European Journal of Pharmacology 921 (2022) 174862

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Association of *PDYN* 68-bp VNTR polymorphism with sublingual buprenorphine/naloxone treatment and with opioid or alcohol use disorder: Effect on craving, depression, anxiety and age onset of first use

Dilek Kaya-Akyüzlü [a,*], Selin Özkan-Kotiloğlu [b], Şafak Yalçın-Şahiner [c,1], Ece Ağtaş-Ertan [d], İnci Özgür-İlhan [d]

[a] Ankara University, Institute of Forensic Sciences, Ankara, Turkey
[b] Kırşehir Ahi Evran University, Faculty of Science and Art, Department of Molecular Biology and Genetics, Kırşehir, Turkey
[c] Ankara Training and Research Hospital AMATEM Clinic, Ankara, Turkey
[d] Ankara University, Faculty of Medicine, Department of Mental Health and Diseases, Ankara, Turkey

ARTICLE INFO

Keywords:
Alcohol
Heroin
Buprenorphine
PDYN 68-bp VNTR
Age onset of first use

ABSTRACT

In this case-control study (423 Turkish subjects), the functional pro-dynorphin (*PDYN*) 68-bp VNTR polymorphism was genotyped in opioid users receiving sublingual buprenorphine/naloxone treatment (SBNT; n = 129, 119 males and 10 females), in opioid users (OUD; n = 99, 90 males and 9 females), in alcohol users (AUD; n = 75, 75 males) and in controls (n = 120, 109 males and 11 females) to determine the effect of this polymorphism on different treatment responses, heroin or alcohol dependence as well as age onset of first use. The *PDYN* 68-bp alleles were determined based on the number of repeats and genotypes were classified as "short/short (SS)", "short-long (SL)" and "long-long (LL)". The intensity of craving, withdrawal, depression and anxiety were measured by the Substance Craving Scale (SCS), the Clinical Opiate Withdrawal Scale (COWS), the Beck Depression Inventory-II (BDI-II) and Beck Anxiety Inventory (BAI), respectively. Healthy controls (5.5 ± 5.8) had significantly lower levels of depressive symptoms compared to OUD (25.4 ± 13.5), AUD (22.5 ± 11.3) and SBNT (19.29 ± 12.2) groups. In OUD group, the LL genotype was associated with decreased intensity of anxiety and depressive symptoms than the SS+SL genotype. The BDI-II scores for *PDYN* VNTR genotypes within the 4 groups were analysed by two-way ANOVA and statistical differences were found for the groups. SBNT group had significantly lower COWS score than OUD group (1.00 versus 3.00). There were statistically significant differences in the median BAI (11 versus 24) and BDI-II scores (17.5 versus 25) between OUD and SBNT groups, supporting the antidepressant and anxiolytic effects of SBNT in persons with OUD.

1. Introduction

Buprenorphine (BUP) is a Food and Drug Administration (FDA)-approved drug for the treatment of opioid use disorder (OUD) in combination with naloxone (Suboxone) (U.S. Food and Drug Administration, 2002). BUP has a complex pharmacological property. It was described as a partial mu-opioid receptor agonist by Martin et al. (1976). Later studies demonstrated that BUP can also act as an antagonist at delta- and kappa-opioid receptors and an agonist at nociceptin receptor (Leander, 1987; Sadée et al., 1982; Negus et al., 2002). Among these receptors, the activation of kappa-opioid receptors by their endogenous ligands, the dynorphins (DYN), produce an aversive state in experimental animals and dysphoria in humans (Mucha and Herz, 1985; Pfeiffer et al., 1986; Bals-Kubik et al., 1993; Shippenberg et al., 1993; Knoll and Carlezon, 2010). DYNs are a class of opioid peptides that are derived from the precursor protein PDYN (Chavkin et al., 1982). DYN is considered as an integral part of the brain's stress response system and its release is increased during painful, noxious, or stressful conditions (Corbett et al., 1982; Chavkin, 2013; Nabeshima et al., 1992). Furthermore, during acute intoxication stage of the cycle of dependence, excess activation of the dopamine receptors in the nucleus accumbens (NAc) leads to increased production of DYNs. This negative feedback on dopamine

* Corresponding author. Institute of Forensic Sciences, Ankara University, Dikimevi, 06590, Ankara, Turkey. .
E-mail addresses: kaya@ankara.edu.tr, kayadilek79@gmail.com (D. Kaya-Akyüzlü).
[1] Kütahya Health Sciences University, Faculty of Medicine, Department of Mental Health and Diseases, Kütahya, TURKEY.

https://doi.org/10.1016/j.ejphar.2022.174862
Received 18 December 2021; Received in revised form 17 February 2022; Accepted 22 February 2022
Available online 7 March 2022

release in the mesolimbic system results in anhedonia, depressive symptomatology and reduced subjective experience of reward (Butelman et al., 2012; Koob et al., 2014). Evidences from animal studies suggested that dysregulation of dynorphin/kappa-opioid receptor system may contribute to the compulsive drug-seeking behaviour (Graziane et al., 2013; Zhou et al., 2013; Xi et al., 1998), which is compelling motivation for relapse to the most illicit substances (Anderson et al., 2013; Weiss et al., 2001). Altogether, the functional status of the endogenous dynorphin/kappa-opioid receptor system is a crucial feature to understand the underlying mechanisms of the drug withdrawal associated with dysphoria and anhedonia, stress-induced relapse-like behaviour and drug seeking behaviour.

In spite of the great efficacy of BUP in the treatment of opioid dependence, most sublingual BUP/naloxone-treated (SBNT) heroin patients (>70%) experience relapse to drug-seeking behaviour (Northrup et al., 2015). A growing body of the data from animal models suggested that the inhibition of neurons in NAc mediated by kappa-opioid receptor contributes to relapse (Graziane et al., 2013; Zhou et al., 2013). In the present study, *PDYN* gene encoding DYN neuropeptides which contributes to drug-seeking behaviour and stress-induced relapse was selected (Lalanne et al., 2014) to find out the individual differences in the treatment responses. Increasing our understanding of these underlying mechanisms might help us to design better treatment strategies in order to maintain prolonged abstinence, possibly throughout life.

The vulnerability to dependence is related to biological factors including genetic variations (Heath et al., 1997; Bierut et al., 1998; Kendler et al., 1999). It is suggested that genetic factors account for approximately 50% of the risk for dependence (Heath et al., 1997; Verhulst et al., 2015; Deak and Johnson, 2021). The contribution of genetics to dependence is generally thought to be polygenic (Goldman et al., 2005) and the exact role of some gene polymorphisms in this complex disorder has been characterized (Bierut et al., 2012; Vandenbergh et al., 1997; Jugurnauth et al., 2011). However, many genes contributing the genetic vulnerability to dependence have not been identified yet. The PDYN is expressed in brain regions relevant for drug taking or drug withdrawal and the chronic administration of addictive substances alters the activity of PDYN in the brain (Clarke et al., 2009). Thus, to date, various studies have examined the association of the *PDYN* gene polymorphisms with vulnerability to substance use disorders (SUDs) (Flory et al., 2011; Williams et al., 2007; Wei et al., 2011; Yuferov et al., 2019; Saify et al., 2014a; Nomura et al., 2006). Among *PDYN* polymorphisms, 68-base pair (bp) repeat polymorphism within the core promoter region of the human *PDYN* gene has an important role in transcriptional activation associated with copies of the tandem repeats (Saify et al., 2014). Previous genetic association studies of *PDYN* 68-bp VNTR polymorphism with SUD in different ethnic groups have produced conflicting results. Most of these studies have been focused on OUD. Some have shown an association between a 68-bp VNTR promoter polymorphism in *PDYN* and OUD in African-American population (Ray et al., 2005), in Chinese individuals with OUD receiving treatment in the Methadone Maintenance Treatment Program (Wei et al., 2011; Yuanyuan et al., 2018), and in Iranian population with a male limited pattern (Saify and Saadat, 2014). However, some of which did not find an association between this polymorphism and heroin abuse in a German (Zimprich et al., 2000), in an Iranian (Hashemi et al., 2018; Esfahani and Saremi, 2020) and in a Caucasian (Yuferov et al., 2019) population. As for alcohol dependence, Williams et al. (2007) reported a significant difference in grouped genotype frequency between controls and the cocaine/alcohol co-dependent group in African Americans, but not in Caucasians or Hispanics. Similarly, Flory et al. (2011) did not find an association between the diagnosis of alcohol dependence and the *PDYN* polymorphism in Caucasians. According to these previous studies, ethnicity seems to be critical when considering the role of *PDYN* VNTR polymorphism in SUD. Thus, in the present study we re-analysed the effect of a 68-bp repeat polymorphism of the human *PDYN* gene on heroin or alcohol dependence in a Turkish population. In addition, we examined the effect of this polymorphism on the intensity of depression and age of onset of first use in a sample of persons with alcohol use disorder (AUD). Furthermore, to the best of our knowledge, we analysed for the first time whether there was an association of variants of the *PDYN* 68-bp tandem repeats with craving, withdrawal, anxiety and depression in persons with OUD receiving SBNT in Caucasian volunteers.

2. Materials and methods

2.1. Study population

This study consists of 423 volunteers, which were divided into 4 groups depending on the history of dependence. These groups were as follows:

1) Controls: who declared that they had no diagnosis of past or current alcohol and/or SUD (n = 120). These healthy volunteers were recruited from the hospital staff and their first or second-degree relatives. Their blood samples for genetic analysis were taken after they voluntarily agreed to participate in the study when they came to the hospital for routine health check-up.
2) OUD: Individuals (n = 99) attended to Ankara Training and Research Hospital (AMATEM Clinic) in Ankara, Turkey and had opioid use disorder diagnosis according to The Diagnostic and Statistical Manual of Mental Disorders (DSM–5) diagnostic criteria. Subjects diagnosed with alcohol and substance use disorders other than heroin (e.g., cocaine, marijuana) were excluded from this group. Blood samples were taken when they were admitted to the hospital for opioid maintenance treatment.
3) SBNT: Individuals had an opioid use disorder by DSM–5 criteria and had been receiving sublingual BUP/naloxone for at least 2 months at AMATEM in Ankara, Turkey (n = 129). Subjects with SUDs other than heroin and nicotine dependence were excluded from this group, proved by the urine drug test performed by the routine laboratory analysis of AMATEM. They were included in the study when they came to AMATEM for routine control by clinicians.
4) AUD: Patients (n = 75) affected by current alcoholism according to International Classification of Diseases-10 (ICD-10) diagnostic criteria and also fulfilled the DSM-5 criteria in Ankara University, Faculty of Medicine Department of Mental Health and Diseases. Subjects with substance use disorders other than alcohol and nicotine dependence (e.g., heroin, cocaine, marijuana) were excluded from this group. The volunteers of this group were recruited into this study when they were admitted to the hospital just beginning of the detoxification treatment.

