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**Article**

# Effect of *PDYN* and *OPRK1* polymorphisms on the risk of alcohol use disorder and the intensity of depressive symptoms

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## Abstract

**Aims: The dynorphin (DYN)/Kappa Opioid Receptor (KOR) system has been suggested to be involved in both negative affective states and the action of alcohol. The present study was undertaken to explore whether the DYN/KOR system genes, *PDYN* and *OPRK1*, influence on individual differences in the intensity of depressive symptoms at admission as well as the risk of alcohol use disorder (AUD) risk in a sample of 101 individuals with AUD and 100 controls. Methods: *PDYN* (rs2281285, rs2225749 and rs910080) and *OPRK1* (rs6473797, rs963549 and rs997917) polymorphisms were analyzed by PCR-RFLP. The intensity of depressive and anxiety symptoms and craving were measured by the Beck Depression Inventory-II (BDI-II), Beck Anxiety Inventory (BAI), and Penn Alcohol Craving Scale, respectively. Results: A significant association between the risk of AUD and *OPRK1* rs6473797 ($P < 0.05$) at the gene level. *OPRK1* rs6473797 CC genotype was found to lead to a 3.11 times greater alcohol dependence risk. In addition, the BDI-II score of the *OPRK1* rs963549 CC genotype was found to be significantly lower ($20.9 \pm 11.2$, min: 1.0, max: 48.0) than that of the CT + TT genotypes ($27.04 \pm 12.7$, min: 0.0, max: 49.0) ($t$: −2.332, $P = 0.022$). None of the *PDYN* polymorphisms were associated with BDI-II score. Conclusion: Variations in the KOR are associated with the risk of AUD and the intensity of depressive symptoms at admission at the gene level in Turkish males. On the other hand, *PDYN* gene seemed not to be associated with AUD, depression, anxiety, and craving.**

**Keywords:** alcohol use disorder; kappa-opioid receptor; individual difference; *OPRK1* rs6473797; *OPRK1* rs963549

## Introduction

Alcohol use disorder (AUD) is a relapsing brain disorder leading to emotional, physical, and social problems and resulting in harmful drinking behavior (Edenberg and Foroud 2013, Kaya *et al.* 2017). Individuals with AUD have impaired control over alcohol consumption despite the severely damaging costs to their health (Carvalho *et al.* 2019). In addition, AUD leads to social and economic problems (Arunachalam and Chandrasekaran 2020). AUDs are common in all developed countries. According to the data of the 'Global Status Report of Health and Alcohol-2018' published by World Health Organization (WHO) in 2018, worldwide 43% of the population (2.348 billion people) are current drinkers and 3 million deaths globally in 2016 was due to the harmful use of alcohol (WHO 2018). The rates of AUD are low in Mediterranean countries such as Greece, Italy, and Israel, whereas its rate is high in north and east Europe such as Russia and Scandinavia (Saxena 1997). Chronic alcohol use has been shown to lead to negative affective states by modulating reward and anti-reward neurocircuitries (Khantzian 1990, Markou *et al.* 1998, Koob and Le Moal 2008, Sureshkumar *et al.* 2022). Increased negative affective states serve as a negative reinforcer and contribute to the alcohol withdrawal that is one of the distinguishing signs of alcoholism (Zywiak *et al.* 2003, Zywiak *et al.* 2006). The dynorphin (DYN)/kappa opioid receptor (KOR) system in the nucleus accumbens has been suggested to be involved in negative affective states (Walker and Koob 2008). Alcohol can induce neuroadaptations in the DYN/KOR system in nucleus accumbens, central nucleus of the amygdala and the bed nucleus of the stria terminalis within nuclei of the extended amygdala in site-specific (Nealey *et al.* 2011, Kissler *et al.* 2014, Rose *et al.* 2016, Erikson *et al.* 2018, Siciliano *et al.* 2018). The DYN/KOR system modulates behavioral, reward and mood processes and several neuroendocrine functions by interacting prominently with dopaminergic circuits (Butelman *et al.* 2012). Activation of this system causes dysphoria and sedation in humans, depressant-like effects and anhedonia in rodents, which can be blocked by KOR antagonists. After exposure to drug of abuse or to stress, dynorphin mRNA levels upregulated. Increased activation of KOR by its ligand dynorphin can cause neuropsychiatric adverse events such as depression and anhedonia (Lalanne *et al.* 2014). There have also been studies regarding the co-morbidity of these psychiatric disorders and drugs of abuse (Regier *et al.* 1990, Logrip *et al.* 2012, Jeanblanc 2015).

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Despite severe public health and economic damages of alcoholism, there are a few FDA-approved pharmacotherapies to treat AUD (Bloodgood *et al.* 2021). In addition to these pharmacotherapies, kappa opioid receptor (KOR)-specific antagonists are currently being considered for the treatment of AUD due to the evidence showing that KOR antagonism in the central nucleus of the amygdala decreases alcohol consumption in animal models (Kissler *et al.* 2014, Anderson *et al.* 2019). There have been also preclinical data suggesting KOR antagonists have therapeutic effects in humans (Carlezon Jr and Krystal 2016). Furthermore, KOR antagonists such as nor-binaltorphimine (Portoghese *et al.* 1987, Walker and Koob 2008, Schank *et al.* 2012), arodyn (Bennett *et al.* 2002), zyklophin (Aldrich *et al.* 2009), and JDTic (Deehan Jr *et al.* 2012, Schank *et al.* 2012) have been assessed on alcohol related behaviors. Taken together, KOR antagonists are suggested to be a highly promising target for the treatment of several psychiatric disorders including substance use disorder, anxiety, and depression (Carlezon Jr and Krystal 2016). On the other hand, the therapeutic effects of KOR antagonists could differ from person to person based on gene polymorphisms or epigenetic mechanisms influencing *OPRK1* gene function. Thus, individual differences in negative mood among individuals with AUD need to be clarified. To date, only Karpyak *et al.* (2013) investigated the effect of single nucleotide polymorphisms in the *PDYN* and *OPRK1* genes on the intensity of depressive symptoms and negative craving in alcohol-dependent subjects. Here, we focused on gene polymorphism of the DYN/KOR system genes and choose to analyze a collection of candidate polymorphisms that have been previously shown to associate with AUD. Additionally, polymorphisms with greater than 10% heterozygosity were preferentially selected. We studied whether the selected polymorphisms of the *PDYN* and *OPRK1* genes affect (i) the risk of AUD, (ii) AUD individuals' intensity of depression, anxiety and craving at admission in Turkish males.

