significantly different ($t=3535$ $p<0.05$).

Distribution of *OPRM1* A118G genotype in patients and control subjects

Upon completion of genotyping analysis in the AD and healthy control samples, three unique genotypes were identified at the *OPRM1* A118G SNP. When the AD and control groups were evaluated together the genotype distribution corresponded to Hardy-Weinberg equilibrium (The expected genotype distribution in the study was 185.29, 49.41, 3.29, and the observed results in this study were 185, 50, 3 $p>0.05$). Evaluating all samples (AD group and control group together), the frequency of the wild type allele (A) was 88.24% and the frequency of the variant allele (G) was 11.76% (wild type allele was 89.26% and variant allele was 10.8% in the AD group; wild-type allele was 87.18% and the variant allele was 12.82% in the control group). *OPRM1* A118G genotype distribution was not significantly different between the AD and control groups (Table 1). Also, analysis performed on samples grouped according to the presence of the wild A allele and variant G allele did not reveal a significant difference between the AD and control groups (Table1).

Association between *OPRM1* A118G genotypes and the age of onset of alcohol use problems

The evaluation of age of onset for problematic alcohol use was accurate in 108 patients. Using a cut-off point of 20 years in 108 individuals diagnosed with AD, the number of early onset individuals was 33 (30.6%) whereas the number of late onset individuals was 75 (69.4%). When the cut-off point was set at 25 years, 66 (61.1%) individuals were classified as early onset and 42 (38.9%) individuals were classified in the late-onset group. There was no significant difference in genotype distribution between early-onset AD and late-onset AD (Table 2).

When groups with accurate age of onset data and inexact age of onset were compared, there was no significant difference in age ($t=0.804$, $df=226$, $p=0.422$), MAST points ($U=496.5$, $p=0.632$), maximum consumption ($U=424.0$, $p=0.546$), or average daily alcohol consumption ($U=653.0$, $p=0.724$).

Table 1. *OPRM1* A118G genotype distribution in Alcohol Dependence (AD) and control groups

<i>OPRM1</i> A118G	AD Group n (%)	Control Group n (%)	<i>p</i>
A/A	97 (80.2)	88 (75.2)	0.504*
A/G	22 (18.2)	28 (23.9)	
G/G	2 (1.6)	1 (0.9)	
A/A	97 (80.2)	88 (75.2)	0.359**
A/G + G/G	24 (19.8)	29 (24.8)	
A/A + A/G	119 (98.4)	116 (99.1)	0.513***
G/G	2 (1.6)	1 (0.9)	
Total	121	117	

* Fisher's Exact Chi-Square test, $\chi^2=1.499$ $df=2$,

** Pearson Chi-Square test $\chi^2=0.843$ $df=1$, OR=0.75 CI (%95)=0.41-1.39

*** Fisher's Exact Chi-Square test, $\chi^2=0.304$ $df=1$, OR=1.95 CI (%95)=0.17-21.79

Table 2. Distribution of *OPRM1* genotypes according to Alcohol Dependence (AD) age of onset (n=108)

AD age of onset	A118G SNP AA N (%)	A118G AG + GG N (%)	χ^2 , <i>df</i>	<i>p</i>
<20 years	24 (72.7)	9 (27.3)	1396.1	0.237*
>20 years	62 (82.7)	13 (17.3)		
<25 years	50 (75.8)	16 (24.2)	1569.1	0.210**
>25 years	36 (85.7)	6 (14.3)		

* $\chi^2=1396$, $df=1$, ** $\chi^2=1569$, $df=1$

Association between *OPRM1* A118G genotypes and alcohol dependence severity

When comparing individuals bearing the *OPRM1* A118G variant allele and individuals bearing the wild type allele in the AD group, MAST scores were significantly higher among individuals bearing the wild type allele (Table 3). When comparing MAST scores among AD patients with homozygous (GG), heterozygous (AG) and wild type homozygous (AA) genotypes, MAST scores increased significantly as the number of variant alleles increased ($H=6469$, $df=2$, $p=0.039$, Figure 1). On the other hand, we observed no significant interaction between *OPRM1* A118G and maximum and daily alcohol consumption in the last 6 months among the patients with AD (Table 3).

