Environmental Toxicology and Pharmacology 95 (2022) 103979

Contents lists available at ScienceDirect

Environmental Toxicology and Pharmacology

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

Sublingual buprenorphine/naloxone treatment is not affected by OPRM1 A118G and BDNF Va66Met polymorphisms, but alters the plasma beta-endorphin and BDNF levels in individuals with opioid use disorder☆

Dilek Kaya-Akyüzlü [a,*], Selin Özkan-Kotiloğlu [b], Ceylan Bal [c], Gamze Avcıoğlu [c], Şafak Yalçın-Şahiner [d,1], İsmail Volkan Şahiner [d,1]

[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 Yıldırım Beyazıt University, Department of Medical Biochemistry, Ankara, Turkey
[d] Ankara Training and Research Hospital AMATEM Clinic, Ankara, Turkey

ARTICLE INFO

Edited by Malcolm D. Tingle

Keywords:
Beta-endorphin
BDNF
Buprenorphine
Pharmacogenetics

ABSTRACT

The study aimed to examine the genetic contribution to buprenorphine (BUP) treatment in individuals with opioid use disorder (OUD), with a specific focus on *BDNF* and *OPRM1* genes. A total of 113 controls and 111 OUD patients receiving sublingual BUP/naloxone were enrolled. *OPRM1* A118G and *BDNF* Val66Met polymorphisms were investigated by PCR-FRLP. Plasma BDNF and beta-endorphin levels were assessed by ELISA kits in both groups. Blood BUP levels were measured by LC-MS/MS and normalized with daily BUP dose (BUP/D). *OPRM1* A118G and *BDNF* Val66Met polymorphisms didn't have an effect on plasma beta-endorphin and BDNF levels in OUD patients, respectively. Interestingly, OUD patients had significantly higher plasma BDNF and lower beta-endorphin levels compared to the controls ($p < 0.001$). A negative and significant correlation between plasma BUP/D and BDNF levels was found. Age onset of first use was associated with *OPRM1* A118G polymorphism. The findings indicated that sublingual BUP/naloxone may increase plasma BDNF levels, but may decrease beta-endorphin levels in individuals with OUD. Plasma BDNF level seemed to be decreased in a BUP/D concentration-dependent manner.

1. Introduction

Buprenorphine (BUP) is used in the treatment of opioid use disorder (OUD) and the management of moderate to severe pain (Madison and Shoshana Eitan, 2020; Xhakaza et al., 2021). BUP's pharmacological properties are different from other opioids such as methadone and naltrexone being a partial mu-opioid receptor agonist (Martin et al., 1976), delta- and kappa-opioid receptor antagonist and nociceptin receptor agonist (Leander, 1987; Negus et al., 2002). BUP is generally preferred over naltrexone and methadone due to reduced abusive potential and lower risk of overdose death and toxicity (Madison et al., 2020; Xhakaza et al., 2021). BUP is approved by the Food and Drug Administration (FDA) for the treatment of opioid use disorders in combination with naloxone (Suboxone) and alone (Subutex) (U.S. FDA, 2002). Although both drugs found to be relatively effective in the treatment of opioid addiction in some persons, the rate of treatment failure including relapse and/or dropout is high among opioid users, which leads significant costs to society in view of health care and the criminal justice system (Crist et al., 2018; Wang et al., 2019; Randesi et al., 2020). It is estimated that the efficacy of pharmacotherapies for OUD is between 60% and 70% (Taqi et al., 2019). A recently completed 24-week, randomized, open-label trial of methadone and BUP/naloxone combination for the treatment of opioid dependence stated that patients treated with BUP were significantly more likely to drop out of the study than those treated with methadone (Crist et al., 2018). In order to boost the effectiveness of treatment and reduce the high rates of dropout, more detailed understanding of the patients' characteristics such as genetic profile is essential (Crist et al., 2018; Randesi et al., 2020). Some studies

☆ The manuscript was partially presented in the 1. INTERNATIONAL ADDICTION CONGRESS 24–25 JULY 2021/ Ankara, Turkey orally.

* Correspondence to: 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.etap.2022.103979
Received 23 June 2022; Received in revised form 21 September 2022; Accepted 22 September 2022
Available online 26 September 2022

demonstrated that there is an interaction between responsiveness to treatment and genetic variants regulating the metabolism, mechanism of action and transport of drugs (Wang et al., 2019). Therefore, patient-treatment matching would be one approach to improve the treatment outcomes and, also, facilitate longer periods of abstinence in addicts with high-risk of treatment failure (Crist et al., 2018; Wang, 2019; Randesi et al., 2020). Furthermore, Hjelmström et al. (2020) suggested that the BUP dose should be individualized in treatment of OUD and further studies are needed to understand the relationship between BUP dose and efficacy following the systematic review of the opioid receptor blockade studies.