## Methods

### Study population

A total of 101 males affected by current alcoholism according to ICD-10 (International Classification of Diseases-10) diagnostic criteria and 100 males without alcohol problems (comparison group) were enrolled in the current investigation. All individuals with AUD were outpatients admitted to the alcohol dependence unit of the Psychiatric Department of Ankara University Cebeci Hospital in Türkiye. Patients were eligible for inclusion in the study if (i) they were at least 18 years of age, (ii) they had no clinically significant comorbid psychiatric illness such as depression, (iii) they have not been administered drugs for psychiatric illness, (iv) they had no history of substance use disorders other than alcohol and nicotine, (v) they did not receive any detoxification treatment yet. For comparison, a total of 100 male blood donors who declared that they had no diagnosis of past or current alcohol problems were included in the study. They were recruited from the volunteers who were admitted to Blood Donation Center of Ankara University and were matched to patients with AUD for gender and smoking habits. The study design was approved by Ankara University Faculty of Medicine Human Research Ethics Committee (Approval No: I3-220-21 in 2021). Samplings were performed in accordance with the principles of The Declaration of Helsinki. Written informed consent was obtained from each group of participants who was eligible for the study. A questionnaire regarding age, marital, education and employment status was given to all subjects.

### Determination of the *PDYN* and *OPRK1* polymorphisms

About 5 ml of blood sample was taken from all subjects of study and comparison groups into tubes with EDTA for Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) method used for genotyping of *PDYN* (rs910080, rs2281285 and rs2235749) and *OPRK1* (rs6473797, rs963549 and rs997917) single nucleotide polymorphisms (SNPs). Firstly, genomic DNA was isolated from blood samples using GeneJET™ Whole Blood Genomic DNA Purification Mini Kit (Thermo Fisher Scientific, Waltham, MA, USA), as recommended by the manufacturer. PCR amplification was conducted on a Techne Tc 512 PCR System in a 25-$\mu$l reaction mixture containing 6.25 $\mu$M of dNTPs, 1 pmol each of the forward (F) and reverse (R) primers, 1.25 U of Hot Star Taq DNA polymerase (Ampliqon, UK), 5X PCR buffer (Ampliqon, UK) and 50 ng of genomic DNA. The PCR cycling conditions consisted of an initial denaturation step at 94°C for 10 min, followed by 35 cycles of 94°C for 1 min, 60°C for 1 min, 72°C for 1 min, and a final extension step at 72°C for 10 min. Then, the PCR products were digested in a reaction containing 5 U of restriction enzyme. The undigested and digested PCR products were separated by gel electrophoresis on a 3% agarose gel, visualized by ethidium bromide staining under an UV illuminator, and then scanned and photographed using the Syngene Monitoring System. Primer sequences, restriction enzymes, conditions of PCR and enzyme restriction as well as the lengths of PCR products and restriction fragments were given in Supplementary Table S1.

### Measurements

To measure alcohol craving and the intensity of depression and anxiety, individuals with AUD were assessed by Penn Alcohol Craving Scale (PACS), Beck Depression Inventory-II (BDI-II) and Beck Anxiety Inventory (BAI), respectively. PACS evaluates the desire for alcohol for the previous week. The validity and reliability of a Turkish version of the SCS was demonstrated (Evren *et al.* 2008). BDI-II, a Likert-type instrument, consists of 21 item and was developed to measure the symptoms of depression (mood, sense of failure, guilt, punishment, suicidal ideas, irritability, social withdrawal, indecisiveness, work difficulty, fatigability, and somatic preoccupation) in both normal and psychiatric individuals. Hisli (1989) demonstrated the validity and reliability of a Turkish version of the BDI-II. BAI is also a Likert-type tool and has 21 items. It was developed to identify anxiety symptoms and to quantify their intensity in adults and adolescents. The validity and reliability of a Turkish version of the BAI was demonstrated by Ulusoy *et al.* 1998. The total score is calculated by the sum of item scores (ranging from 0 to 63). The total score of 0–7 is interpreted as minimal, 8–15 as mild, 16–25 as moderate and 30–63 as severe.

### Statistical analysis

The Statistical Package for Social Sciences (SPSS) version 26.0 software for Windows was used for the statistical analyses. The normality of numerical variables was examined by the Kolmogorov–Smirnov test. Data were presented as the mean and standard deviation (SD) or median and the interquartile

range (IQR) according to the normality of the data. The frequencies of the *PDYN* (rs910080, rs2281285 and rs2235749) and *OPRK1* (rs6473797, rs963549 and rs997917) alleles and genotypes were obtained by direct count, and the departure from the Hardy–Weinberg equilibrium ($p^2+2pq+q^2=1$) was evaluated by the chi-square test. The relationship between the *PDYN* and *OPRK1* polymorphisms, and AUD was modeled by binary logistic regression analysis. *PDYN* and *OPRK1* genotypes were subdivided in three groups (homozygote wild type, heterozygote and homozygote variant type) and statistically compared according to co-dominant, major/minor allele-dominant and -recessive models in view of the patients' total scores of BDI-II, PACS and BAI using appropriate statistical tests (independent *t*-test, One-Way ANOVA, Mann Whitney test or Kruskal-Wallis test). Linkage disequilibrium (LD) and haplotype analysis were performed using SHEsis-Plus software to evaluate the combined effect of all polymorphisms (Shi and He 2005, Li *et al.* 2009). Haplotypes with frequencies <3% were omitted. Linkage disequilibrium was followed by the $D'$ statistic, and a $D'$ value of ≥0.8 indicated that related SNPs formed one block. $P<0.05$ was considered as statistically significant.

## Results

### Characteristics of subjects

A total of 201 individuals with (study group, $n=101$; 45.14 ± 9.48 years) and without (comparison group, $n=100$; 39.59 ± 9.4 years) alcohol problems enrolled in the current study. All the individuals with AUD were outpatients and smokers. The characteristics of study and comparison groups were presented on Supplementary Table S2. The mean ages of the two groups at the time of ascertainment were significantly different ($P=0.001$). However, there were no statistically significant differences in age between the *PDYN* and *OPRK1* genotype subgroups in individuals with AUD ($P>0.05$) (Supplementary Table S3). There was a significant difference in marital status between the study and comparison groups ($P=0.001$), with 45.5% of individuals with AUD being married, 23.8% single, and 30.7% widow/divorced. By contrast, 66.0% of individuals in the comparison group were married, 30.0% were single, and 4.0% were widow/divorced. The median first age-onset of alcohol use and smoking were 17.0 years (IQR:15.0–20.0 years) and 19.0 years (IQR:16.0–24.0 years). The Spearman's correlation test revealed that there was a significant and positive correlation between the duration of smoking and the duration of alcohol dependence ($r=+0.334$, $P=0.001$).