Association between *OPRM1* A118G genotypes and family history of alcohol dependence

Genotype distributions were analyzed in the context of positive family history of alcohol dependence and increased risk for alcohol dependence development. Accurate family history assessments could be carried out in a total of 205 participants (101 patients and 104 controls). When all samples were taken together, there was no significant association between positive family history and *OPRM1* A118G genotype distribution ($\chi^2=1099$ $df=2$ $p=0.577$), A allele frequency ($\chi^2=1080$ $df=1$ $p=0.364$), or G allele frequency ($\chi^2=1010$ $df=1$).

Table 3. *OPRM1* genotype, *OPRM1* G variant allele dominant genotypes, features of alcohol use and MAST scores

	<i>OPRM1</i> A/A	<i>OPRM1</i> A118G A/G + G/G	U	p
Average daily alcohol use, last 6 months (Unit)				
Mean $\pm$ SD	30.2 $\pm$ 13.8	32.8 $\pm$ 15.8	905.5	0.583
Median (IQR)	30 (15.6)	30 (15.5)		
Maximum daily alcohol use, last 6 months (Unit)				
Mean $\pm$ SD	45.8 $\pm$ 16.8	51.14 $\pm$ 27.8	901.0	0.858
Median (IQR)	45 (30)	45 (26)		
MAST				
Mean $\pm$ SD	29.9 $\pm$ 12.2	37.0 $\pm$ 9.8	683.5	0.027
Median (IQR)	30.5 (19)	38 (14)		

IQR: Inter-quartile range

SD: Standard Deviance

MAST: Michigan Alcoholism Screening Test

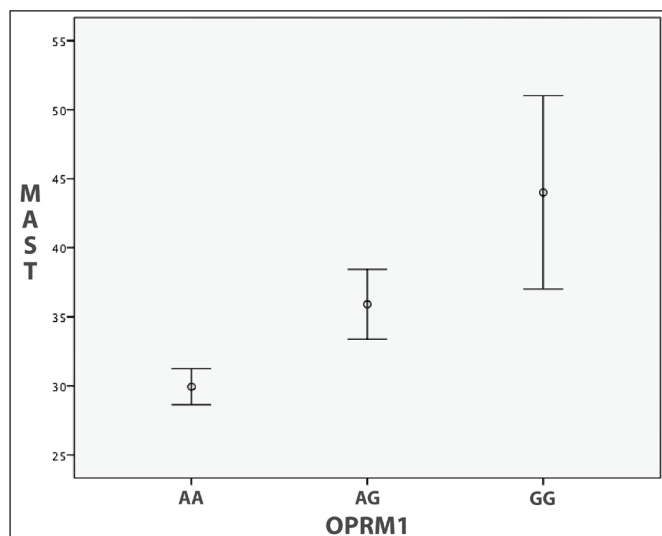


Figure 1. *OPRM1* A118G genotype and average MAST scores
MAST: Michigan Alcoholism Screening Test average $\pm$ Standard Error
OPRM1: Alcohol Dependence group *OPRM1* genotypes
Kruskal-Wallis H= 6469 df=2 p=0.039

p=0.659). When in-group analysis were conducted, there was no significant difference in genotype distribution ($\chi^2=1182$ df=2 p=0.568), A allele frequency ($\chi^2=0.059$ df=1 p=0.661) or G allele frequency ($\chi^2=1176$ df=1 p=0.261) within the AD group. Similarly, genotype ($\chi^2=1182$ df=2 p=0.568), A allele frequency ($\chi^2=0.059$ df=1 p=0.661), and G allele frequency ($\chi^2=1176$ df=1 p=0.261) were not significantly associated with positive family history of AD among the control subjects. When groups with accurate family history data and inexact family history were compared there was no significant difference in age ($t=0.917$ df=117 p=0.365), MAST score (U=528.0 p=0.531), maximum daily alcohol consumption (U=438.0 p=0.458) or average daily alcohol consumption (U= 603.5 p=0.893).