The exclusion criteria for all groups were: (i) subjects with clinically significant comorbid psychiatric illness such as any psychotic disorders, schizophrenia, mental retardation, bipolar disorder and severe depression, (ii) subjects administered either drugs for physical diseases or psychiatric illness such as depression and anxiety.

A small questionnaire used to gather socio-demographic information on social factors such as marital, education and employment status, past and present substance use, family history of substance use disorder, age onset of alcohol or heroin dependence, and times and doses of sublingual BUP/naloxone was given to the individuals. Each subject who were eligible for the study including controls provided written informed consent and approval (approval numbers and years: 19-1300-18 in 2018; I4-207-20 in 2020; I8-509-20 in 2020) for the use of human subjects was obtained from the institutional ethics committee. Samplings were performed in accordance with the principles of The Declaration of Helsinki. There was not any financial reward for study participation. Only individuals stating themselves as Turkish were included in the study.

2.2. Measurements

All volunteers enrolled in this study (n = 423) were administered Beck Depression Inventory (BDI) in order to investigate the effect of *PDYN* 68-bp repeat polymorphism on the intensity of depression symptoms. Hisli (1989) demonstrated the validity and reliability of a Turkish version of the BDI-II. Individuals in OUD and SBNT groups were also administered Clinical Opiate Withdrawal Scale (COWS), Beck Anxiety Inventory (BAI) and Substance Craving Scale (SCS) to examine the effect of this polymorphism on withdrawal, anxiety and craving, respectively. The validities and reliabilities of Turkish versions of these scales were demonstrated (Canan et al., 2015; Ulusoy et al., 1998; Evren et al., 2011).

2.3. Genotyping of the 68-bp repeat polymorphism within the core promoter region of the human PDYN gene

Two ml of venous blood was taken from each individual into tubes with ethylenediaminetetraacetic acid (EDTA) for DNA isolation and were kept at −20 °C while they were inactive use. Genomic DNAs were extracted from 200 μl whole blood samples using the QIAamp DNA blood-kit (Qiagen, Hilden, Germany) according to the method recommended by the manufacturer. DNA concentration was determined using the PicoGreen dsDNA quantitation kit (Molecular Probes, Eugene, OR) according to the manufacturer's instructions.

PDYN VNTR polymorphism were analysed by PCR method, as previously described (Saify et al., 2014). Amplification was conducted on a Techne Tc 512 PCR system in a 25-μl reaction mixture containing 200 μM of dNTPs, 10 pmol each of the forward and reverse primers, 1 U of Hot Star Taq DNA polymerase (New England Biolabs), 5X PCR buffer (New England Biolabs) and 50 ng of genomic DNA. The PCR cycling conditions consisted of an initial denaturation step at 95 °C for 5 min, followed by 35 cycles of 94 °C for 1 min, 60 °C for 1 min, 72 °C for 1 min, and final extension step at 72 °C for 5 min. The following primers were used: forward primer 5′-AGCAATCAGAGGTTGAAGTTGGCAGC-3′ and reverse primer 5′-GCACCAGGCGGTTAGGTAGAGTTGTC-3′, as previously described (Saify and Saadat, 2014). The PCR products were separated by gel electrophoresis on a 3% agarose gel, visualized by ethidium bromide staining under an ultraviolet illuminator, and then scanned and photographed using Syngene Monitoring System (Fig. 1.). Alleles containing 1, 2, 3, 4, or 5 repeats produced PCR amplicons of 379, 447, 515, 583, and 651 bp, respectively. The alleles of the *PDYN* 68-bp repeat polymorphism were then grouped as short/short "SS"(1,1; 1,2; 2,2 copies), short/long "SL" (1,3; 1,4; 2,3; 2,4 copies), and long/long "LL" (3,3; 3,4; 4,4 copies) repeat alleles.

2.4. Statistical analyses

The Statistical Package for Social Sciences (SPSS) version 21.0 software for Windows was used for the statistical analyses. All categorical data were shown as numbers, percentages and 95% confidence interval. For numerical data, the mean and standard deviation (SD) or median and the interquartile range (IQR) were given according to the normality of the data examined by the Kolmogorov-Smirnov test. The frequencies of the *PDYN* 68-bp VNTR alleles and genotypes were obtained by direct counting, and departure from the Hardy-Weinberg equilibrium was evaluated by the chi-square test. The relationship between the *PDYN* 68-bp VNTR and heroin or alcohol dependence was modelled by binary logistic regression analysis. In the exploratory analysis, data showed non-normal distribution such as age of onset of first use and total SCS score the Kruskal-Wallis test or Mann-Whitney *U* test was used. Data showing normal distribution such as total BDI-II scores were analysed with Student's t-test or one-way ANOVA, as appropriate. $p < 0.05$ was considered as statistically significant.

3. Results

3.1. Sample characteristics and demographics

In total 120 controls (109 males and 11 females, median ages 34 years), 99 persons with OUD diagnosis (90 males and 9 females, median ages 27 years) who referred to AMATEM, 129 opioid users (119 males and 10 females, median ages 27 years) in current SBNT and 75 persons (75 males, median ages 47 years) with AUD diagnosis were included in the study. No significant difference was found between the groups regarding sex ($p = 0.007$). The median ages of the groups at the time of ascertainment were significantly different ($p = 0.001$). The other characteristics (e.g., occupation, education and marital status) of the 4 groups included in the study were presented in Table 1.

3.2. Genotype distribution of the PDYN 68-bp VNTR polymorphism

Table 2 showed the genotype distribution with frequencies and 95% confidence interval of the *PDYN* 68-bp VNTR polymorphism between 4 groups. It may be noted that none of the controls had 1-repeat allele, 5-repeat allele was not detected in any of the groups and the most frequent 68-bp repeats were "2" and "3" repeats in our population.

According to the S and L allele groups, the genotype frequencies,

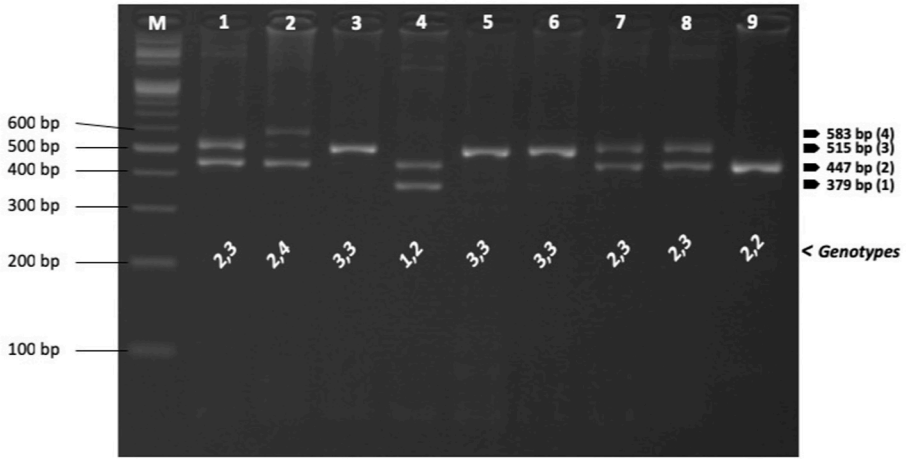

Fig. 1. Agarose gel electrophoresis showing the sizes of PCR products (M: 100 bp ladder; Lanes 1,7,8: alleles containing 2 and 3 repeats (447 and 515 bp); Lane 2: alleles containing 2 and 4 repeats (447 and 583 bp); Lanes 3,5,6: alleles containing 3 repeats (515 bp); Lane 4: alleles containing 1 and 2 repeats (379 and 447 bp); Lane 9: 2 repeats (447 bp).

Table 1
Demographics of the groups included in the study.

Parameters	OUD (n = 99)		SBNT (n = 129)		AUD (n = 75)		Controls (n = 120)		p-value
Age (years) $\tilde{x}$ (IQR)	27.0 (25.0–30.0)		27.0 (24.0–31.0)		47.0 (40.0–54.0)		34.0 (26.0–42.0)		0.001
Weight (kg) $\tilde{x}$ (IQR)	66.0 (60.0–75.0)		65.0 (57.0–77.5)		75.0 (65.5–87.5)		82.5 (74.0–92.0)		0.001
Height (cm) $\tilde{x}$ (IQR)	174.0 (170.0–180.0)		175.0 (170.0–180.0)		174.0 (170.0–180.0)		177.0 (170.3–180.0)		0.392
Education	n	% frequency (95% CI)	n	% frequency (95% CI)	n	% frequency (95% CI)	n	% frequency (95% CI)	0.001
Primary	18	18.2 (10.6–25.8)	20	15.5 (9.3–21.7)	20	26.7 (16.7–36.7)	10	8.3 (3.4–13.2)	
Secondary	56	56.6 (46.8–66.3)	59	45.7 (37.1–54.3)	–	–	18	15.0 (8.6–21.4)	
High School	25	25.3 (16.7–33.9)	44	34.1 (25.9–42.3)	28	37.3 (26.4–48.2)	63	52.5 (43.6–61.4)	
Under-graduate	–	–	6	4.7 (1.0–8.4)	21	28.0 (17.8–38.2)	26	21.7 (19.1–35.1)	
Graduate	–	–	–	–	6	8.0 (1.9–14.1)	3	2.5 NA	
Occupation	n	% frequency (95% CI)	n	% frequency (95% CI)	n	% frequency (95% CI)	n	% frequency (95% CI)	0.001
Working	45	45.5 (35.7–55.3)	84	65.1 (56.9–73.3)	37	49.3 (38.0–60.6)	106	88.3 (82.5–94.1)	
Not working	54	54.5 (44.7–64.3)	45	34.9 (26.7–43.1)	38	50.7 (39.4–62.0)	14	11.7 (5.9–17.5)	
Marital status	n	% frequency (95% CI)	n	% frequency (95% CI)	n	% frequency (95% CI)	n	% frequency (95% CI)	0.001
Single	69	69.7 (60.6–78.8)	86	66.7 (58.6–74.8)	16	21.3 (12.0–30.6)	48	40.0 (31.2–48.8)	
Married	29	29.3 (20.3–38.3)	37	28.7 (20.9–36.5)	38	50.7 (39.4–62.0)	71	59.2 (50.4–68.0)	
Widow/Divorced	1	1.0 NA*	6	4.7 (1.0–8.4)	21	28.0 (17.8–38.2)	1	0.8 NA*	
The onset age of first substance use (years) $\tilde{x}$ (IQR)	21.00 (18.00–23.00)		21.00 (19.00–26.00)		17.00 (15.00–20.00)		–		0.001

n: sample size, CI: Confidence Interval, $\tilde{x}$: median, IQR: Interquartile range, OUD: individuals with opioid use disorder diagnosis, SBNT: opioid users had been receiving sublingual BUP/naloxone, AUD: individuals with alcohol use disorder diagnosis. *NA: non-available because calculation of CI requires n ≥ 5.