### Genotype distribution of the *PDYN* and *OPRK1* polymorphisms

The genotype and allele frequencies of *PDYN* (rs910080, rs2281285 and rs2235749) and *OPRK1* (rs6473797, rs963549 and rs997917) polymorphisms in individuals with ($n=101$) and without ($n=100$) AUD were presented in Table 1. For individuals with and without AUD, the genotype and allele frequencies of studied *PDYN* and *OPRK1* polymorphisms were consistent with Hardy–Weinberg equilibrium ($P>0.05$). Detected minor allele frequencies for the tested *PDYN* and *OPRK1* SNPs were in good correlation with minor allele frequencies reported for Caucasians (dbSNP/NCBI). The relationships between the *PDYN* (rs910080, rs2281285 and rs2235749) and *OPRK1* (rs6473797, rs963549 and rs997917), and AUD were examined by logistic regression analysis. *OPRK1* rs6473797 CC genotype was found to lead to a 3.11 times greater alcohol dependence risk (OR = 3.111; 95% CI = 1.208–8.015). *OPRK1* rs963549 and rs997917, and *PDYN* rs910080, rs2281285 and rs2235749 were not found to be associated with the risk of AUD (Table 1). Haplotypes with frequencies >3% in both individuals with and without AUD were calculated by SHEsis-Plus and shown in Table 2. None of the *PDYN* (A-T-G, G-C-A, G-T-A) and *OPRK1* (C-C-C, T-C-T, C-T-C) haplotypes showed a significant association with the risk for AUD ($P>0.05$). Linkage disequilibrium (LD) test by SHEsis-Plus showed strong LD ($r^2>0.8$) for rs910080 and rs2235749 in *PDYN* and for rs6473797 and rs997917 in *OPRK1*. Figures 1 and 2 showed the patterns of LD in the *PDYN* and *OPRK1* genes, with their $|D'|$ and $r^2$ values, respectively.

### The total score of BDI-II in AUD, across *PDYN* and *OPRK1* genotypes

Table 3 showed the BDI-II total scores of individuals with AUD according to *PDYN* (rs910080, rs2281285 and rs2235749) and *OPRK1* (rs6473797, rs963549 and rs997917) genotypes of co-dominant, major/minor allele-dominant and -recessive models. It was found that the BDI-II score of the *OPRK1* rs963549 CC genotype was significantly lower (20.9 ± 11.2, min: 1.0, max: 48.0) than that of the CT + TT genotypes (27.04 ± 12.7, min: 0.0, max: 49.0) ($t$: −2.332, $P=0.022$). There was no significant difference between genotypes of *PDYN* rs910080, rs2281285 and rs2235749 and *OPRK1* rs6473797 and rs997917 in view of the total scores of BDI-II ($P>0.05$). However, as it was seen in Table 3, *PDYN* rs910080 GG (19.8 ± 11.9), *PDYN* rs2281285 TT (21.6 ± 11.5), *PDYN* rs2235749 AA (18.5 ± 12.8), *OPRK1* rs6473797 TT (20.9 ± 10.7), and *OPRK1* rs997917 TT (20.8 ± 10.6) genotypes had lower BDI-II total scores as compared to *PDYN* rs910080 AA+AG (22.7 ± 11.9), *PDYN* rs2281285 TC + CC (24.0 ± 12.4), *PDYN* rs2235749 GG + GA (22.7 ± 11.8), *OPRK1* rs6473797 TC + CC (24.0 ± 12.8), and *OPRK1* rs997917 CC + CT (23.9 ± 12.7), respectively.

### The total scores of PACS and BAI in AUD, across *PDYN* and *OPRK1* genotypes

The PACS and BAI scores of individuals with AUD ($n=45$) were also compared according to major/minor-dominant, and -recessive models of *PDYN* rs910080, rs2281285 and rs2235749, and *OPRK1* rs6473797, rs963549 and rs997917 polymorphisms (Table 4). The median BAI score of the *OPRK1* rs963549 CC genotype was significantly lower (12.0, IQR:5.0–20.75) than that of the CT + TT genotypes (25.0, IQR:14.5–40.5) ($Z=-2.857$, $P=0.001$). In addition, AUD patients with *OPRK1* rs997917 TT genotype (12.0, IQR:4.75–18.25) had significantly lower median BAI score as compared to *OPRK1* rs997917 CC + CT genotype (20.0, IQR:7.0–39.0) ($Z=-2.022$, $P=0.04$). *OPRK1* rs6473797 variation showed also a trend association with increased anxiety symptoms ($P=0.09$, Table 4). On the other hand, there was not significant differences between *PDYN* rs910080, rs2281285 and rs2235749 genotype subgroups in view of the total score of BAI ($P>0.05$). In addition, *OPRK1* rs6473797, rs963549 and rs997917 variations showed also a trend association with increased negative craving symptoms ($P=0.06$, $P=0.09$ and $P=0.06$, respectively, Table 4). When

**Table 1.** Genotype frequencies of *PDYN* and *OPRK1* polymorphisms in individuals with AUD and comparison group

| | AUD ($n=101$) | | Comparison group ($n=100$) | | *P*-value | Odds Ratio (95% CI) |
|---|---|---|---|---|---|---|
| | *n* | % | *n* | % | | |
| ***PDYN* rs910080 genotypes** | | | | | | |
| AA | 42 | 41.6 | 45 | 45.0 | | Reference |
| AG | 50 | 49.5 | 48 | 48.0 | 0.824 | 1.38 (0.471–4.03) |
| GG | 9 | 8.9 | 7 | 7.0 | | 1.23 (0.43–3.58) |
| Variant allele freq. | 34% | | 31% | | | |
| HWE *P*-value | $\chi^2=1.19$; $P=0.28$ | | $\chi^2=1.49$; $P=0.22$ | | | |
| ***PDYN* rs2281285 genotypes** | | | | | | |
| TT | 63 | 62.4 | 72 | 72.0 | | Reference |
| TC | 36 | 35.6 | 23 | 23.0 | | 0.457 (0.086–2.439) |
| CC | 2 | 2.0 | 5 | 5.0 | 0.10 | 0.256 (0.046–1.429) |
| Variant allele freq. | 20% | | 17% | | | |
| HWE *P*-value | $\chi^2=1.51$; $P=0.22$ | | $\chi^2=2.73$; $P=0.09$ | | | |
| ***PDYN* rs2235749 genotypes** | | | | | | |
| GG | 39 | 38.6 | 47 | 47 | | Reference |
| GA | 54 | 53.5 | 45 | 45 | 0.461 | 0.904 (0.289–2.827) |
| AA | 8 | 7.9 | 8 | 8 | | 0.603 (0.195–1.864) |
| Variant allele freq. | 35% | | 31% | | | |
| HWE *P*-value | $\chi^2=3.29$; $P=0.07$ | | $\chi^2=0.38$; $P=0.54$ | | | |
| ***OPRK1* rs6473797 genotypes** | | | | | | |
| TT | 48 | 47.5 | 38 | 38.0 | | Reference |
| TC | 37 | 36.6 | 54 | 54.0 | **0.012** | 1.469 (0.567–3.805) |
| CC | 16 | 15.8 | 8 | 8.0 | | **3.111 (1.208–8.015)** |
| Variant allele freq. | 34% | | 35% | | | |
| HWE *P*-value | $\chi^2=3.48$; $P=0.06$ | | $\chi^2=3.48$; $P=0.06$ | | | |
| ***OPRK1* rs963549 genotypes** | | | | | | |
| CC | 75 | 74.3 | 68 | 72.0 | | Reference |
| CT | 22 | 21.8 | 29 | 23.0 | 0.488 | 1.209 (0.261–5.597) |
| TT | 4 | 4.0 | 3 | 5.0 | | 1.758 (0.356–8.673) |
| Variant allele freq. | 15% | | 18% | | | |
| HWE *P*-value | $\chi^2=1.94$; $P=0.16$ | | $\chi^2=0.002$; $P=0.97$ | | | |
| ***OPRK1* rs997917 genotypes** | | | | | | |
| CC | 16 | 15.8 | 11 | 11.0 | | Reference |
| TC | 39 | 38.6 | 57 | 57.0 | 0.979 | 0.988 (0.406–2.408) |
| TT | 46 | 45.5 | 32 | 32.0 | | 2.101 (1.144–3.858) |
| Variant allele freq. | 35% | | 40% | | | |
| HWE *P*-value | $\chi^2=2.36$; $P=0.12$ | | $\chi^2=3.71$; $P=0.05$ | | | |