Distribution of *OPRM1* C17T genotypes in patient and control groups

While the wild-type homozygous allele (CC) and the heterozygous (CT) genotypes for *OPRM1* C17T single nucleotide polymorphisms were observed in both the control and AD groups, the variant homozygous allele (TT) was not detected. One heterozygous individual was detected in the AD group (n=121) and 2 heterozygous individuals were detected in the control group (n=117). Due to low T allele frequency in the AD and control groups, 0.004 and 0.009 respectively, no further statistical analyses were carried out for this SNP.

DISCUSSION

In this study, we evaluated *OPRM1* polymorphisms in a population of Turkish AD patients for the first time. Although we observed no significant difference in genotype distribution, alcohol dependence was more severe among A118G SNP G allele carriers.

The primary finding of our study is that the G allele of the *OPRM1* A118G polymorphism is associated with higher MAST scores. The difference in MAST scores may reflect the possible causal effects of the G allele on the severity of alcohol dependence. This effect may be associated with the affinity increase to the endogenous opiates in the μ -opioid receptor (Bond et al. 1998). Alternatively, increased AD severity may be due to increased alcohol consumption. However, we did not observe any difference in the level of alcohol consumption between G and A allele carriers in our sample population. On the other hand, the G allele is associated with decreased response to opioids. In human studies, G allele carriers required higher doses of opioid agonists to elicit miosis, respiratory depression and analgesia, the effects of which are dependent on the μ -opioid receptors (Oertel et al. 2006). Similarly, some effects of alcohol may differ between G and A allele carriers. Interestingly, when the effects of alcohol were investigated in non-dependent individuals, G allele carriers reported higher levels of 'vigor' and subjective 'euphoria' (Ray et al. 2010a, Ray et al. 2010b). The risk of alcohol dependence and mean alcohol consumption did not differ among *OPRM1* A118G genotypes and alleles. If G allele carriers experience more alcohol-related effects compared to A allele carriers with a similar amount of alcohol exposure, then both alcohol use patterns as well as problematic behaviors associated with the alcohol use may differ between individuals with different genotypes. The subjective feelings of intoxication may increase the tendency towards risky behaviors and the resulting legal and social complications. That may explain the discrepancy between the significant increase in MAST scores seen in G allele carriers without a significant increase in the quantity of alcohol consumed. However, as a

point of limitation, it is important to mention that MAST scores are only an indirect measure of dependence severity.

Contradicting findings exist regarding the association between *OPRM1* rs1799971 (A118G) polymorphisms and alcohol dependence. There are both positive (Miranda et al. 2010) and negative studies (Bergen et al. 1997; Gscheidel et al. 2000; Kim et al. 2004a; Loh et al. 2004; Sander et al. 1998), including the current study. Our negative results may be attributable to a variety of factors. The influence of the variant G allele on alcohol dependence may be too subtle to be detected in our study population. Negative results may be related to the specific characteristics of the subjects. As an example, *OPRM1* polymorphisms are known to be associated with the response to naltrexone, an opioid antagonist, in alcohol dependent individuals. (Chamorro et al. 2012). The response to naltrexone is also associated with other factors such as male gender, the severity of craving, and the amount of alcohol use (Garbutt et al. 2014). Therefore, variance in the above mentioned characteristics could have caused a confounding effect on the observed genotype distributions for the *OPRM1* A118G SNP. Due to the design of our study, the age of onset and the amount of alcohol consumption was evaluated retrospectively based on the patients' statements and no significant associations between these parameters and *OPRM1* A118G SNP. Since other factors such as tobacco use and treatment response were not assessed, it is not possible to speculate on these elements which can also affect the genotype distribution.