Table 2
Genotypic distribution of *PDYN* 68-bp VNTR polymorphism in controls and in SBNT, OUD and AUD groups.

PDYN VNTR Geno-types	Controls (n = 120)		OUD (n = 99)		SBNT (n = 129)		AUD (n = 75)	
	n	% frequency (95% CI)	n	% frequency (95% CI)	n	% frequency (95% CI)	n	% frequency) (95% CI)
1,2	0	0 NA*	2	2.0 NA*	1	0.8 NA*	1	1.3 NA*
1,3	0	0 NA*	1	1.0 NA*	1	0.8 NA*	1	1.3 NA*
2,2	10	8.3 (3.15–12.9)	10	10.1 (4.16–16.0)	15	11.6 (6.07–17.1)	9	12.0 (4.6–19.4)
2,3	59	49.2 (40.3–58.1)	34	34.3 (24.9–43.7)	59	45.7 (37.1–54.3)	34	45.3 (34.0–56.6)
2,4	2	1.7 NA*	3	3.0 NA*	3	2.3 NA*	1	1.3 NA*
3,3	42	35.0 (26.5–43.5)	43	43.4 (33.6–53.2)	45	34.9 (26.7–43.1)	26	34.7 (23.9–45.5)
3,4	7	5.8 (1.62–9.98)	6	6.1 (1.4–10.8)	5	3.9 (0.6–7.2)	3	4.0 NA*

n: sample size, CI: Confidence Interval, OUD: individuals with opioid use disorder diagnosis, SBNT: opioid users had been receiving sublingual BUP/naloxone, AUD: individuals with alcohol use disorder diagnosis. *NA: non-available because calculation of CI requires n ≥ 5.

95% confidence interval and Hardy–Weinberg equilibrium (HWE) were given in Table 3. For each group, the distribution for the genotypes of the *PDYN* 68-bp VNTR was in HWE. There was no statistically significant difference between the groups analysed in the study in view of the frequencies of the genotypes (SS, SL and LL) ($p > 0.05$). The relationship between the *PDYN* 68-bp VNTR genotypes and heroin or alcohol dependence was examined by logistic regression analysis and none of the *PDYN* 68-bp VNTR genotypes were found to be associated with

Table 3
The genotype frequencies, 95% confidence interval and Hardy–Weinberg equilibrium (HWE) according to the S and L allele groups.

PDYN VNTR Genotypes	OUD (n = 99)		SBNT (n = 129)		AUD (n = 75)		Controls (n = 120)	
	n	% frequency (95% CI)	n	% frequency (95% CI)	n	% frequency (95% CI)	n	% frequency (95% CI)
LL	49	49.5 (39.6–59.3)	50	38.8 (30.4–47.2)	29	38.7 (27.7–49.7)	49	40.8 (32.0–49.6)
SL	38	38.4 (28.8–47.9)	63	48.8 (40.1–57.4)	36	48.0 (36.7–59.3)	61	50.8 (41.9–59.7)
SS	12	12.1 (5.7–18.5)	16	12.4 (6.7–18.1)	10	13.3 (5.6–20.9)	10	8.3 (3.4–13.2)
Variant allele freq.	31%		37%		37%		34%	
HWE	$\chi2 = 1.15$; $p = 0.28$		$\chi2 = 0.32$; $p = 0.57$		$\chi2 = 0.05$; $p = 0.82$		$\chi2 = 2.24$; $p = 0.13$	
Logistic regression analysis	$p^* > 0.05$		$p^* > 0.05$		$p^* > 0.05$			
Odds Ratio (95% CI)	LL: reference		LL: reference		LL: reference			
	SL: 0.62 (0.35–1.1)		SL: 1.01 (0.6–1.72)		SL: 0.99 (0.54–1.85)			
	SS: 1.2 (0.47–3.03)		SS: 1.57 (0.65–3.8)		SS: 1.7 (0.63–4.55)			

n: sample size, CI: Confidence Interval, OUD: individuals with opioid use disorder diagnosis, SBNT: opioid users had been receiving sublingual BUP/naloxone, AUD: individuals with alcohol use disorder diagnosis. *Compared with the control group.

heroin or alcohol dependence ($p > 0.05$).

3.3. Substance craving, opioid withdrawal, depression, anxiety and treatment duration in SBNT group, across genotype

Table 4 showed the intensity of the drug craving (reflected by an elevated SCS score), opioid withdrawal (reflected by an elevated COWS score), anxiety (reflected by an elevated BAI score) and depressive symptoms (reflected by an elevated Beck Depression Inventory-II score) according to *PDYN* 68-bp VNTR genotypes in SBNT group. Mann-Whitney U tests conducted for a L-recessive (LL versus SS+SL) or a L-dominant model (SS versus SL + LL) did not show significant associations of the *PDYN* 68-bp repeat genotype with the scores of SCS, COWS, BDI-II and BAI. However, opioid users in SBNT with the LL genotype had lower SCS, BDI-II and BAI scores compared to those with the SS+SL genotype (4.0 versus 6.0; 14.0 versus 20.0; 11.0 versus 11.5, respectively). When COWS score was compared according to *PDYN* 68-bp VNTR genotype subgroups, it was seen that all genotype subgroups had similar low median COWS scores, most probably due to the chronic buprenorphine treatment. In addition, we examined the treatment duration (months), across genotype (LL versus SS+SL). This analysis indicated that individuals in SBNT group with the LL genotype had higher treatment duration, compared with those with the SS + SL genotype (median 13.5 versus 12 months); but this difference was not statistically significant (Mann-Whitney *U* test, $p = 0.683$).

3.4. Age onset of first heroin use, the intensity of depression and anxiety in individuals with OUD, across genotype

In OUD group, the intensity of the opioid withdrawal, anxiety and depressive symptoms were also compared according to *PDYN* 68-bp VNTR genotypes and were presented in Table 5. Mann-Whitney U tests conducted for a L-recessive (LL versus SS+SL) model showed significant associations of the *PDYN* 68-bp repeat genotype with the scores of BAI and BDI-II ($p = 0.003$ and $p = 0.009$, respectively). Individuals with OUD having LL genotype had lower BDI-II and BAI scores compared with those with the SS+SL genotype (23.43 ± 15.30 versus 27.28 ± 11.23 and 24.9 ± 15.18 versus 25.4 ± 12.6) (Fig. 2 and Fig. 3, respectively). There was not a statistically significant difference between genotypes in view of the COWS score reflecting the opioid withdrawal. In addition, we examined the age of heroin first use (years), across genotype (SS versus SL+LL) and found that persons with OUD with SL+LL genotype (20 years, IQR:18–23) had an earlier age of heroin first use compared to those with the SS genotype (21.5 years, IQR:18.25–29.25) (Mann-Whitney *U* test, $p = 0.347$).

3.5. Age onset of first alcohol use and the intensity of depression in individuals with AUD, across genotype

Table 6 showed that the intensity of the depressive symptoms and the age onset of first alcohol use across *PDYN* 68-bp VNTR genotype in persons with AUD. Although independent *t*-test showed that there was not a statistically significant difference in the mean BDI-II scores between the *PDYN* 68-bp VNTR genotype subgroups ($p = 0.226$), BDI-II score was lower in SS genotype subgroup when compared to SL+LL (17.57 ± 14.28 versus 23.05 ± 10.85). The age of first alcohol use was also analysed across genotype (SS versus SL+LL). This analysis indicated that alcohol dependent cases with SL+LL genotype (17 years, IQR:15–20) had an earlier age of alcohol first use compared to those with the SS genotype (18, IQR:15–20), but this difference was not statistically significant ($p = 0.668$).

3.6. The intensity of depression and anxiety in controls and in SBNT, OUD and AUD groups

The mean BDI-II scores of controls (5.5 ± 5.8) was significantly lower than that of AUD group (22.5 ± 11.3), OUD group (25.4 ± 13.5) and SBNT group (19.29 ± 12.2) ($p < 0.001$). The scores of BDI-II found for *PDYN* VNTR genotypes (LL versus SL+SS; L-recessive model) within the 4 study groups analysed by two-way ANOVA can be seen in Table 7 and Fig. 4. Statistical differences were found for the groups ($p < 0.001$). No differences were found for *PDYN* VNTR genotypes alone ($p = 0.692$), or the interaction between main factors ($p = 0.133$). Mann Whitney U tests showed that there were statistically significant differences in the median BAI (11 versus 24) and BDI-II scores (17.5 versus 25) between OUD and SBNT groups ($p < 0.001$). It may be noted that SBNT group had significantly lower COWS score than OUD group (1.00 versus 3.00, $p < 0.001$), supporting the pharmacological effect of SBNT that reduce withdrawal symptoms.