*n*: sample size, AUD: Alcohol Use Disorder, CI: Confidence Interval, HWE: Hardy–Weinberg Equilibrium, bold values indicate statistically significance.

the mean PACS scores of *PDYN* rs910080, rs2281285 and rs2235749 genotypes were compared, *PDYN* rs910080 AA ($15.7\pm6.5$), *PDYN* rs2281285 TT ($16.2\pm8.1$) and *PDYN* rs2235749 GG ($16.2\pm6.6$) genotypes had lower PACS score as compared to *PDYN* rs910080 AG + GG ($18.7\pm10.3$), *PDYN* rs2281285 TC + CC ($19.0\pm9.8$), and *PDYN* rs2235749 GA + AA ($18.1\pm10.1$). However, these differences were not statistically significant ($P>0.05$).

## Discussion

This study investigated the associations between six polymorphisms of the DYN/KOR system genes *PDYN* and *OPRK1* and the risk of AUD in Turkish males with AUD. There was a significant difference between genotype distributions of *OPRK1* rs6473797 among individuals with and without alcohol problems. *OPRK1* rs6473797 CC genotype was found to lead to a 3.11 times greater alcohol dependence risk. Previous studies in humans regarding the role of *OPRK1* in AUD had inconsistent findings (Table 5). Of these only Xuei *et al.* (2006) demonstrated an association between *OPRK1* rs6473797 and alcohol dependence in a large ($n=1860$), family-based study of European-Americans, while others in different populations (Taiwanese Han, Croatian or Korean population) reported no genetic effect of *OPRK1* in alcoholism (Loh *et al.* 2004, Cupic *et al.* 2013, Park *et al.* 2020). It seems that the effect of *OPRK1* rs6473797 on AUD could depend on ethnicity.

**Table 2.** Overall haplotype associations of SNPs of *PDYN* or *OPRK1* according to SHEsis-Plus software

| | | | AUD *n* (freq) | Comparison group *n* (freq) | Chi$^2$ | Fisher's *P* | Pearson's *P* | Odds ratio (95% CI) |
|---|---|---|---|---|---|---|---|---|
| ***PDYN* haplotypes** | | | | | | | | |
| rs910080 | rs2281285 | rs2235749 | | | | | | |
| A | T | G | 126 (0.623) | 135 (0.675) | 1.158 | 0.297 | 0.281 | 0.798 (0.529–1.203) |
| G | C | A | 31 (0.153) | 28 (0.14) | 0.145 | 0.778 | 0.702 | 1.113 (0.64–1.936) |
| G | T | A | 34 (0.168) | 32 (0.16) | 0.05 | 0.893 | 0.821 | 1.062 (0.626–1.801) |
| ***OPRK1* haplotypes** | | | | | | | | |
| rs6473797 | rs963549 | rs997917 | | | | | | |
| C | C | C | 41 (0.202) | 42 (0.21) | 0.03 | 0.902 | 0.861 | 0.958 (0.59–1.552) |
| T | C | T | 125 (0.618) | 120 (0.6) | 0.149 | 0.759 | 0.699 | 1.082 (0.724–1.615) |
| C | T | C | 24 (0.118) | 29 (0.145) | 0.602 | 0.463 | 0.437 | 0.795 (0.445–1.42) |

*n*: sample size, AUD: Alcohol Use Disorder, CI: Confidence Interval

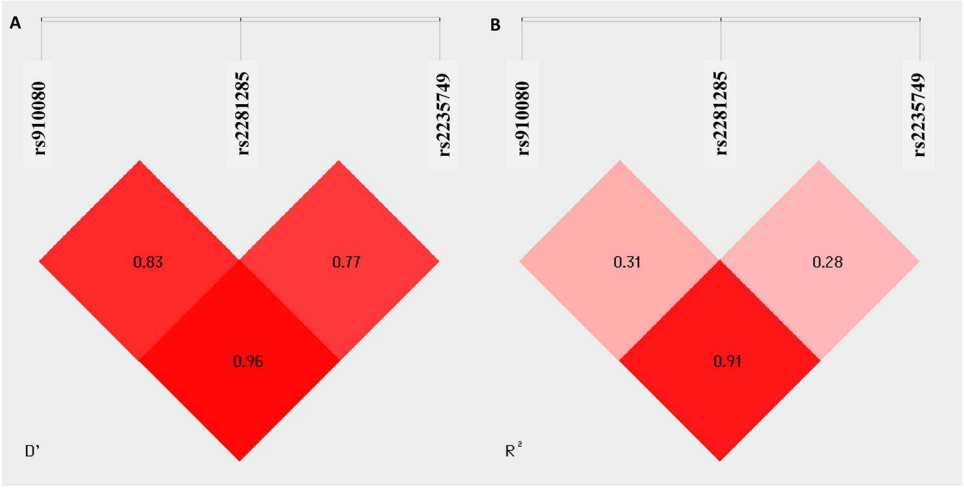


**Figure 1.** (A) Linkage disequilibrium (*D'*) and (B) correlation coefficient ($r^2$) of 3 studied *PDYN* gene variants