Pharmacotherapy outcomes can be affected by pharmacogenetic variants altering pharmacokinetics and/or pharmacodynamics (Crist et al., 2018; Randesi et al., 2020). One of the pharmacodynamic genes that could affect the mechanism of BUP action is *OPRM1* encoding the endogenous mu-opioid receptors (MORs). MORs those are located in cerebral cortex and thalamus bind endorphins, stimulating euphoria, physical dependence and respiratory depression. MORs located in the nucleus accumbens and basolateral amygdala trigger the incentive properties of rewarding stimuli. Repeated abuse of opioids causes neurobiological changes in brain pathways and the increase in tolerance of opioid receptors heightens the risk of relapse. One reason of continuous drug dependence is to stop the withdrawal symptoms such as abdominal pain, diarrhea and sweating. Opioid agonist and/or antagonist medications with an effect on MOR can reduce this intensity of withdrawal and craving (Wang et al., 2019). In the brain, BUP exerts its effects by binding to MORs partially and typical opioid effects such as euphoria and decreased pain are experienced (Coe et al., 2019; Xhakaza et al., 2021). Due to this partial agonism at the MOR, BUP has a ceiling effect and, thus, is safer than other full-opioid agonists. BUP's high binding affinity to MORs leads to a precipitated withdrawal and craving as well as a reduced illicit opioid use in individuals physically dependent on opioids (Coe et al., 2019; Buresh et al., 2020). In addition, the blockade of MORs by BUP blocks the effects of exogenous opioids (Hjelmström et al., 2020). BUP has low intrinsic activity at MORs and can displace other full agonists such as morphine from MORs. On the other hand, full opioid agonists cannot displace BUP and, thus, BUP can blunt the high of heroin (Zoorob et al., 2018). Moreover, BUP has a long half-time due to its slow-dissociation kinetics, which allows for once or twice daily dosing in the treatment of OUD (Kumar et al., 2021).

Upon binding of BUP to the MORs, neurotransmitters (e.g., gamma-aminobutyric acid, dopamine, serotonin and glutamate) changes seen in opioid addiction are antagonized by BUP and, thus, the stimulation associated with drug use reduced. This reduction occurs since these neurotransmitters play an important role in drug addiction as well as learning and memory, mood and locomotor activity. In addition, transcription factors such as brain-derived neurotrophic factor (BDNF) can modify the levels of these neurotransmitters (Xhakaza et al., 2021). Therefore, variants in *BDNF* gene could be good candidates to predict the treatment failure. BDNF is a neurotrophic peptide modulating the neuronal survival, neurotransmission and plasticity (Bawor et al., 2015; Xhakaza et al., 2021). Recently, Xhakaza and co-workers (2021) investigated the pharmacodynamic effects of intranasally administered BUP on *BDNF* expression in a rodent model and found that BUP administration contributed to significant upregulation of BDNF, with the highest changes at 8 h following drug dosing. However, to the best of our knowledge, there has not been any human study examining the association between BUP treatment response (dose-adjusted plasma BUP levels) and *BDNF* gene variants, although evidence suggesting its contribution to OUD and drug seeking (Greenwald et al., 2013; Bawor et al., 2015; Roviš et al., 2018).

Hitherto, little is known about pharmacogenetic variants that would affect the bioavailability of the sublingual BUP/naloxone combination in the body of individuals with OUD. A limited number of papers examined some polymorphisms on genes such as *OPRM1* (Crist et al., 2018; Randesi et al., 2020), *OPRD1* (Kranzler et al., 2021), *PDYN* and *OPRK1* (Randesi et al., 2020) and *CYP3A4* (Ettienne et al., 2019) in patients with OUD receiving sublingual BUP/naloxone combination treatment. These existing genetic studies have explored the therapeutic response to BUP, with a focus on dropout rate, opioid-positive urine drug screens, failure to receive first or last doses and BUP dosing. However, plasma BUP concentrations inversely associated with withdrawal symptoms and MOR saturation were not measured in none of these studies. Higher agonistic substitution of the MOR by increasing the plasma BUP levels is required for higher clinical efficacy of BUP for the treatment of OUD (Santiago et al., 2020). Laib et al. (2013) reported that 40% and 50% of the patients were above or below the therapeutic reference range, respectively, and only 10% exhibited plasma levels within the therapeutic reference range. In addition, Chawarski et al. (1999) observed a wide range of intra- and inter-subject variability in plasma BUP concentrations of opioid dependent subjects following sublingual BUP administration. Therefore, the present study aimed to determine the genetic contribution to BUP treatment response (dose-adjusted plasma BUP levels, craving and withdrawal) among a cohort of subjects with OUD, with a specific focus on *OPRM1* and *BDNF* genes. In addition, the effect of BUP on plasma BDNF and beta-endorphin levels compared to controls was evaluated in humans.