4. Discussion

To the best of our knowledge, this is the first study evaluating the effect of a 68-bp repeat polymorphism in *PDYN* gene on treatment responses such as drug craving and withdrawal in heroin dependent cases receiving SBNT. Since drug craving is critical for the risk of relapse, reducing craving with pharmacological therapies such as BUP and/or with non-pharmacological treatments would improve the life quality of patients. These pharmacological treatments have also positive impacts on comorbid anxiety and depressive symptoms associated with higher craving (Latif et al., 2019; Fatseas et al., 2018). However, a variety of pharmacological and individual factors could limit the effectiveness of the pharmacological and behavioral interventions. Hence, some patients

Table 4
Parameters of persons with opioid use disorder had been receiving sublingual BUP/naloxone according to *PDYN* 68-bp VNTR genotypes.

Parameters	Treatment duration (month)	SCS score	COWS score	BDI-II score	BAI score
	Median (IQR)				
***PDYN* VNTR genotypes (Co-dominant model)**					
SS (n = 16)	16.5 (5.3–33.0)	9.0 (1.5–18.8)	1.0 (0.25–2.5)	15.5 (8.5–28.5)	13.0 (6.0–29.0)
SL (n = 63)	12.0 (5.0–24.0)	5.0 (0.0–12.0)	1.0 (0.0–2.0)	20.5 (9.75–29.75)	10.5 (4.0–27.25)
LL (n = 50)	13.5 (6.0–34.5)	4.0 (0.0–11.25)	1.0 (0.0–3.0)	14.0 (7.25–25.5)	11.0 (5.0–23.0)
Kruskal-Wallis test	$\chi2 = 0.263$ p = 0.877	$\chi2 = 2.367$ p = 0.306	$\chi2 = 0.240$ p = 0.887	$\chi2 = 1.617$ p = 0.445	$\chi2 = 0.527$ p = 0.306
***PDYN* VNTR genotypes (L-Dominant model)**					
SL+LL* (n = 113)	12.0 (6.0–30.0)	5.0 (0.0–11.5)	1.0 (0.0–2.75)	17.5 (8.0–27.0)	11.0 (4.0–27.0)
Mann-Whitney *U* test	U = 864.0 p = 0.861	U = 712.0 p = 0.166	U = 832.0 p = 0.632	U = 837.5 p = 0.755	U = 788.5 p = 0.469
	Z = −0.175	Z = −1.385	Z = −0.478	Z = −0.312	Z = −0.724
***PDYN* VNTR genotypes (L-Recessive model)**					
SL+SS** (n = 79)	12.0 (5.0–24.0)	6.0 (1.0–13.0)	1.0 (0.0–2.0)	20.0 (9.75–29.0)	11.50 (4.75–27.25)
Mann-Whitney *U* test	U = 1814.0 p = 0.683	U = 1759.0 p = 0.292	U = 1927.5 p = 0.967	U = 1656.5 p = 0.279	U = 1855.5 p = 0.783
	Z = −0.409	Z = −1.054	Z = −0.041	Z = −1.083	Z = −0.275

n: sample size, IQR: Interquartile range, SCS: Substance Craving Scale, COWS: Clinical Opiate Withdrawal Scale, BAI: Beck Anxiety Inventory, BDI-II: Beck Depression Inventory II, *Compared with the SS genotype, ** Compared with the LL genotype.

Table 5
Comparison of persons with opioid use disorder according to *PDYN* 68-bp VNTR genotypes.

Parame-ters	Heroin dependence (years)	Age onset of heroin (years)	Amount of heroin used (g/day)	COWS score	BAI score	BDI-II score
	Median (IQR)				Mean ± S.D.	
***PDYN* VNTR genotypes (Co-dominant model)**						
SS (n = 12)	6.0 (3.0–7.75)	21.5 (18.25–29.25)	2.0 (1.63–3.0)	3.0 (0.25–6.75)	25.0 ± 8.99	26.3 ± 9.57
SL (n = 38)	6.0 (4.88–7.25)	20.0 (17.75–23.25)	2.0 (1.0–3.25)	3.5 (0.0–6.0)	25.6 ± 13.6	27.58 ± 11.81
LL (n = 49)	7.0 (4.0–9.5)	21.0 (18.0–23.0)	3.0 (1.0–5.0)	3.0 (0.0–6.0)	24.9 ± 15.18	23.43 ± 15.30
Kruskal-Wallis test	$\chi2 = 1.211$ p = 0.546	$\chi2 = 0.889$ p = 0.641	$\chi2 = 1.941$ p = 0.379	$\chi2 = 0.019$ p = 0.991	F = 0.02 p = 0.980	F = 1.052 p = 0.353
***PDYN* VNTR genotypes (L-Dominant model)**						
SL+LL (n = 87)	7.0 (4.0–8.0)	20.0 (18.0–23.0)	2.0 (1.0–5.0)	3.0 (0.0–6.0)	25.18 ± 15.89	25.24 ± 13.96
Mann-Whitney *U* test	U = 436.0 p = 0.353 Z = −0.930	U = 434.5 p = 0.347 Z = −0.941	U = 513.0 p = 0.922 Z = −0.098	U = 513.5 p = 0.926 Z = −0.092	F = 6.353 ***p=0.013**	F = 2.877 p = 0.093
***PDYN* VNTR genotypes (L-Recessive model)**						
SL+SS (n = 50)	6.0 (4.0–7.25)	21.0 (18.0–24.0)	2.0 (1.0–3.0)	3.5 (0.0–6.0)	25.4 ± 12.6	27.28 ± 11.23
Mann-Whitney *U* test	U = 1099.0 p = 0.374 Z = −0.889	U = 1183.5 p = 0.771 Z = −0.291	U = 1039.0 p = 0184 Z = −1.329	U = 1216.5 p = 0.952 Z = −0.06	F = 9.124 ***p=0.003**	F = 7.007 ***p=0.009**

n: sample size, IQR: Interquartile range, S.D.: standard deviation, COWS: Clinical Opiate Withdrawal Scale, BAI: Beck Anxiety Inventory, BDI-II: Beck Depression Inventory II, *Compared with the SS genotype, ** Compared with the LL genotype.

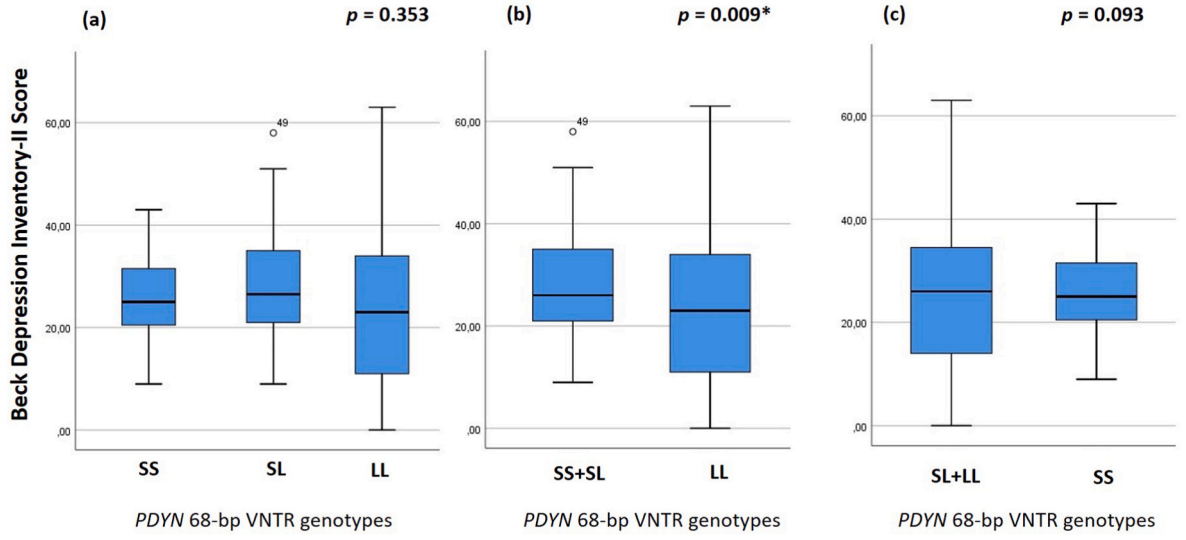

Fig. 2. (**a**) The One-Way ANOVA test revealed a non-significant difference in total scores of Beck Depression Inventory-II (BDI-II) in each genotype group (SS, SL (n = 38), and LL) for the *PDYN* 68-bp VNTR polymorphism (p = 0.353) in persons with opioid use disorder. (**b**) The independent *t*-test revealed a significant difference in the mean BDI-II scores between the SS+SL (n = 50) group and LL (n = 49) group (p = 0.009). (**c**) This difference was not significant between SL+LL (n = 87) group and SS (n = 12) group (p = 0.093).

continue to use heroin or misuse other substances or drop out from opiate maintenance treatment or experience worsening psychiatric distresses (Mysels et al., 2011; Dean et al., 2006). In a follow-up study with opioid users randomized to 12 weeks of treatment with BUP/naloxone, improvements in symptoms of anxiety, depression or insomnia and reductions in the use of illicit substances were shown (Latif et al., 2019). Consistent with Latif's findings, Zaaijer et al. (2015) and Mysels et al. (2011) observed a significant improvement in depressive symptoms with naltrexone treatment. On the other hand, there have been studies observing no improvement in anxiety symptoms (Mysels et al., 2011; Dean et al., 2006), in the depression and anxiety scores in Opiate Maintenance Treatment group (Ravndal and Lauritzen, 2017). These inconsistent effects of treatments on psychiatric distresses could be reflection of patients' genetic background. This hypothesis was tested in the present study. Although there were not statistically differences between *PDYN* 68-bp repeat genotype subgroups; the median scores of SCS, BAI and BDI-II were found higher in SBNT group with *PDYN* 68-bp SS genotypes than those with SL+LL genotypes, indicating that S allele of the *PDYN* 68-bp polymorphism could increase drug craving and the intensity of anxiety and depressive symptoms. Extended treatment duration may be required to treat these patients with S allele compared to L allele. A Kruskal-Wallis test showed no statistical difference between genotypes in view of the duration of treatment (months) at the time of ascertainment. However, as we expected, patients with S allele had higher median treatment duration than those with L allele (SL+LL) (16.5 versus 12 months). Taken together, our results suggested that S allele of the *PDYN* 68-bp polymorphism might negatively contribute to the BUP treatment for opioid dependence by increasing the intensity of negative craving and the severity of anxiety and depressive symptoms. In our opinion, this finding warrants further investigation for individualized treatment of OUD.