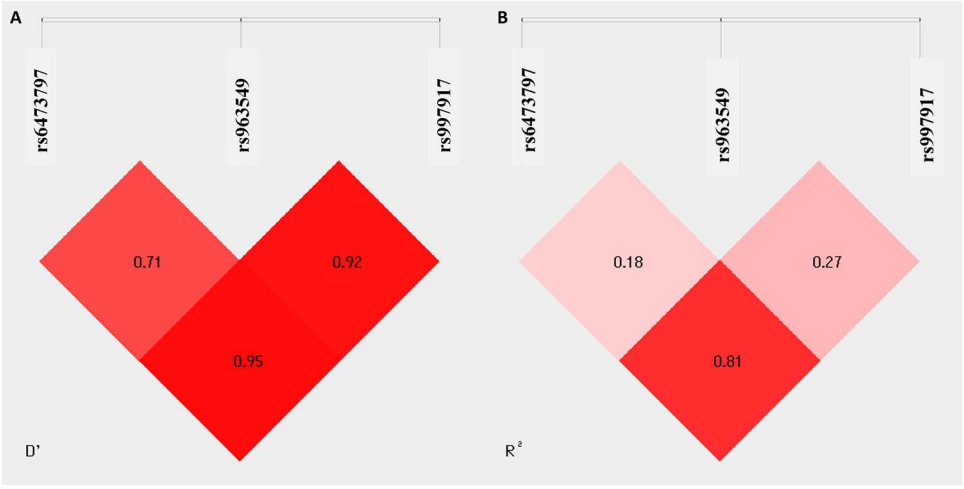


**Figure 2.** (A) Linkage disequilibrium (*D'*) and (B) correlation coefficient ($r^2$) of 3 studied *OPRK1* gene variants

**Table 3.** Comparison of individuals with AUD according to the PDYN and *OPRK1* genotypes in view of the *BDI-II* scores

| ***PDYN* rs910080 genotypes Co-dominant model** | | | | ***PDYN* rs910080 genotypes G-dominant model** | | ***PDYN* rs910080 genotypes G-recessive model** | |
|---|---|---|---|---|---|---|---|
| BDI-II Mean ± SD (min.–max.) | AA ($n=42$) | AG ($n=50$) | GG ($n=9$) | AA ($n=42$) | AG + GG ($n=59$) | AA+AG ($n=92$) | GG ($n=9$) |
| | 23.1 ± 11.4 (1.0–48.0) | 22.4 ± 12.3 (0.0–49.0) | 19.8 ± 11.9 (2.0–35.0) | 23.1 ± 11.4 (1.0–48.0) | 22.0 ± 12.2 (0.0–49.0) | 22.7 ± 11.9 (0.0–49.0) | 19.8 ± 11.9 (2.0–35.0) |
| *P*-value | $F=0.29$, $P=0.75$ | | | $t=0.442$, $P=0.659$ | | $t=0.715$, $P=0.476$ | |
| ***PDYN* rs2281285 genotypes Co-dominant model** | | | | ***PDYN* rs2281285 genotypes T-recessive model** | | ***PDYN* rs2281285 genotypes T-dominant model** | |
| BDI-II Mean ± SD (min.–max.) | TT ($n=63$) 21.6 ± 11.5 (0.0–48.0) | TC ($n=36$) 23.9 ± 12.5 (2.0–49.0) | CC ($n=2$) 26.0 ± 12.7 (17.0–35.0) | TT ($n=63$) 21.6 ± 11.5 (0.0–48.0) | TC + CC ($n=38$) 24.0 ± 12.4 (2.0–49.0) | – – | – – |
| *P*-value[a] | – | | | $t=-1.006$, $P=0.317$ | | – | – |
| ***PDYN* rs2235749 genotypes Co-dominant model** | | | | ***PDYN* rs2235749 genotypes G-recessive model** | | ***PDYN* rs2235749 genotypes G-dominant model** | |
| BDI-II Mean ± SD (min.–max.) | GG ($n=39$) 23.8 ± 11.4 (1.0–48.0) | GA ($n=54$) 21.9 ± 12.1 (0.0–49.0) | AA ($n=8$) 18.5 ± 12.8 (2.0–34.0) | GG ($n=39$) 23.8 ± 11.4 (1.0–48.0) | GA + AA ($n=62$) 21.6 ± 12.1 (0.0–49.0) | GG + GA ($n=93$) 22.7 ± 11.8 (0.0–49.0) | AA ($n=8$) 18.5 ± 12.8 (2.0–34.0) |
| *P*-value | F = 0.65, $P=0.52$ | | | $t=0.923$, $P=0.358$ | | $t=0.847$, $P=0.399$ | |
| ***OPRK1* rs6473797 genotypes Co-dominant model** | | | | ***OPRK1* rs6473797 genotypes T-recessive model** | | ***OPRK1* rs6473797 genotypes T-dominant model** | |
| BDI-II Mean ± SD (min.–max.) | TT ($n=48$) 20.9 ± 10.7 (2.0–43.0) | TC ($n=37$) 25.0 ± 12.2 (1.0–49.0) | CC ($n=16$) 21.6 ± 14.1 (0.0–48.0) | TT ($n=48$) 20.9 ± 10.7 (2.0–43.0) | TC + CC ($n=53$) 24.0 ± 12.8 (0.0–49.0) | TT + TC ($n=85$) 22.6 ± 11.4 (1.0–49.0) | CC ($n=16$) 21.6 ± 14.1 (0.0–48.0) |
| *P*-value | F = 1.304, $P=0.276$ | | | $t=-1.305$, $P=0.195$ | | $t=0.312$, $P=0.756$ | |
| ***OPRK1* rs963549 genotypes Co-dominant model** | | | | ***OPRK1* rs963549 genotypes C-recessive model** | | ***OPRK1* rs963549 genotypes C-recessive model** | |
| BDI-II Mean ± SD (min.–max.) | CC ($n=75$) 20.9 ± 11.2 (1.0–48.0) | CT ($n=22$) 26.9 ± 12.8 (0.0–49.0) | TT ($n=4$) 27.5 ± 13.8 (13.0–45.0) | CC ($n=75$) 20.9 ± 11.2 (1.0–48.0) | CT + TT ($n=26$) 27.04 ± 12.7 (0.0–49.0) | – – | – – |
| *P*-value[a] | – | | | **$t=-2.332$, $P=0.022$** | | – | |
| ***OPRK1* rs997917 genotypes Co-dominant model** | | | | ***OPRK1* rs997917 genotypes C-recessive model** | | ***OPRK1* rs997917 genotypes C-dominant model** | |
| BDI-II Mean ± SD (min.–max.) | CC ($n=16$) 23.4 ± 13.7 (0.0–48.0) | TC ($n=39$) 24.1 ± 12.4 (1.0–49.0) | TT ($n=46$) 20.8 ± 10.6 (2.0–43.0) | CC ($n=16$) 23.4 ± 13.7 (0.0–48.0) | TC + TT ($n=85$) 22.3 ± 11.5 (1.0–49.0) | CC + TC ($n=55$) 23.9 ± 12.7 (0.0–49.0) | TT ($n=46$) 20.8 ± 10.6 (2.0–43.0) |
| *P*-value | $F=0.903$, $P=0.409$ | | | $t=0.353$, $P=0.725$ | | $t=1.337$, $P=0.184$ | |

[a]No comparison due to the frequency < 5 AUD: Alcohol Use Disorder, BDI-II: Beck Depression Inventory, *n*: sample size, S.D.; Standard Deviation, min.: minimum, max.: maximum, IQR: Interquartile Range, bold values indicate statistically significance.