2. Patients and methods

2.1. Study population

A total of 111 individuals who had an opioid use disorder by the Diagnostic and Statistical Manual of Mental Disorders (DSM–5) criteria and had been receiving sublingual BUP/naloxone treatment for at least 10 days for steady-state plasma BUP concentrations at AMATEM in Ankara were enrolled in this study. Patients were eligible for inclusion in the study if (i) they were at least 18 years of age, (ii) they have not been receiving any drugs that might interact with the metabolism of BUP, and (iii) they had no acute health problems. Subjects with active drug addiction during BUP management therapy, with substance use disorders other than heroin and nicotine dependence such as other opioids, alcohol and/or benzodiazepines, and with impaired renal or hepatic function were excluded from the study. Urine drug test performed by the routine analysis laboratory of AMATEM was used to confirm the presence of substances other than BUP.

For comparison, a total of 113 controls who declared that they had no diagnosis of past or current OUD were included in the study. These healthy volunteers were recruited from the volunteers who admitted to Blood Donation Center of Ankara University. Controls were matched to individuals with OUD for gender and smoking habits.

Subjects with clinically significant co-morbid psychiatric illness (e.g., any psychotic disorders, schizophrenia, mental retardation, bipolar disorder and severe depression), and subjects administered either drugs for physical diseases or psychiatric illness such as depression and anxiety were also excluded from both groups.

All subjects completed a small questionnaire used to gather socio-demographic information regarding age, marital, education and employment status, duration of heroin use, age at onset of first heroin use, family history of illicit substance use, and times and doses of sublingual BUP/naloxone. Written informed consent was obtained from each participant who was eligible for the study. Samplings were performed in accordance with the principles of The Declaration of Helsinki. The study design was approved by the institutional ethics committee (Approval No: 07–536–19 in 2019).

2.2. Determination of the OPRM1 A118G and BDNF Val66Met polymorphisms

Venous blood samples (approximately 2–4 ml) were taken from each subject into tubes with EDTA for DNA isolation and were kept at − 20 °C until the polymerase chain reaction (PCR) was performed. Genomic

DNA was extracted from 200 µl whole blood samples using a Qiagen QIAamp DNA Blood Kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions.

OPRM1 A118G (rs1799971) and *BDNF* Val66Met (rs6265) polymorphisms were analyzed by PCR followed by restriction fragment length polymorphism (RFLP), as previously described by Cheng et al. (2019) and Skibinska et al. (2018), respectively (Cheng et al., 2019; Skibinska et al., 2018). The forward and reverse primers used by Skibinska et al. (2018) were modified in order to increase the length of PCR product, whereas the same restriction enzyme was used (Fig. 1). PCR 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.

For detection of the A118G SNP of the *OPRM1* gene, 300-bp fragment was amplified by PCR using the following primers: forward: 5'-CTGACGCTCCTCTCTGTCTCA-3' and reverse: 5'-TTCGGACCG-CATGGGTCGGACAGGT-3'. The PCR cycling conditions consisted of an initial denaturation step of 10 min at 94 °C for; 35 cycles of 1 min of denaturation at 94 °C, 1 min of annealing at 60 °C, and 1 min of extension at 72 °C; and a final extension of 10 min at 72 °C. A total of 5 microliters of PCR product (300 bp) was then digested by *Sau3AI* (New England Biolabs, Hertfordshire, UK). To screen for Val66Met polymorphism of *BDNF*, 660-bp fragment containing the Val66Met polymorphic site was amplified by PCR using the following primers: forward 5'-TCTGTCTTGTTTCTGCTTTCTC-3' and reverse 5'-AATGCCTTTTGTCTATGCCC-3'. The PCR cycling conditions consisted of an initial denaturation step at 95 °C for 15 min, followed by 36 cycles of 94 °C for 1 min, 55 °C for 1 min 30 s, 72 °C for 1 min, and final extension step at 72 °C for 10 min. Then, the PCR product (660 bp) was digested with *Eco72I* restriction enzyme (New England Biolabs, Hertfordshire, UK).

The undigested and digested DNA fragments were separated by electrophoresis on a 2% agarose gel, visualized by ethidium bromide staining under an UV illuminator, and then scanned and photographed using the Syngene Monitoring System.

2.3. Determination of plasma BDNF and beta-endorphin levels

Blood samples were also collected into serum separation tubes from each subject. The tubes were allowed to clot for approximately 30 min. Then, they were centrifuged for 15 min at 3500 rpm. The supernatant was collected and was kept at − 20 °C until analysis.

Plasma BDNF levels were determined using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Human BDNF ELISA Kit, Elabscience, USA). Plasma beta-endorphin levels were determined by using Human beta-endorphin ELISA Kit (Elabscience, USA). Samples, reagents and standards were prepared according to the manufacturer's instructions. The levels of plasma BDNF and beta-endorphin levels were expressed in pg/ml.