The allelic distribution in our study population (A allele $f=0.87$; G allele $f=0.13$ in controls; A allele $f=0.89$, G allele $f=0.11$ in the AD group) was similar to other Caucasian populations, but different from Asian populations (Chen et al. 2012). In our previous work with an overlapping sample, a polymorphism affecting aldehyde dehydrogenase, rs671 was evaluated and the absence of the wild type allele was similar to European Caucasians, whereas contrary to the findings with Asian populations (Ayhan et al. 2014). Therefore, this finding of the current study is consistent with our previous findings on grounds of ethnic similarities. Although we did not evaluate genetic markers related to ethnic origin, both studies suggest that the sample population characteristics were more similar to European rather than the Near Eastern populations.

When the whole sample was assessed, the frequency of the variant T allele (0.006) was very low. Similarly, in previous research on *OPRM1* the C17T SNP variant allele is rare in different ethnicities with the exception of samples with African descent (Gelertner et al. 1999, Tan et al. 2003, Crowley et al. 2003, Isaza et al. 2013). Although no other genetic markers related to the ethnic origin were evaluated, the results suggest that the study sample differed from African populations with respect to *OPRM1* C17T SNP allele frequency.

The major limitations of our study are related to the characteristics of the sample population. Although we evaluated parental native language and only included participants who reported Turkish as the sole answer, we were not able to determine the ethnic background of the participants using a genetic method. Therefore it is possible that participants from different ethnic backgrounds were included, hence affecting the *OPRM1* genotypic distribution. Heterogeneity of the sample may have been a confounding factor in determining phenotypes affected by *OPRM1* polymorphisms. Different variants of the gene have been associated with psychiatric phenotypes such as suicidal behavior (Arias et al. 2012), treatment response in major depression (Garriock et al. 2010), and nicotine dependence (Zhang et al. 2006). We have not evaluated whether these factors confound our results. Given the possibility of multiple confounders, it may be better to study the association between genotype and AD phenotype in a larger sample; hence the sample size is an important limitation of our study. This is especially evident in the age of onset analyses in which the power of the statistical analyses is low. In addition, the small sample size prevented us from applying further analyses on the effect of observed significant differences between the AD and control groups, namely age and educational differences which are discussed below.

The participants in the control group are younger than the AD patients in our sample population. Therefore, it is possible that given additional time (for example, until the mean age of the AD group), some of these individuals would develop alcohol dependence. However, 61.1% of cases developed AD before age 25 and the mean age of the control participants at the time of the study was 34, suggesting that the age of onset of alcohol dependence was lower than the mean age of the controls in our sample. Thus in our sample as a whole, if the majority of the individuals had developed AD by the age of 34, one may expect that the majority of cases should have been identified at the time of assessment. However the significant age difference between the study groups constitute a major limitation of this dataset.

Other sociodemographic differences between the groups are related to educational status. We were not able to find a study regarding an association between opioid receptor polymorphisms and academic performance. On the other hand, opioid receptor polymorphisms have been associated with antisocial behavior, which may be related to poor academic performance (Corley 2008). However in the present study, there was no difference in genotypic distribution between the groups, suggesting the educational differences may be independent of *OPRM1* polymorphism.

Our sample consisted of male participants only. A previous study conducted with a female-only sample found an increased risk of alcohol dependence in individuals carrying the homozygous TT genotype for C17T polymorphism (Crystal

et al. 2012). Thus it may be more appropriate to evaluate the effects of C17T polymorphism in a sample containing female participants.

In conclusion, our study is the first to evaluate the association between *OPRM1* polymorphisms and alcohol dependence in a Turkish population. We found increased severity of dependence among G allele carriers of the A118G polymorphism. To better understand the relationship between *OPRM1* polymorphisms and alcohol dependence, ethnic background differences should be taken into account and the design of future studies should include the role of possible confounders as well as the evaluation of additional characteristics of alcohol dependence.

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