Although Park and co-workers (2020) could not show the effect of *OPRK1* on AUD, they reported that AUD patients with *OPRK1* rs6473797 minor C allele showed more harmful drinking behavior and severe withdrawal symptoms with higher Alcohol Use Disorders Identification Test (signifies more harmful drinking behavior) and Obsessive–Compulsive Drinking Scale (suggests more severe craving symptoms) scores. Considering the results of Xuei *et al.* (2006) and Park *et al.* (2020) in conjunction with ours, *OPRK1* rs6473797 allele C could be determined as a risk factor for AUD in Caucasian. The human *OPRK1* gene, located on chromosome 8q11.2, contains four exons and three introns. *OPRK1* rs6473797 polymorphism is in intron 2 of *OPRK1* gene. The functional significance of this SNP is not known clearly. Recently, an enhancer region was identified in intron 2 region of *OPRK1*, and it is suggested that gene expression of *OPRK1* could be regulated in a DNA methylation-dependent manner by binding of the glucocorticoid receptor complex (GR) to this enhancer region (Lutz *et al.* 2018). Furthermore, *OPRK1* rs6473797 is believed to form CpG sites. Methylation of cytosine at a CpG site is an epigenetic mechanism regulating gene expression (Yuferov *et al.* 2022).

**Table 4.** Comparison of individuals with AUD according to the *PDYN* and *OPRK1* rs963549 genotypes in view of the PACS and BAI total scores

| ***PDYN* rs910080 genotypes/G-dominant model** | | | ***OPRK1* rs6473797 genotypes/T-recessive model** | | |
|---|---|---|---|---|---|
| PACS<br>Mean ± S.D.<br>(min.–max.) | AA<br>($n = 20$)<br>15.7 ± 6.5<br>(7.0–27.0) | AG + GG<br>($n = 25$)<br>18.7 ± 10.3<br>(0.0–30.0) | PACS<br>Mean ± S.D.<br>(min.–max.) | TT<br>($n = 24$)<br>15.1 ± 8.1<br>(0.0–29.0) | TC + CC<br>($n = 21$)<br>20.0 ± 9.2<br>(4.0–30.0) |
| *P*-value | $t = -1.139$, $P = 0.261$ | | *P*-value | $t = -1.911$, $P = 0.06$ | |
| BAI<br>Median (IQR) | 15.0<br>(7.0–26.25) | 14.0<br>(6.0–28.0) | BAI<br>Median (IQR) | 12.5<br>(5.5–20.75) | 20.0<br>(7.0–37.0) |
| *P*-value | $U = 246.5$; $Z = -0.08$, $P = 0.936$ | | *P*-value | $U = 179.5$; $Z = -1.651$, $P = 0.09$ | |
| ***PDYN* rs2281285 genotypes/T-recessive model** | | | ***OPRK1* rs963549 genotypes/T-recessive model** | | |
| PACS<br>Mean ± S.D.<br>(min.–max.) | TT<br>($n = 26$)<br>16.2 ± 8.1<br>(0.0–30.0) | TC + CC<br>($n = 19$)<br>19.0 ± 9.8<br>(3.0–30.0) | PACS<br>Mean ± S.D.<br>(min.–max.) | CC<br>($n = 32$)<br>15.9 ± 8.6<br>(0.0–30.0) | CT + TT<br>($n = 13$)<br>20.8 ± 8.8<br>(8.0–30.0) |
| *P*-value | $t = -1.050$, $P = 0.299$ | | *P*-value | $t = -1.709$, $P = 0.09$ | |
| BAI<br>Median (IQR) | 15.5<br>(7.75–28.0) | 13.0<br>(5.0–25.0) | BAI<br>Median (IQR) | 12.0<br>(5.0–20.75) | 25.0<br>(14.5–40.5) |
| *P*-value | $U = 227.0$; $Z = -0.46$, $P = 0.646$ | | *P*-value | $U = 94.0$; $Z = -2.857$, $P = \mathbf{0.001}$ | |
| ***PDYN* rs2235749 genotypes/G-recessive model** | | | ***OPRK1* rs997917 genotypes/C-dominant model** | | |
| PACS<br>Mean ± S.D.<br>(min.–max.) | GG<br>($n = 18$)<br>16.2 ± 6.6<br>(7.0–27.0) | GA + AA<br>($n = 27$)<br>18.1 ± 10.1<br>(0.0–30.0) | PACS<br>Mean ± S.D.<br>(min.–max.) | CC + TC<br>($n = 23$)<br>19.8 ± 8.8<br>(4.0–30.0) | TT<br>($n = 22$)<br>14.9 ± 8.4<br>(0.0–29.0) |
| *P*-value | $t = 0.710$, $P = 0.482$ | | *P*-value | $t = 1.916$, $P = 0.06$ | |
| BAI<br>Median (IQR) | 15.5<br>(10.75–28.0) | 13.0<br>(4.0–25.0) | BAI<br>Median (IQR) | 20.0<br>(7.0–39.0) | 12.0<br>(4.75–18.25) |
| *P*-value | $U = 204.0$; $Z = -0.904$, $P = 0.366$ | | *P*-value | $U = 164.0$; $Z = -2.022$, $P = \mathbf{0.04}$ | |

AUD: Alcohol Use Disorder, PACS: Penn Alcohol Craving Scale, BAI: Beck Anxiety Inventory, *n*: sample size, S.D.; Standard Deviation, min.: minimum, max.: maximum, IQR: Interquartile Range, bold values indicate statistically significance.

We can speculate that if variant C base was methylated, kappa-opioid receptor upregulation could be inhibited and receptor signaling efficiency could be reduced. According to this speculation, *OPRK1* rs6473797 allele C could be a risk factor for AUD. The findings of the studies examining the effect of KOR antagonists on the alcohol consumption were contradictory. It should be noted that overarching weight of these contradictory results showed that KOR antagonists reduce withdrawal- or stress-mediated alcohol intake, but generally do not reduce non-dependent alcohol intake (Walker and Koob 2008, Walker *et al.* 2011, Nealey *et al.* 2011, Deehan Jr *et al.* 2012, Schank *et al.* 2012, Kissler *et al.* 2014, Kissler and Walker 2016, Rose *et al.* 2016, Erikson *et al.* 2018). On the other hand, only Mitchell *et al.* (2005) reported that blocking the KOR system with a highly selective KOR antagonist (nor-BNI), increases ethanol self-administration in male Lewis rats, which supported our speculation that *OPRK1* rs6473797 allele C seems to be a risk factor for AUD. However, Lewis rats are an 'addiction prone' line of animals with unusual dopaminergic characteristics that potentially underlie dramatically altered drug self-administration behavior. Thus, further human studies with larger sample sizes in different populations with different ethnicities should be needed to clearly confirm the impact of *OPRK1* rs6473797 allele C in AUD.