2.4. Measurement of plasma buprenorphine

In order to measure the steady-state concentration of plasma buprenorphine level, 4 ml venous blood sample from each OUD patient was also collected into another tube with EDTA before taking daily BUP dose. All samples were stored at − 20 °C until analysis in the biochemistry laboratory. Plasma BUP concentrations were measured by Liquid Chromatography Tandem Mass Spectrometry (LC–MS/MS) using a triple quadrupole SCIEX 5500 Qtrap (AB Sciex, Darmstadt, Germany). A Shimadzu Prominence system with a RRHD Eclipse Plus C18 (95 Å, 1,8 µm, 2,1 ×50 mm, Zorbax, USA) column was used to separate substances. Liquid–liquid extraction was performed using "Abuse Drugs in Blood by LC/MS" (Eureka Lab Division). The retention time for BUP was 6.52 min. The LOD and LOQ for BUP were 0.02 ng/ml and 0.125 ng/ml, respectively. Precision of the method was evaluated with the intra- and inter-day variations which were < 10% for both compounds.

2.5. Measurements

To investigate the effects of *OPRM1* A118G and *BDNF* Val66Met polymorphisms on the intensity of craving and withdrawal in OUD patients were assessed by the Substance Craving Scale (SCS) and Clinical Opiate Withdrawal Scale (COWS). The validities and reliabilities of Turkish versions of these scales were demonstrated (Evren et al., 2011; Canan et al., 2015).

2.6. Statistical analyses

The Statistical Package for Social Sciences (SPSS) version 21.0 software for Windows was used for the statistical analyses. The normality of numerical variables was examined using the Kolmogorow-Smirnow test. All data were shown as median and interquartile range (IQR) due to the non-normal distribution of numerical data. Numbers, percentages and 95% confidence interval were given for categorical data. The frequencies of the *OPRM1* A118G and *BDNF* Val66Met alleles and genotypes were obtained by direct count, and the departure from the Hardy-Weinberg equilibrium ($p2 + 2pq + q2 = 1$) was evaluated by the chi-square test. Plasma BUP values were normalized by adjusting with patients' daily dose. Dose-normalized BUP concentration (BUP/D) was calculated using the following equations: BUP concentration (ng/ml)/

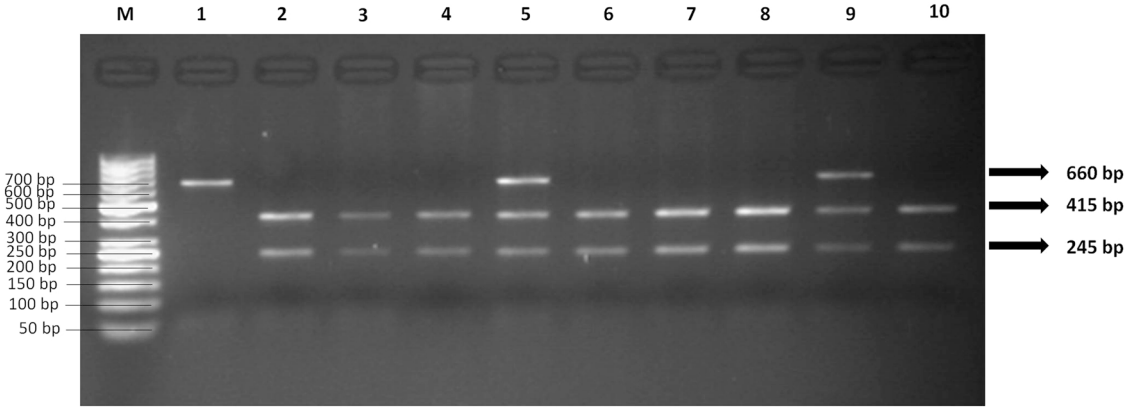

Fig. 1. A representative agarose gel image of digested PCR products (660 bp) with *Eco72I* for the *BDNF* Val66Met polymorphism. M: 50 bp ladder; Lanes 2–4, 6–8 and 10: homozygous wild-type genotype (Val/Val) (415 bp and 245 bp bp); Lanes 5 and 9: heterozygote genotype (Val/Met) (660 bp, 415 bp and 245 bp); Lane 1: homozygous variant genotype (Met/Met) (660 bp).

BUP daily dose (mg/day). For each polymorphism, genotypes were subdivided in 3 groups (homozygote wild type, heterozygote and homozygote variant type) and statistically compared. In addition, the parameters such as plasma BDNF and beta-endorphin levels, daily BUP dosing, BUP/D value, measures' scores (craving and withdrawal) and age onset of first heroin use (years) were compared according to *OPRM1* A-recessive and *BDNF* Val-recessive models, but not for co-dominant and dominant models (A-dominant and Val-dominant, respectively) due to the low frequency of GG and Met/Met genotypes, respectively, as shown in Tables 3–5. Genotypes were compared using Mann Whitney test or Kruskal-Wallis test, as appropriate. The correlations between scores of measures and plasma BDNF and beta-endorphin levels were analyzed by the Spearman correlation test. Comparisons of controls vs. OUD patients receiving BUP were also conducted with the ANCOVA test using age as covariate. $p < 0.05$ was considered as statistically significant.