Heroin dependent persons are more likely to develop depressive symptoms compared to general population to improve their negative mood (Lutz et al., 2014). There have been many studies confirming such relationship between negative mood states and SUD (Gros et al., 2013; Burns et al., 2019; Hall et al., 2009). Consistent with these previous

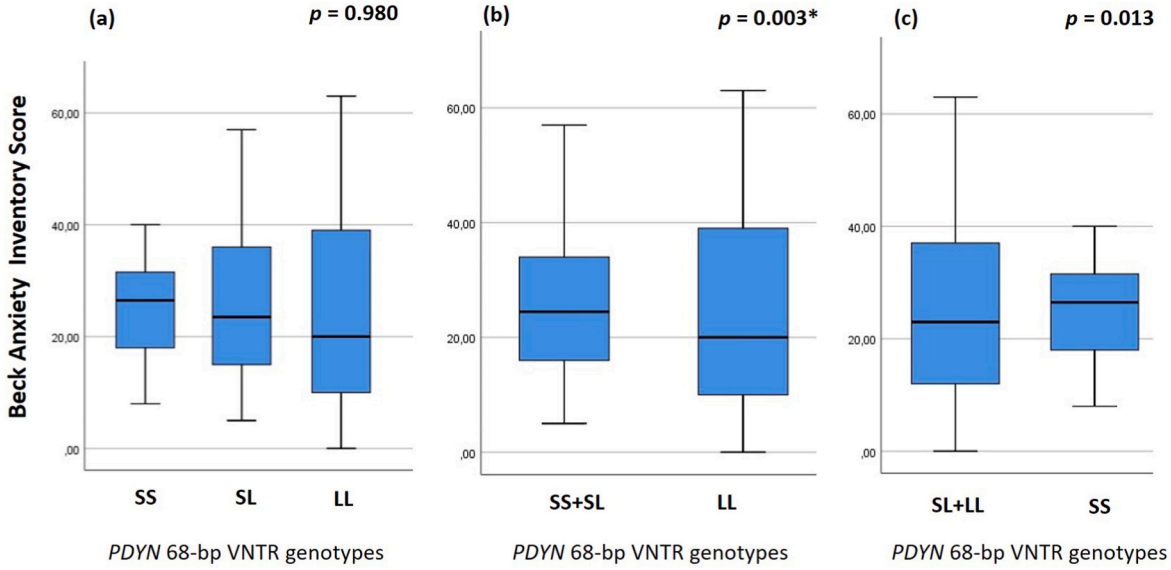

Fig. 3. (**a**) The One-Way ANOVA test revealed a non-significant difference in total scores of Beck Anxiety Inventory (BAI) in each genotype group (SS, SL (n = 38), and LL) for the *PDYN* 68-bp VNTR polymorphism (p = 0.980) in persons with opioid use disorder. (**b**) The independent *t*-test revealed a significant difference in the mean BAI scores between the SS+SL (n = 50) group and LL (n = 49) group (p = 0.003). (**c**) This difference was also significant between SL+LL (n = 87) group and SS (n = 12) group (p = 0.013).

Table 6
Comparison of persons with alcohol use disorder according to the *PDYN* 68-bp VNTR genotypes in view of age onset and the intensity of depressive symptoms.

Parameters	*PDYN* VNTR genotypes (Co-dominant model)			*PDYN* VNTR genotypes (L-Dominant model)	*PDYN* VNTR genotypes (L-Recessive model)
	SS (n = 10)	SL (n = 36)	LL (n = 29)	SL+LL (n = 65)	SL+SS (n = 46)
Age onset of alcohol (years) (Median, IQR)	18.0 (15.0–20.0)	17.0 (15.0–20.0)	17.0 (15.0–20.0)	17.0 (15.0–20.0)	17.0 (15.0–20.0)
Kruskal-Wallis test	$\chi2 = 0.185$ p = 0.912			U = 258.5 p = 0.668[a] Z = −0.429	U = 615.0 p = 0.922[b] Z = −0.098
BDI-II score Mean ± S.D.	17.57 ± 14.28	22.66 ± 10.48	23.52 ± 11.45	23.05 ± 10.85	21.74 ± 11.21
One-Way Anova Test	F = 0.779 p = 0.463			t = 1.222[a] p = 0.226	t = −0.627 p = 0.533[b]

n: sample size, S.D.: standard deviation, BDI-II: Beck Depression Inventory II.
[a] Compared with the SS genotype.
[b] Compared with the LL genotype.

Table 7
Two-way ANOVA for the group (according to the history of dependence), *PDYN* VNTR genotypes and the interaction.

Two-way ANOVA	SS	df	MS	F	p-value
Intercept	122648.696	1	3633.677	1017.884	0.001
Groups	23628.374	3	7876.125	65.365	**0.001**
Genotypes	18.980	1	18.980	0.158	0.692
Groups*Genotypes Interaction	679.219	3	226.406	1.879	0.133
Error	47956.548	398	120.494		

SS: Sum of Squares, df: degrees of freedom; MS: Mean Square.

studies, healthy controls had significantly lower levels of anxiety and depressive symptoms compared to OUD group. Understanding the risk factors contributing this comorbidity between depression/anxiety and heroin dependence is critical to prevent the increased risk for continued heroin use as well as to reduce the higher levels of relapse risk following detoxification (Moustafa et al., 2020). In the present study, individuals of OUD group with LL genotype had lower BAI and BDI-II scores compared to those with the SL+SS genotype (24.9 ± 15.18 versus 25.4

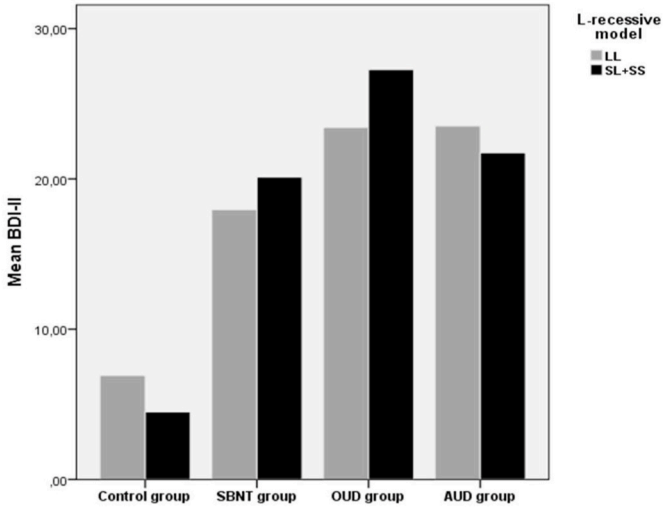

Fig. 4. Effect of *PDYN* VNTR genotypes according to L-recessive model (LL versus SL+SS) on the mean scores of BDI-II in all 4 groups.

± 12.6 and 23.42 ± 15.30 versus 27.28 ± 11.23, respectively). This finding suggested for the first time that *PDYN* 68-bp repeat polymorphism may have an effect on the depressive-anxious symptomatology in heroin dependent cases by changing the expression level of the gene. In consistent with our finding, Femenía et al. (2011) and Bilkei-Gorzo et al. (2008) showed that increased anxiety-like behaviors were associated with the deletion/ablation of prodynorphin gene/prodynorphin in mice. Inconsistent with these observations, Kuzmin et al. (2006) reported an induced anxiolytic-like behavior in mice by a prodynorphin-derived precursor peptide. These discrepancies among animal studies indicated that more research is necessary to determine the functional importance of the prodynorphin on anxiety and depression in human studies. Furthermore, Wittmann et al. (2009) suggested that the discrepancies might also be due to the differences of genetic background. With our study, the effect of genetic variation in *PDYN* gene on the anxiety was shown, but should be replicated.

Additionally, statistically significant differences in the median BAI and BDI-II scores between OUD and SBNT groups supported the antidepressant and anxiolytic effects of BUP in persons with OUD, which was previously shown in preclinical studies with rodents (Falcon et al., 2015 and Falcon et al., 2016) and in several patient populations (Emrich et al., 1982; Bodkin et al., 1995; Nyhuis et al., 2008; Norelli et al., 2013; Karp et al., 2014; Schatzberg, 2015; Yovell et al., 2015; Kosten, 2016). This pharmacological property of BUP is mediated by kappa-opioid receptors located in dopaminergic neurons. Kappa-receptor agonists triggers stress and dysphoria through inhibiting dopamine release and excessive stress can also reinforce substance-seeking behaviour (Butelman et al., 2012; Koob et al., 2014). BUP exerts its effect by antagonizing kappa-opioid receptors and, thus, the dopamine level in the NAc becomes normal, so the mood and impulsive behaviour tendency improve (Segui et al., 2020).

When the stage or severity of opiate withdrawal was examined with COWS in OUD and SBNT groups, mild level of withdrawal was seen in both groups, most probably due to the effect of heroin or BUP at that moment. Thus, the effect of *PDYN* 68-bp repeat polymorphism on heroin withdrawal symptoms could not be detected in either of the groups. To examine this effect, studies with individuals withdraw from heroin should be planned.

The effect of *PDYN* 68-bp repeat polymorphism on the intensity of depressive symptoms was found different in persons with OUD or AUD. Individuals in OUD and SBNT groups with SL+SS genotypes had higher BDI-II score than those with LL genotype. On the other hand, patients with AUD with LL genotypes had higher BDI-II score than those with SL+SS genotypes, suggested that the effect of this polymorphism may be dependent on the consumed substances. The effects of regulatory variants may be dependent on the precise environmental context (Cirulli and Goldstein, 2007). Pro-dynorphin can alter its expression level under pathophysiologically important conditions such as intake of substance of abuse. Zimprich et al. (2000) suggested that several brain functions could be affected by the allelic variation of the stimulus-induced PDYN transcription. In consistent with this suggestion, allelic variation of the prodynorphin transcription may be induced differently by alcohol (a psychotropic depressant) or heroin (narcotic).