The current study also investigated the association of variability in *PDYN* and *OPRK1* genes with the intensity of depressive and anxiety symptoms at admission, since kappa-opioid receptors are widely distributed in the brain regions implicated in the regulation of stress-related disorders such as depression and anxiety (Xuei *et al.* 2006). To the best of our knowledge, this is the first report showing a statistically significant association of the *OPRK1* rs963549 with the intensity of depressive and anxiety symptoms measured by BDI-II and BAI, respectively ($P = 0.022$ and $P = 0.001$, respectively) in individuals with AUD. Previously, Arias *et al.* (2008) examined the effects of some *OPRM1*, *OPRD1* and *OPRK1* polymorphisms on therapeutic effects of the opioid antagonist nalmefene in individuals with AUD. They also measured the intensity of depressive symptoms; however, they did not examine the effect of these polymorphisms on BDI-score. In literature, only Karpyak *et al.* (2013) investigated the effect of single nucleotide polymorphisms in the *PDYN* and *OPRK1* genes on the intensity of depressive symptoms and negative craving in alcohol-dependent subjects. They reported no association between BDI scores and *OPRK1* polymorphisms including rs963549. Thus, it seems that further studies need to clarify this inconsistency and the functional effect of this *OPRK1* polymorphism (Mayer and Hollt 2006). Our results regarding the effect of *OPRK1* rs963549 polymorphism on the intensity of depressive symptoms indicated that this polymorphism seemed to have a functional importance. The *OPRK1* rs963549 CC genotype (20.9 ± 11.2 and 12.0, respectively)

**Table 5.** Studies concerning the relationship between opioid receptor genes and the risk of AUD/SUD and related phenotypes

| Study | Demographics | Measurement | Polymorphism | Findings |
|---|---|---|---|---|
| Loh *et al.* (2004) | $n = 158$ alcohol-dependent subjects and $n = 149$ controls.<br>Taiwanese Han | Alcohol dependence | 20 SNPs in *OPRM1*, *OPRD1*, and *OPRK1* genes | No significant association between cases and controls in view of allele frequency and genotype distribution of the SNPs. |
| Xuei *et al.* (2006) | $n = 1860$<br>European-Americans from 219 multiplex alcohol dependent families | Alcohol dependence | *PDYN* (18 SNPs) and *OPRK1* (13 SNPs) | *PDYN* and *OPRK1* genes are associated with alcoholism. |
| Arias *et al.* (2008) | $n = 272$, 80% were male<br>$n = 106$ subjects from the placebo group<br>$n = 166$ subjects from the nalmefene group<br>Caucasian and Finnish ancestry | Depressive symptoms | *OPRM1* (2 SNPs)<br>*OPRD1* (2 SNPs)<br>*OPRK1* (1 SNP) | Variation in *OPRM1*, *OPRD1* and *OPRK1* genes did not moderate the response to nalmefene. |
| Zhang *et al.* (2008) | $n = 1063$<br>$n = 557$ with alcohol dependence<br>$n = 225$ with cocaine dependence<br>$n = 111$ with opioid dependence<br>$n = 443$ controls<br>European Americans | Substance dependence | *OPRK1*<br>*OPRD1* | *OPRD1* G80T variant in exon 1 may be associated with opioid dependence.<br>The minor C allele of *OPRK1* rs997917 might play a protective role for alcohol use disorder.<br>A specific *OPRD1* haplotype GCAACT could be a risk factor for substance dependence. |
| Clarke *et al.* (2009) | $n = 484$ opioid dependents<br>$n = 374$ controls<br>Chinese population | Opioid dependence | *PDYN* | The association of *PDYN* rs1997794 and rs1022563 polymorphisms with opioid dependence in only Chinese females. |
| Ashenhurst *et al.* (2012) | $n = 40$ heavy drinkers (12 female) who underwent naltrexone treatment | self-reported alcohol-induced stimulation, sedation, craving | *OPRK1*<br>*OPRD1* | Reduced naltrexone-induced alcohol sedation in homozygous *OPRK1* rs997917 TT as compared to C allele carriers.<br>*OPRD1* rs4654327 was associated with alcohol-induced stimulation and craving |
| Karpyak *et al.* (2013) | $n = 816$ alcohol-dependent subjects<br>$n = 1248$ controls | Alcohol dependence<br>Depressive symptoms Negative craving | *OPRK1*<br>*PDYN* | No association of *OPRK1* rs997917 with negative craving or the risk of alcohol use disorder.<br>Single SNP and haplotypes of *PDYN* was associated with alcohol dependence (rs2281285 or rs6045868–rs2235751–rs2281285 haplotype), negative craving (rs2281285 or rs2281285–rs1997794 haplotype) and depression (rs6041859). |
| Cupic *et al.* (2013) | $n = 354$ male alcohol-dependent<br>$n = 357$ male control subjects<br>Croatian | Alcohol dependence<br>Type-1 and Type-2 alcoholism according to Cloninger's criteria | *OPRM1/POMC*<br>*OPRK1/PDYN* | None of the selected SNPs was associated with Type-1 or Type-2 alcoholism. |
| Winham *et al.* (2015) | $n = 816$ alcohol-dependent cases $n = 1248$ controls<br>European ancestry | Alcohol dependence and related phenotypes | *PDYN* | rs2281285 was associated with increased negative craving and increased risk of post-treatment relapse in females, but not males.<br>The rs6045868-rs2235751-rs2281285 haplotype was associated with alcohol dependence in only males. |
| Masih and Verbeke (2019) | $n = 2986$ healthy subjects Caucasian | Positive and negative moods | *OPRM1*<br>*OPRD1*<br>*OPRK1* | Variations in *OPRM1*, *OPRD1* and *OPRK1* genes were associated with positive and negative moods. |
| Park *et al.* (2020) | $n = 314$ male patients with AUD<br>$n = 324$ male controls<br>Korean | Drinking behavior, severe withdrawal symptoms | *OPRM1* (3 SNPs)<br>*OPRK1* (2 SNPs) | No significant effect of single SNPs or haplotypes on AUD.<br>AUD patients with *OPRK1* rs6473797 minor C allele showed more harmful drinking behavior and severe withdrawal symptoms<br>A haplotype containing rs6473797 was associated with drinking behavior. |
| Bloodgood *et al.* (2021) | *pdyn* and *oprk1* knockout male and female mice | Alcohol consumption | *OPRK1*<br>*PDYN* | Manipulations of *PDYN* and *OPRK1* caused an increase in ethanol consumption in a sex-specific manner mouse models |
| Yuferov *et al.* (2022) | 577 subjects ($n = 152$ control, $n = 142$ persons with opioid dependence, $n = 283$ subjects with cocaine dependence.<br>African ancestry | Dimensional assessment a subject's exposure to heroin and cocaine | Five SNPs in intron 2 of *OPRK1* | *OPRK1* rs997917 and rs10111937 were associated cocaine dependence.<br>*OPRK1* rs10111937 was associated with opioid dependence. |
| Lepreux *et al.* (2023) | Male and female transgenic Cre-Lox (a technology inserted Cre-recombinase at tyrosine hydroxylase promotor region) rats | Excessive alcohol consumption<br>Negative emotional state<br>Executive function | Oprk1 mRNA expression | VTA Oprk1 overexpression recapitulates escalated alcohol self-administration and depressive-like behavior, but not working memory performance. |
| Present study (2023) | Turkish males ($n = 101$) and without ($n = 100$) AUD, Caucasian | Alcohol dependence, craving, depression, anxiety | *OPRK1* rs997917 | *OPRK1* rs6473797 CC genotype was found to lead to a 3.11 times greater alcohol dependence risk. |