3. Results

3.1. Characteristics and demographics of subjects

Demographic characteristics of controls and individuals with OUD receiving sublingual BUP/naloxone treatment were given in Table 1. In total 113 controls (103 males and 10 females, median ages 32 years) and 111 individuals with OUD diagnosis (101 males and 10 females, median ages 27 years) were included in the study. No significant difference was found between the groups regarding sex ($p = 0.967$). All subjects in both the controls and OUD patients were smokers. The median ages and the weight of the groups at the time of ascertainment were significantly different ($p = 0.001$). However, there was no statistically significant difference in age between both the *OPRM1* A118G and *BDNF* Val66Met genotype subgroups ($p > 0.05$).

Table 1
Socio-demographics characteristics of OUD patients and controls.

Parameter	OUD (n = 111)		Controls (n = 113)	
Age (years)	27.00		32	
$\tilde{x}$ (IQR)	(25.00–32.00)		(26.00–42.00)	
Weight (kg)	65.00		80.00	
$\tilde{x}$ (IQR)	(57.00–78.00)		(74.50–92.00)	
Height (cm)	175.00		178.00	
$\tilde{x}$ (IQR)	(169.00–180.00)		(172.00–180.00)	
Education	n	% frequency (95% CI)	n	% frequency (95% CI)
Primary	20	18.0 (10.9–25.1)	11	9.7 (4.2–15.2)
Secondary	46	41.4 (32.2–50.6)	18	15.9 (9.15–22.6)
High School	39	35.1 (26.2–44.0)	61	54.0 (44.8–63.2)
Undergraduate	6	5.4 (1.2–9.6)	21	18.6 (11.4–25.8)
Graduate	0	0 (NA)	2	1.8 (NA)
Occupation	n	% frequency (95% CI)	n	% frequency (95% CI)
Working	71	64.0 (55.1–72.9)	100	88.5 (82.6–94.4)
Not working	40	36.0 (27.1–44.9)	13	11.5 (5.62–17.4)
Marital status	n	% frequency (95% CI)	n	% frequency (95% CI)
Single	70	63.1 (54.1–72.1)	45	39.8 (30.8–48.8)
Married	35	31.5 (22.9–40.1)	67	59.3 (58.3–75.7)
Widow/Divorced	6	5.4 (1.2–9.6)	1	0.9 (NA)
Living	n	% frequency (95% CI)	n	% frequency (95% CI)
Alone	6	5.4 (1.2–9.6)	13	11.5 (5.6–17.4)
With a partner	3	2.7 (NA)	0	0 (NA)
With husband/ wife	31	27.9 (19.6–36.2)	65	57.5 (48.4–66.6)
With a family	71	64.0 (55.1–72.9)	35	31.0 (22.5–39.5)
Incarceration	n	% frequency (95% CI)	n	% frequency (95% CI)
Yes	25	22.5 (14.7–30.3)	4	3.5 (0.1–6.9)
No	86	77.5 (69.7–85.3)	109	96.5 (93.1–99.9)
Probation	n	% frequency (95% CI)	n	% frequency (95% CI)
Yes	20	18.0 (10.9–25.1)	0	0 (NA)
No	91	82.0 (74.9–89.1)	113	100

OUD: Individuals with opioid use disorder receiving sublingual buprenorphine/naloxone treatment, $\tilde{x}$: median, IQR: Interquartile range, n: sample size, CI: confidence interval.

Since OUD patients were significantly younger and lighter than controls ($p < 0.05$), we controlled for age and weight using non-parametric ANCOVA (Quade's) due to the non-normal distribution of these parameters, revealing that age ($p = 0.14$ and $p = 0.827$, respectively) and weight was not significantly associated the plasma BDNF and beta-endorphin levels ($p = 0.29$ and $p = 0.636$, respectively).

3.2. Genotype distribution of the OPRM1 A118G and BDNF Val66Met polymorphisms

The genotype and minor allele frequencies, 95% confidence interval and Hardy-Weinberg equilibrium (HWE) of the *OPRM1* A118G and *BDNF* Val66Met polymorphisms in controls and OUD group were presented in Table 2. The genotype frequencies of these two polymorphisms in all samples were consistent with Hardy–Weinberg equilibrium ($p > 0.05$). The relationship between the *OPRM1* and *BDNF* genotypes and OUD was examined by logistic regression analysis and none of the polymorphisms were found to be associated with the vulnerability to OUD (Table 2).