SUD is widespread among youth and is generally initiated in early adulthood before the age of 20. Early age onset of first drug use is related to an increased risk of drug dependence, school problems, conduct disorder and risky sexual behaviours. Individuals who initiate drug use earlier are also more likely to have legal, social and family problems compared to their counterparts with late onset (Poudel and Gautam, 2017). Furthermore, earlier studies implicated that individuals with early onset experience more and longer episodes of relapse and more dependence symptoms (Hingson et al., 2006). Several previous studies showing that different genotypic groups of genetic polymorphisms have significant effects on early age onset of first use indicated that genetic predisposition could be a risk factor (Cheng et al., 2005; Sartor et al., 2009; Hou et al., 2010). The possible effect of *PDYN* 68-bp repeat polymorphism on early age onset of alcohol first use was demonstrated for the first time. Patients in AUD group with SL+LL genotypes had an earlier age of alcohol first use compared to those with the SS genotype. Hitherto, only one study examined the effect of this polymorphism in patients with AUD demonstrating that the "low" expressing S allele of the *PDYN* gene was associated with disinhibited behavior due to more likely preference for heavy drinking and related risky behavior (Flory et al., 2011). According to this study and our results, it could be suggested that patients diagnosed with AUD who had at least one S allele may have highest level of disinhibited behavior due to initiating alcohol use early. As for the age of heroin first use, recently Yuferov et al. (2018, 2019) reported that the *PDYN* 68-bp LL genotype was associated with later age of first use of cannabis and heroin in African-American males and in Caucasians, respectively. On the contrary, we suggested that the *PDYN* 68-bp SL+LL genotypes might be a risk factor for early-onset alcohol or opioid use disorders in Caucasian subjects. It is plausible to suppose that high *PDYN* expression due to at least one L allele may cause alterations in mood, which may result in earlier age of alcohol or heroin first use.

The aforementioned findings indicated that *PDYN* 68-bp VNTR appears to be a functional polymorphism affecting the treatment outcomes such as craving and depression and age onset of first substance use. This polymorphism, located in 1250 bp upstream of exon 1, consists of 1–5 repeats of a 68-bp sequence in the core promoter region of the *PDYN* gene. Zimprich and coworkers (2000), who was first described this polymorphism, demonstrated that each repeat element contains a transcription factor activator protein (AP)-1 binding site and promoter activity is dependent on the number of repeats. Previous *in vitro* and *in vivo* studies have implicated that alleles with 3 or 4 copies of the repeat were associated with increased expression, whereas alleles with 1 or 2 copies associate with lower expression (Zimprich et al., 2000; Nikoshkov et al., 2008; Babbitt et al., 2010). Inconsistent with these studies, Cirulli and Goldstein (2007) did not confirm this effect of *PDYN* 68-bp VNTR polymorphism, which might be result of many factors such as cell type, brain region, stage of dependence cycle such as withdrawal and gender. Our findings revealed that both S and L allele of *PDYN* 68-bp seemed to be a risk factor for different dimensions, supporting two models suggested by Flory et al. (2011) regarding the contradictory effects of "low" or "high" expressing alleles. According to the first model, low levels of dopamine release due to "high" expressing allele of *PDYN* may lead to compensatory behaviors (e.g., early age onset of first use). High dopamine release related to "low" expressing allele may increase the likelihood of behaviors causing reward (e.g., craving). Our results, showing an association between the "low" expressing allele and craving, anxiety and depression in OUD supported the latter model. Whereas our results showing an association between the "high" expressing allele and early age onset of first alcohol use supported the first model.

A limitation of our study is that it has been conducted on a limited number of women participants as the prevalence of heroin use was lower in females compared to males in Turkey due to social and economic reasons. However, there was not a significant difference between the analysed groups in view of gender. A clear generalized statement about the role of the *PDYN* 68-bp VNTR polymorphism can be done with a larger sample including women substance users. Second, the findings of this genetic association study are descriptive and correlational, but do not establish causality. Third, the median ages of the analysed groups (controls, OUD, SBNT and AUD) were statistically different. In our opinion, this third limitation did not affect the findings since genetic background examined in the present study do not change during lifetime. Despite these limitations, our study brings to attention an inter-individual variability in craving, anxiety, depression and age of onset of first use, which all are important in treatment of SUDs, due to the *PDYN* 68 bp VNTR polymorphism.

In conclusion, our results showed for the first time an association of *PDYN* 68-bp VNTR variations with the intensity of depressive and

anxiety symptoms as well as negative craving in opioid users treated with sublingual BUP/naloxone combination. The current study also reported the first genetic association of the age onset of first alcohol use with the pro-dynorphin promoter 68-bp repeats in Caucasians with alcohol dependence.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

CRediT authorship contribution statement

Dilek Kaya-Akyüzlü: Conceptualization, Methodology, Formal analysis, Investigation, Writing – review & editing. **Selin Özkan-Kotiloğlu:** Conceptualization, Methodology, Investigation, Writing – review & editing. **Şafak Yalçın-Şahiner:** Resources. **Ece Ağtaş-Ertan:** Resources. **İnci Özgür-İlhan:** Resources, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Anderson, S.A.R., Michaelides, M., Zarnegar, P., Ren, Y., Fagergren, P., Thanos, P.K., Wang, G.-J., Bannon, M., Neumaier, J.F., Keller, E., Volkow, N.D., Hurd, Y.L., 2013. Impaired periamygdaloid-cortex prodynorphin is characteristic of opiate addiction and depression. J. Clin. Invest. 123, 5334–5341. https://doi.org/10.1172/JCI70395.

Babbitt, C.C., Silverman, J.S., Haygood, R., Reininga, J.M., Rockman, M.V., Wray, G.A., 2010. Multiple functional variants in cis modulate PDYN expression. Mol. Biol. Evol. 27, 465–479. https://doi.org/10.1093/molbev/msp276.

Bals-Kubik, R., Ableitner, A., Herz, A., Shippenberg, T.S., 1993. Neuroanatomical sites mediating the motivational effects of opioids as mapped by the conditioned place preference paradigm in rats. J. Pharmacol. Exp. Therapeut. 264, 489–495.

Bierut, L.J., Dinwiddie, S.H., Begleiter, H., Crowe, R.R., Hesselbrock, V., Nurnberger Jr., J.I., Porjesz, B., Schuckit, M.A., Reich, T., 1998. Familial transmission of substance dependence: alcohol, marijuana, cocaine, and habitual smoking: a report from the Collaborative Study on the Genetics of Alcoholism. Arch. Gen. Psychiatry. 55, 982–988. https://doi.org/10.1001/archpsyc.55.11.982.

Bierut, L.J., Goate, A.M., Breslau, N., Johnson, E.O., Bertelsen, S., Fox, L., Agrawal, A., Bucholz, K.K., Grucza, R., Hesselbrock, V., Kramer, J., Kuperman, S., Nurnberger, J., Porjesz, B., Saccone, N.L., Schuckit, M., Tischfield, J., Wang, J.C., Foroud, T., Rice, J. P., Edenberg, H.J., 2012. ADH1B is associated with alcohol dependence and alcohol consumption in populations of European and African ancestry. Mol. Psychiatr. 17, 445–450. https://doi.org/10.1038/mp.2011.124.

Bilkei-Gorzo, A., Racz, I., Michel, K., Mauer, D., Zimmer, A., Klingmüller, D., Zimmer, A., 2008. Control of hormonal stress reactivity by the endogenous opioid system. Psychoneuroendocrinology 33, 425–436. https://doi.org/10.1016/j.psyneuen.2007.12.010.

Bodkin, J.A., Zornberg, G.L., Lukas, S.E., Cole, J.O., 1995. Buprenorphine treatment of refractory depression. J. Clin. Psychopharmacol. 15, 49–57. https://doi.org/10.1097/00004714-199502000-00008.

Burns, J.A., Kroll, D.S., Feldman, D.E., Kure Liu, C., Manza, P., Wiers, C.E., Volkow, N.D., Wang, G.-J., 2019. Molecular imaging of opioid and dopamine systems: insights into the pharmacogenetics of opioid use disorders. Front. Psychiatr. 10, 626. https://doi.org/10.3389/fpsyt.2019.00626.

Butelman, E.R., Yuferov, V., Kreek, M.J., 2012. Kappa-opioid receptor/dynorphin system: genetic and pharmacotherapeutic implications for addiction. Trends Neurosci. 35, 587–596. https://doi.org/10.1016/j.tins.2012.05.005, 2012.

Canan, F., Kuloglu, M., Guven, M., Gecici, Ö., 2015. Reliability and validity of the Turkish version of the clinical opiate withdrawal scale (COWS). Bull. Clin. Psychopharmacol. 25, 209–320. https://doi.org/10.5455/bcp.20150404070711.

Chavkin, C., James, I.F., Goldstein, A., 1982. Dynorphin is a specific endogenous ligand of the kappa opioid receptor. Science 215, 413–415. https://doi.org/10.1126/science.6120570.

Chavkin, C., 2013. Dynorphin–still an extraordinarily potent opioid peptide. Mol. Pharmacol. 83, 729–736. https://doi.org/10.1124/mol.112.083337.

Cheng, C.Y., Hong, C.J., Yu, Y.W., Chen, T.J., Wu, H.C., Tsai, S.J., 2005. Brain-derived neurotrophic factor (Val66Met) genetic polymorphism is associated with substance abuse in males. Mol. Brain Res. 140, 86–90. https://doi.org/10.1016/j.molbrainres.2005.07.008.

Cirulli, E.T., Goldstein, D.B., 2007. In vitro assays fail to predict in vivo effects of regulatory polymorphisms. Hum. Mol. Genet. 16, 1931–1939. https://doi.org/10.1093/hmg/ddm140.

Clarke, T.K., Krause, K., Li, T., Schumann, G., 2009. An association of prodynorphin polymorphisms and opioid dependence in females in a Chinese population. Addiction Biol. 14, 366–370. https://doi.org/10.1111/j.1369-1600.2009.00151.x.

Corbett, A.D., Paterson, S.J., McKnight, A.T., Magnan, J., Kosterlitz, H.W., 1982. Dynorphin and dynorphin are ligands for the kappa-subtype of opiate receptor. Nature 299, 79–81. https://doi.org/10.1038/299079a0.

Deak, J.D., Johnson, E.C., 2021. Genetics of substance use disorders: a review. Psychol. Med. 51, 2189–2200. https://doi.org/10.1017/S0033291721000969.