had a lower BDI-II and BAI total scores than CT + TT genotypes (27.04 ± 12.7 and 25.0, respectively), indicating that *OPRK1* rs963549 would be associated with individual differences in negative mood that is an addiction-related phenotype. In accordance with our findings, Masih and Verbeke (2019) reported that *OPRK1* rs963549 was positively correlated with negative mood (acute and chronic depression, and anxiety) in a healthy Caucasian population, but not with the positive mood. *OPRK1* rs963549 is an upstream and synonymous variation at chromosome 8 (Masih and Verbeke 2019). It has been suggested that synonymous variations may cause changes in gene expression, translation efficiency and/or alterations in mRNA folding or stability (Duan *et al.* 2003, Capon *et al.* 2004). Furthermore, it is reported that *OPRK1* rs963549 had undergone selection due to its high $F_{ST}$ (a measure of population differentiation) (Gelernter *et al.* 2007).

Previously, Zhang *et al.* (2008) found that the minor C allele of *OPRK1* rs997917 might play a protective role for alcohol use disorder. Similarly, Xuei *et al.* (2006) demonstrated an association between *OPRK1* rs997917 and alcohol dependence in European-Americans. On the other hand, Karpyak *et al.* (2013) found no association of *OPRK1* rs997917 with negative craving or the risk of alcohol use disorder. Ashenhurst *et al.* (2012) reported reduced naltrexone-induced alcohol sedation in *OPRK1* rs997917 homozygous TT as compared to C allele carriers. Due to these few inconsistent results, *OPRK1* rs997917 that is located on the second intron of *OPRK1* gene was also analyzed in the current study. It was found that the *OPRK1* rs997917 was not associated with alcohol dependence in Turkish males, consistent with Karpyak *et al.* (2013). However, it may be associated with individual differences in the intensity of anxiety symptoms in AUD patients due to the statistical difference between *OPRK1* rs997917 genotypes in view of the intensity of anxiety symptoms measured by BAI at admission (Table 4). The results of our study demonstrating an association correspond with Xuei's findings indicating that the strongest association in *OPRK1* was with SNPs in intron 2. Therefore, our results warrant further investigations to confirm the observed associations with larger sample size.

In the current study none of the candidate SNPs (rs910080, rs2281285 and rs2235749) or haplotypes of the *PDYN* gene correlated with the risk of AUD or the intensity of depression and anxiety. On the other hand, Karpyak *et al.* (2013) found that single SNP and haplotypes of *PDYN* was associated with alcohol dependence (rs2281285 or rs6045868–rs2235751–rs2281285 haplotype), negative craving (rs2281285 or rs2281285-rs1997794 haplotype) and depression (rs6041859). In addition, Xuei *et al.* (2006) reported an association of selected *PDYN* SNP with alcoholism. It is possible that we were unable to detect association of *PDYN* SNPs with the risk of AUD due to the modest sample size and gender of the participants who were all male. Recently, Bloodgood *et al.* (2021) reported that the manipulations of *PDYN* and *OPRK1* caused an increase in ethanol consumption in a sex-specific manner in pdyn or oprk1 knock-out mouse models. Following alcohol drinking, reductions in PDYN neuron excitability was found in only female mice, whereas this reduction in KOR was seen in male mice. Furthermore, Lepreux *et al.* (2023) showed that alcohol self-administration was significantly increased in female transgenic Cre-Lox (a technology inserted Cre-recombinase at tyrosine hydroxylase promotor region) rat as compared to males following VTA viral infusions in the 5th and 6th week, but overall no sex differences were reported in acquired alcohol self-administration. Consistent with Lepreux's and Bloodgood's results, Clarke *et al.* (2009) reported the association of *PDYN* rs1997794 and rs1022563 polymorphisms with opioid dependence in only Chinese females. Furthermore, when Winham *et al.* (2015) examined gender-dependent associations of *PDYN* rs2281285 with alcohol related phenotypes in European ancestry, they found that rs2281285 was associated with increased negative craving in females. Thus, *PDYN* SNPs examined in the current study (rs910080, rs2281285 and rs2235749) should be studied in additional females with AUD.

The limitation of the present study is that no women were included since the prevalence of alcohol use was lower in females compared to males in Turkey due to social and economic reasons. With a larger sample, including women with AUD and social drinkers, a clear generalized statement about the role of the *PDYN* and *OPRK1* polymorphisms can be done. Second, the findings of this study are descriptive and correlational, but do not establish causality. Though its limitations, this study contributes additional evidence suggesting the effects of *PDYN* and *OPRK1* polymorphisms on AUD and AUD-related phenotypes.

In conclusion, we have demonstrated that variations in the kappa-opioid system are associated with the risk of AUD and the intensity of depressive symptoms at admission at the gene level in Turkish males, which may be useful for treatment failures of individuals with AUD due to their *OPRK1* rs6473797 and rs963549 genotypes. Despite the modest number of subjects evaluated, the findings in this study further strengthen the role of the *OPRK1* rs6473797 in AUD and support the studies searching for KOR-specific antagonists to treat AUD. We did not find an association of the *PDYN* gene with AUD, depression, anxiety, and craving.

## Author contributions

S.Ö.K. and D.K.A. conducted the genetic analysis and prepared the manuscript. D.K.A. designed and directed the study. R.Y. and M.A.Y. contributed to laboratory analysis under the supervision of S.Ö.K. İ.Ö.İ. collected venous blood samples and demographic data of all subjects. All authors contributed to and have approved the final manuscript.

## CRediT author statement

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## Supplementary data

Supplementary data is available at *Alcohol and Alcoholism* online.

*Conflicts of interest*: The authors have no conflicts of interest to declare.

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## Data availability

No data was used for the research described in the article.

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