3.3. The median plasma beta-endorphin levels in OUD and control groups, across OPRM1 A118G genotypes

The median plasma beta-endorphin levels of OUD and control groups as well as the median dose-adjusted plasma BUP concentrations (BUP/D) of OUD group were shown in Table 3. The median plasma beta-endorphin levels of OUD group was significantly lower than that of controls (33.47 pg/ml vs. 96.89 pg/ml, $p = 0.001$) (Fig. 2). Table 3 also showed the plasma beta-endorphin levels of both groups according to *OPRM1* A118G genotype subgroups. Because of the small number of individuals ($n < 4$) in *OPRM1* GG genotype, subjects having the GG genotype was merged with those having AG genotype and compared statistically to those having AA. Mann-Whitney U test conducted for this A-recessive model (AA versus AG+GG) did not show significant associations of the *OPRM1* A118G genotype with the plasma beta-endorphin and BUP/D levels in OUD group. However, individuals with OUD with the AA genotype had lower median beta-endorphin level and higher BUP/D level compared to those with the AG+GG genotype (33.47 versus 41.65 pg/ml; 0.042 vs. 0.039 ng/ml/day; $p > 0.05$). As for control group, a statistically significant association was also not found between *OPRM1* A118G genotypes in view of plasma beta-endorphin levels ($p = 0.794$). However, it was detected that controls with the AG+GG genotype had higher median plasma beta-endorphin concentrations compared to those with AA genotype (Table 3; Fig. 2).

3.4. The median plasma BDNF levels in OUD and control groups, across BDNF Val66Met genotypes

Table 4 showed that the median BDNF level of OUD group was significantly higher than that of controls (686.29 pg/ml vs. 59.54 pg/ml, $p = 0.001$), when controlled for age. The plasma BDNF concentrations of OUD group and controls according to *BDNF* Val66Met genotypes were also presented in Table 4. The *BDNF* Met/Met genotype was detected only in three patients with OUD and controls. Thus, subjects having the Met/Met genotype was merged with those having Val/Met genotype and compared statistically to those having Val/Val genotype. Mann-Whitney U test conducted for this Val-recessive model (Val/Val versus Val/Met+Met/Met) did not show significant associations of the *BDNF* Val66Met genotype with the plasma BDNF levels ($p > 0.05$). However, the median plasma BDNF concentration of OUD group with Val/Val genotype was lower (686.29 pg/ml) than that of the Val/Met+Met/Met genotypes (706.4 pg/ml). Similarly, controls with *BDNF* Val66Met genotype had lower BDNF levels (56.67 pg/ml vs. 66.54 pg/ml) than those with Val/Met+Met/Met genotypes (Fig. 2).

Table 2
The genotype frequencies of the *OPRM1* A118G *and BDNF* Val66Met polymorphisms in OUD patients and controls.

OPRM1 A118G Genotypes	OUD patients (n = 111)		Controls (n = 113)		*p*-value*	Odds Ratio (95% CI)
	n	% frequency (95% CI)	n	% frequency (95% CI)		
AA	79	71.2 (62.8–79.6)	81	71.7 (63.4–80.0)	0.735	0.916 (0.553–1.520)
AG	28	25.2 (17.1–33.3)	30	26.5 (18.4–34.6)		
GG	4	3.6 (0.01–7.1)	2	1.8 (NA)		
Total	111	100	113	100		
Variant allele freq.	16%		15%			
HWE p-value	$\chi2 = 0.57$; $p = 0.45$		$\chi2 = 0.16$; $p = 0.68$			
***BDNF* Val66Met Genotypes**	**OUD patients (n = 111)**		**Controls (n = 113)**		***p*-value***	**Odds Ratio (95% CI)**
	n	% frequency (95% CI)	n	% frequency (95% CI)		
Val/Val	83	74.8 (66.7–82.9)	84	74.3 (66.2–82.4)	0.984	1.005 (0.599–1.688)
Val/Met	25	22.5 (14.7–30.3)	26	23.0 (15.2–30.8)		
Met/Met	3	2.7 (NA)	3	2.7 (NA)		
Total	111	100	113			
Variant allele freq.	14%		14%			
HWE *p*-value	$\chi2 = 0.44$; $p = 0.51$		$\chi2 = 0.32$; $p = 0.57$			

OUD: Individuals with opioid use disorder receiving sublingual buprenorphine/naloxone treatment, n: sample size, CI: confidence interval, $\chi2$: chi-square, HWE: Hardy-Weinberg Equilibrium, *the p-value for logistic regression analysis

Table 3
Association of the *OPRM1* A118G polymorphism with plasma beta-endorphin in both OUD patients and the controls and with plasma dose-adjusted BUP concentration (BUP/D) in OUD patients.

SNPs	OUD patients (n = 111)				SNPs	Controls (n = 113)	
	Beta-endorphin levels (pg/ml)		BUP/D ng/ml per mg/day			Beta-endorphin levels (pg/ml)	
	Median	IQR	Median	IQR		Median	IQR
***OPRM1* A118G genotypes (A-Recessive model)**							
AA (n = 79)	33.47	20.8–75.2	0.042	0.021–0.10	AA (n = 81)	94.5	75.4–108.9
AG+GG (n = 32)	41.65	22.7–69.0	0.039	0.02–0.09	AG+GG (n = 32)	100.7	73.8–106.2
Mann-Whitney U test	U= 1223.0 p = 0.790 Z = −0.267		U= 1153.0 p = 0.470 Z = −0.723		Mann-Whitney U test	U= 1255.0 p = 0.794 Z = −0.261	
Total (n = 111)	33.47	20.78–73.61	0.042	0.021–0.094	**Total (n = 113)**	96.89	74.8–108.5
Mann-Whitney U test	U= 1713.0 **p = 0.001** * Z = −9.400						

OUD: Individuals with opioid use disorder receiving sublingual buprenorphine/naloxone treatment, IQR: Interquartile range, n: sample size.
*Comparison of OUD patients with the controls in view of beta-endorphin levels.