Dean, A.J., Saunders, J.B., Jones, R.T., Young, R.M., Connor, J.P., Lawford, B.R., 2006. Does naltrexone treatment lead to depression? Findings from a randomized controlled trial in subjects with opioid dependence. J. Psychiatry Neurosci. 31, 38–45.

Emrich, H.M., Vogt, P., Herz, A., 1982. Possible antidepressive effect of opioids: action of buprenorphine. Ann. N. Y. Acad. Sci. 398, 108–112. https://doi.org/10.1111/j.1749-6632.1982.tb39483.x.

Esfahani, V.R., Saremi, M.A., 2020. Investigation of the relationship between PDYN gene polymorphisms and tendency to heroin use. Personalized Med. J. 1, 4–6. https://doi.org/10.21859/pmj01032.

Evren, C., Gürol, D.T., Ögel, K., 2011. Reliability and validity of the Penn Alcohol Craving Scale (PACS) Revised Version for substance craving in male substance dependent inpatients. Turk. J. Psychiatr. 22, 70–85.

Falcon, E., Maier, K., Robinson, S.A., Hill-Smith, T.E., Lucki, I., 2015. Effects of buprenorphine on behavioral tests for antidepressant and anxiolytic drugs in mice. Psychopharmacology 232, 907–915. https://doi.org/10.1007/s00213-014-3723-y.

Falcon, E., Browne, C., Leon, R., Fleites, V.C., Sweeney, R., Kirby, L.G., Lucki, I., 2016. Antidepressant-like effects of buprenorphine are mediated by kappa opioid receptors. Neuropsychopharmacology 41, 2344–2351. https://doi.org/10.1038/npp.2016.38.

Fatseas, M., Serre, F., Swendsen, J., Auriacombe, M., 2018. Effects of anxiety and mood disorders on craving and substance use among patients with substance use disorder: an ecological momentary assessment study. Drug Alcohol Depend. 187, 242–248. https://doi.org/10.1016/j.drugalcdep.2018.03.008.

U.S. Food and Drug Administation, 2002. Center for drug evaluation and research. https://www.accessdata.fda.gov/drugsatfda_docs/label/2011/020733s007s008lbl.pdf. (Accessed 15 December 2021).

Femenía, T., Pérez-Rial, S., Urigüen, L., Jorge Manzanares, J., 2011. Prodynorphin gene deletion increased anxiety-like behaviours, impaired the anxiolytic effect of bromazepam and altered GABAA receptor subunits gene expression in the amygdala. J. Psychopharmacol. 25, 87–96. https://doi.org/10.1177/0269881110367724.

Flory, J.D., Pytte, C.L., Hurd, Y., Ferrell, R.E., Manuck, S.B., 2011. Alcohol dependence, disinhibited behavior and variation in the prodynorphin gene. Biol. Psychol. 88, 51–56. https://doi.org/10.1016/j.biopsycho.2011.06.007.

Goldman, D., Oroszi, G., Ducci, F., 2005. The genetics of addictions: uncovering the genes. Nat. Rev. Genet. 6, 521–532. https://doi.org/10.1038/nrg1635.

Graziane, N.M., Polter, A.M., Briand, L.A., Pierce, R.C., Kauer, J.A., 2013. Kappa opioid receptors regulate stress-induced cocaine seeking and synaptic plasticity. Neuron 77, 942–954. https://doi.org/10.1016/j.neuron.2012.12.034.

Gros, D.F., Milanak, M.E., Brady, K.T., Back, S.E., 2013. Frequency and severity of comorbid mood and anxiety disorders in prescription opioid dependence. Am. J. Addict. 22, 261–265. https://doi.org/10.1111/j.1521-0391.2012.12008.x.

Hall, W., Degenhardt, L., Teesson, M., 2009. Understanding comorbidity between substance use, anxiety and affective disorders: broadening the research base. Addict. Behav. 34, 526–530. https://doi.org/10.1016/j.addbeh.2009.03.010.

Hashemi, M., Shakiba, M., Sanaei, S., Shahkar, G., Rezaei, M., Mojahed, A., Bahari, G., 2018. Evaluation of prodynorphin gene polymorphisms and their association with heroin addiction in a sample of the southeast Iranian population. Mol. Biol. Res. Commun. 7, 1–6. https://doi.org/10.22099/mbrc.2017.27182.1294.

Heath, A.C., Bucholz, K.K., Madden, P.A., Dinwiddie, S.H., Slutske, W.S., Bierut, L.J., Statham, D.J., Dunne, M.P., Whitfield, J.B., N G Martin, N.G., 1997. Genetic and environmental contributions to alcohol dependence. Psychol. Med. 27, 1381–1396. https://doi.org/10.1017/s0033291797005643.

Hingson, Heeren, Winter, et al., 2006. Age of Alcohol-Dependence Onset: Associations With Severity of Dependence and Seeking Treatment. PEDIATRICS 118, e755–e763. https://doi.org/10.1542/peds.2006-0223.

Hisli, N., 1989. Reliability and validity of Beck Depression Inventory among university students. Türk Psikol. Derg. 7, 3–13.

Hou, H., Qing, Z., Jia, S., Zhang, X., Hu, S., Hu, J., 2010. Influence of brain-derived neurotrophic factor (Val66Met) genetic polymorphism on the ages of onset for heroin abuse in males. Brain Res. 1353, 245–248. https://doi.org/10.1016/j.brainres.2010.07.022.

Jugurnauth, S.K., Chen, C.K., Barnes, M.R., Li, T., Lin, S.K., Liu, H.C., Collier, D.A., Breen, G.A., 2011. COMT gene haplotype associated with methamphetamine abuse. Pharmacogenetics Genom. 21, 731–740. https://doi.org/10.1097/FPC.0b013e32834a53f9.

Karp, J.F., Butters, M.A., Begley, A.E., Miller, M.D., Lenze, E.J., Blumberger, D.M., Mulsant, B.H., Reynolds, C.F., 2014. Safety, tolerability, and clinical effect of low-dose buprenorphine for treatment-resistant depression in midlife and older adults. J. Clin. Psychiatr. 75 (8), e785–793. https://doi.org/10.4088/JCP.13m08725.

Kendler, K.S., Karkowski, L.M., Corey, L.A., Prescott, C.A., Neale, M.C., 1999. Genetic and environmental risk factors in the aetiology of illicit drug initiation and subsequent misuse in women. Br. J. Psychiatry 175, 351–356. https://doi.org/10.1192/bjp.175.4.351.

Knoll, A.T., Carlezon, W.A., 2010. Dynorphin, stress, and depression. Brain Res. 1314, 56–73. https://doi.org/10.1016/j.brainres.2009.09.074.

Koob, G.F., Buck, C.L., Cohen, A., Edwards, S., Park, P.E., Schlosburg, J.E., Schmeichel, B., Vendruscolo, L.F., Wade, C.L., Whitfield, T.W., George, O., 2014.