3.5. Age onset of first heroin use, BUP dosing and the intensity of depression and anxiety in individuals with OUD, across OPRM1 A118G and BDNF Val66Met genotypes

In OUD group, the median age onset of first heroin use was 21.0 years (IQR: 19.0–27.0 years). Daily doses of BUP ranged from 2.0 mg/day to 10.0 mg/day (median 6.0 mg/day; IQR: 4.0–8.0 mg/day). The median withdrawal instances was 1 (IQR: 0–2), with a minimum of zero and a maximum of 4 instances. The median SCS score was 5 (IQR: 0–12) with a minimum of zero and a maximum of 25. The intensity of craving and withdrawal, age onset of first use, the daily sublingual BUP/naloxone amount (mg/day) were also compared according to the *OPRM1* A118G and *BDNF* Val66Met genotypes and were presented in Table 5. For *OPRM1* A118G polymorphism, pairwise comparisons of *OPRM1* A118G subgroups for age onset of first heroin use: GG was significantly different to group AG (15.5 vs. 21.0 years; $p = 0.03$) and to group AA (15.5 vs. 21.0 years; $p = 0.016$). Group AA:AG was not significantly different ($p > 0.05$). There were no significant associations of the *OPRM1* A118G genotype and daily BUP dosing and the scores of SCS and COWS. It may be noted that individuals with OUD having AA genotype had lower SCS and COWS scores compared with those having AG+GG genotypes due to most probably higher plasma BUP/D level (4.0 vs. 5.0; 0.0 vs. 1.0, respectively). As for the effect of *BDNF* Val66Met genotypes SCS score and BUP dosing, individuals with Val/Val genotype had higher SCS score (6.0 vs. 3.5) and BUP dosing (6.0 vs. 4.0 mg/day) compared to those with Val/Met+Met/Met genotypes. However, these differences between genotypes were not statistically significant ($p > 0.05$).

3.6. The effect of the combination of OPRM1 A118G and BDNF Val66Met polymorphisms on plasma beta-endorphin and BDNF levels as well as daily BUP dosing (mg/day), BUP/D, the intensity of craving and withdrawal in OUD group

The subjects were divided into four groups according to genotypes: *OPRM1*G^-/*BDNF*Met^-, *OPRM1*G^+/*BDNF*Met^+, *OPRM1*G^-/*BDNF*Met^+ and *OPRM1*G^+/*BDNF*Met^- (Table 6). Significant associations were not detected between the combination of *OPRM1* A118G and *BDNF* Val66-Met polymorphisms and mentioned parameters above ($p > 0.05$).

Table 4
Association of the *BDNF* Val66Met polymorphism with plasma BDNF levels in both OUD patients and the controls.

SNPs	OUD patients (n = 111)		SNPs	Controls (n = 113)	
	BDNF levels (pg/ml)			BDNF levels (pg/ml)	
	Median	IQR		Median	IQR
BDNF **Val66Met genotypes (Val-Recessive model)**					
Val/Val (n = 83)	686.29	488.4–925.5	Val/Val (n = 84)	56.67	39.9–109.9
Val/Met+ Met/Met (n = 28)	706.4	535.4–972.2	Val/Met+ Met/Met (n = 29)	66.54	48.3–142.6
Mann-Whitney U test	U= 1086.0 p = 0.606 Z = −0.516		Mann-Whitney U test	U= 1061.0 p = 0.302 Z = −1.032	
Total (n = 111)	686.29	493.9–925.5	**Total (n = 113)**	59.54	41.8–120.9
Mann-Whitney U test	U= 531.0 **p = 0.001** * Z = −11.838				

OUD: Individuals with opioid use disorder receiving sublingual buprenorphine/naloxone treatment, IQR: Interquartile range, n: sample size.
*Comparison of OUD patients with the controls in view of plasma BDNF levels.

Table 5
Comparison of OUD patients according to the *OPRM1* A118G and *BDNF* Val66Met genotypes in view of BUP dosing, the scores of craving (SCS) and withdrawal (COWS) scales and age onset of first heroin use.