Addiction as a stress surfeit disorder. Neuropharmacology 76 Pt B, 370–382. https://doi.org/10.1016/j.neuropharm.2013.05.02428.
Kosten, T.R., 2016. An opioid for depression? Am. J. Psychiatr. 173, 446–447. https://doi.org/10.1176/appi.ajp.2016.16010078.
Kuzmin, A., Madjid, N., Terenius, L., Ogren, S.O., Bakalkin, G., 2006. Big dynorphin, a prodynorphin-derived peptide produces NMDA receptor-mediated effects on memory, anxiolytic-like and locomotor behavior in mice. Neuropsychopharmacology 31, 1928–1937. https://doi.org/10.1038/sj.npp.1300959.
Lalanne, L., Ayranci, G., Kieffer, B.L., Lutz, P.E., 2014. The kappa opioid receptor: from addiction to depression, and back. Front. Psychiatr. 5, 170. https://doi.org/10.3389/fpsyt.2014.00170.
Latif, Z.E.H., Benth, J.S., Solli, K.K., Opheim, A., Kunoe, N., Krajci, P., Sharma-Haase, K., Tanum, L., 2019. Anxiety, depression, and insomnia among adults with opioid dependence treated with extended-release naltrexone vs buprenorphine-naloxone: a randomized clinical trial and follow-up study. JAMA Psychiatr. 76, 127–134. https://doi.org/10.1001/jamapsychiatry.2018.3537.
Leander, J.D., 1987. Buprenorphine has potent kappa opioid receptor antagonist activity. Neuropharmacology 26, 1445–1447. https://doi.org/10.1016/0028-3908(87)90112-2.
Lutz, P.E., Ayranci, G., Chu-Sin-Chung, P., Matifas, A., Koebel, P., Filliol, D., Befort, K., Ouagazzal, A.M., Kieffer, B.L., 2014. Distinct mu, delta, and kappa opioid receptor mechanisms underlie low sociability and depressive-like behaviors during heroin abstinence. Neuropsychopharmacology 39, 2694–2705. https://doi.org/10.1038/npp.2014.126.
Martin, W.R., Eades, C.G., Thompson, J.A., Huppler, R.E., Gilbert, P.E., 1976. The effects of morphine- and nalorphine- like drugs in the nondependent and morphine-dependent chronic spinal dog. Pharmacol. Exp. Ther. 197, 517–532.
Moustafa, A.A., Tindle, R., Cashel, S., Parkes, D., Mohamed, E., Hamza, E.A., 2020. Bidirectional relationship between heroin addiction and depression: behavioural and neural studies. Curr. Psychol. https://doi.org/10.1007/s12144-020-01032-4.
Mucha, R.F., Herz, A., 1985. Motivational properties of kappa and mu opioid receptor agonists studied with place and taste preference conditioning. Psychopharmacology (Berl) 86, 274–280. https://doi.org/10.1007/BF00432213.
Mysels, D.J., Cheng, W.Y., Nunes, E.V., Sullivan, M.A., 2011. The association between naltrexone treatment and symptoms of depression in opioid-dependent patients. Am. J. Drug Alcohol Abuse 37, 22–26. https://doi.org/10.3109/00952990.2010.540281.
Nabeshima, T., A Katoh, A., Wada, M., T Kameyama, T., 1992. Stress-induced changes in brain Met-enkephalin, Leu-enkephalin and dynorphin concentrations. Life Sci. 51, 211–217. https://doi.org/10.1016/0024-3205(92)90077-3.
Negus, S.S., Bidlack, J.M., Mello, N.K., Furness, M.S., Rice, K.C., Brandt, M.R., 2002. Delta opioid antagonist effects of buprenorphine in rhesus monkeys. Behav. Pharmacol. 13, 557–570. https://doi.org/10.1097/00008877-200211000-00005.
Nikoshkov, A., Drakenberg, K., Wang, X., Horvath, M.C.s., Keller, E., Hurd, Y.L., 2008. Opioid neuropeptide genotypes in relation to heroin abuse: dopamine tone contributes to reversed mesolimbic proenkephalin expression. Proc. Natl. Acad. Sci. U.S.A. 105, 786–791. https://doi.org/10.1073/pnas.0710902105.
Nomura, A., Ujike, U., Tanaka, Y., Otani, K., Morita, Y., Kishimoto, M., Morio, A., Harano, M., Inada, T., Yamada, M., Komiyama, T., Sekine, Y., Iwata, N., Sora, I., Iyo, M., Ozaki, N., Kuroda, S., 2006. Genetic variant of prodynorphin gene is risk factor for methamphetamine dependence. Neurosci. Lett. 400, 158–162. https://doi.org/10.1016/j.neulet.2006.02.038.
Norelli, L.J., Smith, H.S., Sher, L., Blackwood, T.A., 2013. Buprenorphine in the treatment of non-suicidal self-injury: a case series and discussion of the literature. Int. J. Adolesc. Med. Health 25, 323–330. https://doi.org/10.1515/ijamh-2013-0069.
Northrup, T.F., Stotts, A.L., Green, C., Potter, J.S., Marino, E.N., Walker, R., Weiss, R.D., Trivedi, M., 2015. Opioid withdrawal, craving, and use during and after outpatient buprenorphine stabilization and taper: a discrete survival and growth mixture model. Addict. Behav. 41, 20–28. https://doi.org/10.1016/j.addbeh.2014.09.021.
Nyhuis, P.W., Gastpar, M., Scherbaum, N., 2008. Opiate treatment in depression refractory to antidepressants and electroconvulsive therapy. J. Clin. Psychopharmacol. 28, 593–595. https://doi.org/10.1097/JCP.0b013e31818638a4.
Pfeiffer, A., Brantl, V., Herz, A., Emrich, H., 1986. Psychotomimesis mediated by kappa opiate receptors. Science 233, 774–776. https://doi.org/10.1126/science.3016896.
Poudel, A., Gautam, S., 2017. Age of onset of substance use and psychosocial problems among individuals with substance use disorders. BMC Psychiatr. 17, 10. https://doi.org/10.1186/s12888-016-1191-0.
Ravndal, E., Lauritzen, G., 2017. Rusmisbruk, angst og depresjon etter 10 år: en prospektiv undersøkelse av stoffmisbrukere med og uten LAR-behandling. Nord. Stud. Alcohol Drugs 32, 495–508. https://doi.org/10.1515/nsad-2015-0048.
Ray, R., Doyle, G.A., Crowley, J.J., Buono, R.J., Oslin, D.W., Patkar, A.A., Mannelli, P., DeMaria Jr., P.A., O'Brien, C.P., Berrettini, W.H., 2005. A functional prodynorphin promoter polymorphism and opioid dependence. Psychiatr. Genet. 15, 295–298. https://doi.org/10.1097/00041444-200512000-00013.
Sadée, W., Rosenbaum, J.S., Herz, A., 1982. Buprenorphine: differential interaction with opiate receptor subtypes in vivo. J. Pharmacol. Exp. Therapeut. 223, 157–162.
Saify, K., Saadat, I., Saadat, M., 2014. Association between VNTR polymorphism in promoter region of prodynorphin (PDYN) gene and heroin dependence. Psychiatr. Res. 219, 690–692. https://doi.org/10.1016/j.psychres.2014.06.048.
Saify, K., Saadat, M., 2014. Association between VNTR polymorphism in promoter region of prodynorphin (PDYN) gene and methamphetamine dependence. Open access maced. J. Med. Sci. 3, 371–373. https://doi.org/10.3889/oamjms.2015.079.
Sartor, C.E., Lynskey, M.T., Bucholz, K.K., Madden, P.A.F., Martin, N.G., Heath, A.C., 2009. Timing of first alcohol use and alcohol dependence: evidence of common genetic influences. Addiction 104, 1512–1518. https://doi.org/10.1111/j.1360-0443.2009.02648.x.
Schatzberg, A.F., 2015. Opioids in psychiatric disorders: back to the future? Am. J. Psychiatr. 173, 564–565. https://doi.org/10.1176/appi.ajp.2015.15101354.
Segui, H.A., Melin, K., Quiñones, D.S., Duconge, J., 2020. A review of the pharmacogenomics of buprenorphine for the treatment of opioid use disorder. J. Transl. Genet. Genom 4, 263–277. https://doi.org/10.20517/jtgg.2020.35.
Shippenberg, T.S., Bals-Kubik, R., Herz, A., 1993. Examination of the neurochemical substrates mediating the motivational effects of opioids: role of the mesolimbic dopamine system and D-1 vs. D-2 dopamine receptors. J. Pharmacol. Exp. Therapeut. 265, 53–59.
Ulusoy, M., Şahin, N.H., Erkmen, H., 1998. Turkish version of the Beck Anxiety Inventory: psychometric properties. J. Cognit. Psychother. 12, 163–172.
Vandenbergh, D.J., Rodriguez, L.A., I T Miller, I.T., Uhl, G.R., H M Lachman, H.M., 1997. High-activity catechol-O-methyltransferase allele is more prevalent in polysubstance abusers. Am. J. Med. Genet. 74, 439–442.
Verhulst, B., Neale, M.C., Kendler, K.S., 2015. The heritability of alcohol use disorders: a meta-analysis of twin and adoption studies. Psychol. Med. 45, 1061–1072. https://doi.org/10.1017/S0033291714002165.
Wei, S.G., Zhu, Y.S., Lai, J.H., Xue, H.X., Chai, Z.Q., Li, S.B., 2011. Association between heroin dependence and prodynorphin gene polymorphisms. Brain Res. Bull. 85, 238–242. https://doi.org/10.1016/j.brainresbull.2011.02.010.
Weiss, F., Ciccocioppo, R., Parsons, L.H., Katner, S., Liu, X., Zorrilla, E.P., Valdez, G.R., Ben-Shahar, O., Angeletti, S., Richter, R.R., 2001. Compulsive drug-seeking behavior and relapse. Neuroadaptation, stress, and conditioning factors. Ann. N. Y. Acad. Sci. 937, 1–26. https://doi.org/10.1111/j.1749-6632.2001.tb03556.x.
Williams, T.J., LaForge, K.S., Gordon, D., Bart, G., Kellogg, S., Ott, J., Kreek, M.J., 2007. Prodynorphin gene promoter repeat associated with cocaine/alcohol codependence. Addict. Biol. 12, 496–502. https://doi.org/10.1111/j.1369-1600.2007.00069.x.
Wittmann, W., Schunk, E., Rosskothen, I., Gaburro, S., Singewald, N., Herzog, H., Schwarzer, C., 2009. Prodynorphin-derived peptides are critical modulators of anxiety and regulate neurochemistry and corticosterone. Neuropsychopharmacology 34, 775–785. https://doi.org/10.1038/npp.2008.142.
Xi, X.Z., Fuller, S.A., Stein, E.A., 1998. Dopamine release in the nucleus accumbens during heroin self-administration is modulated by kappa opioid receptors: an in vivo fast-cyclic voltammetry study. J. Pharmacol. Exp. Therapeut. 284, 151–161.
Yovell, Y., Bar, G., Mashiah, M., Baruch, Y., Briskman, I., Asherov, J., Lotan, A., Rigbi, A., Panksepp, J., 2015. Ultra-low-dose buprenorphine as a time-limited treatment for severe suicidal ideation: a randomized controlled trial. Am. J. Psychiatr. 173, 491–498. https://doi.org/10.1176/appi.ajp.2015.15040535.
Yuanyuan, J., Rui, S., Hua, T., Jingjing, C., Cuola, D., Yuhui, S., Shuguang, W., 2018. Genetic association analyses and meta-analysis of Dynorphin-Kappa Opioid system potential functional variants with heroin dependence. Neurosci. Lett. 685, 75–82. https://doi.org/10.1016/j.neulet.2018.08.023.
Yuferov, V., Butelman, E.R., Kreek, M.J., 2018. Gender-specific association of functional prodynorphin 68 bp repeats with cannabis exposure in an African American cohort. Neuropsychiatric Dis. Treat. 14, 1025–1034. https://doi.org/10.2147/NDT.S159954.
Yuferov, V., Randesi, M., Butelman, E.R., van den Brink, W., Blanken, P., van Ree, J.M., Ott, J., Kreek, M.J., 2019. Association of variants of prodynorphin promoter 68-bp repeats in caucasians with opioid dependence diagnosis: effect on age trajectory of heroin use. Neurosci. Lett. 704, 100–105. https://doi.org/10.1016/j.neulet.2019.03.038.
Zaaijer, E.R., van Dijk, L., de Bruin, K., Goudriaan, A.E., Lammers, L.A., Koeter, M.W.J., van den Brink, W., Booij, J., 2015. Effect of extended-release naltrexone on striatal dopamine transporter availability, depression and anhedonia in heroin-dependent patients. Psychopharmacology (Berl) 232, 2597–2607. https://doi.org/10.1007/s00213-015-3891-4.
Zhou, Y., Leri, F., Grella, S.L., Aldrich, J.V., Mary Jeanne Kreek, M.J., 2013. Involvement of dynorphin and kappa opioid receptor in yohimbine-induced reinstatement of heroin seeking in rats. Synapse 67, 358–361. https://doi.org/10.1002/syn.21638.
Zimprich, A., Kraus, J., Wöltje, M., Mayer, P., Rauch, E., Höllt, V., 2000. An allelic variation in the human prodynorphin gene promoter alters stimulus-induced expression. J. Neurochem. 74, 472–477. https://doi.org/10.1046/j.1471-4159.2000.740472.x.