SNPs	BUP dosing (mg/day)	SCS score	COWS score	Age onset of first use (years)
	Median (IQR)			
OPRM1 A118G genotypes (A-Recessive model)				
AA (n = 79)	4.0 (40.−8.0)	4.0 (0.0–13.0)	0.0 (0.0–2.0)	21.0 (19.0–28.0)
AG+GG (n = 32)	6.0 (4.0–8.0)	5.0 (1.5–10.75)	1.0 (0.0–2.0)	20.0 (18.25–25.75)
Mann-Whitney U test	U= 1105.0 p = 0.3284 Z = −1.072	U= 1148.5 p = 0.448 Z = −0.759	U= 1074.5 p = 0.286 Z = −1.066	U= 1100.5 p = 0.286 Z = −1.067
BDNF **Val66Met genotypes (Val-Recessive model)**				
Val/Val (n = 83)	6.0 (4.0–8.0)	6.0 (1.0–13.0)	1.0 (0.0–2.0)	21.0 (19.0–28.0)
Val/Met+ Met/Met (n = 28)	4.0 (4.0–6.0)	3.5 (0.0–10.75)	1.0 (0.0–2.0)	21.0 (19.0–24.38)
Mann-Whitney U test	U= 974.5 p = 0.187 Z = −1.319	U= 971.0 p = 0.190 Z = −1.310	U= 1069.0 p = 0.702 Z = −0.383	U= 1009.0 p = 0.298 Z = −1.042

IQR: Interquartile range, n: sample size.

However, it may be noted that individuals with the *OPRM1*G^-/*BDNF*Met^+ had higher plasma beta-endorphin and BDNF levels (60.13 and 741.8 pg/ml, respectively) than those with *OPRM1*G^-/*BDNF*Met^- (33.47 and 686.3 pg/ml, respectively), *OPRM1*G^+/*BDNF*Met^+ (38.29 and 695.0 pg/ml, respectively) and *OPRM1*G^+/*BDNF*Met^- (41.65 and 681.5 pg/ml, respectively). Individuals with the *OPRM1*G^+/*BDNF*Met^- had the highest daily BUP dosing (7.0 mg) and SCS (7.0) score among all 4 genotype combinations. In addition, individuals with the *OPRM1*G^+/*BDNF*Met^+ had higher plasma BUP/D concentration than those with other genotype combinations (Table 6).

3.7. Correlation analysis

In the present study, it was found that there was a significant and positive correlation between plasma BDNF level and the intensity of craving and withdrawal in OUD group (r^2 =+0.198, p = 0.038; r^2 =+0.245, p = 0.010, respectively). There was a negative and significant correlation between the plasma BUP/D concentration and the intensity of craving (r^2 =−0.279, p = 0.003) and plasma BDNF level (r^2 =−0.290, p = 0.002). In the control group, plasma beta-endorphin level was significantly correlated with plasma BDNF level (r^2 =+0.391, p = 0.001).

4. Discussion

To the best of our knowledge, this is the first study evaluating the effect of sublingual BUP/naloxone treatment on plasma BDNF level in individuals with OUD. Existing studies have correlated BDNF levels to opioid maintenance treatment, with a focus on methadone (Han et al., 2015; Tsai and Huang, 2017; Roviš et al., 2018). Among these previous studies, only Roviš et al. (2018) included OUD patients undergoing BUP/naloxone (24%) or BUP (19.2%) in addition to methadone (43.1%) in their study (Roviš et al., 2018). However, according to Rovis' study, it could not be indicated that BUP could increase serum BDNF levels due to almost half of the patients receiving methadone, a full antagonist to mu-opioid receptor. On the other hand, in the present study, all of the OUD patients (n = 111) had been receiving sublingual BUP/naloxone treatment and plasma BDNF level was found to be significantly increased in these patients compared to controls. Thus, the present study suggested that BUP administration could increase plasma BNDF levels in humans. Our findings supported the Xhakaza's recent study (2021) showing an altered BDNF gene expression in rodent brain following an intranasal administration of BUP. BDNF is a neurotrophic peptide modulating the neuronal survival, neurotransmission and plasticity (Bawor et al., 2015; Xhakaza et al., 2021). In addition, BDNF was shown to modulate the serotonergic, dopaminergic and GABAergic functions in animal models (Hyman et al., 1991; Popova et al., 2017); hence, it is suggested that BDNF involves in the development of drug or alcohol addiction (Hilburn et al., 2011). In striatal neurons, very low levels of BDNF mRNA is expressed under normal conditions (Graham et al., 2007); however, its expression is markedly enhanced by crack-cocaine (Anders et al., 2020), amphetamine (Kim et al., 2005) and opioid exposure (Heberlein et al., 2011). On the other hand, there have been studies showing lower BDNF serum levels in opioid dependent individuals. In one of these studies, Chen et al. (2015) reported that plasma BDNF level was negatively affected by the length of the heroin dependency and they hypothesized that the downregulation of plasma BDNF might be due to the neuronal degeneration in long-term heroin users. Anders et al. (2020) suggested that the increase in BDNF mRNA expression could be related to neuroadaptive mechanisms contributing to excessive dopaminergic function, and craving and relapsing behavior. Furthermore, preclinical studies demonstrated that experimental infusion of BDNF into the ventral tegmental area produced biochemical changes and drug-seeking behavior (Lu et al., 2004; Vargas-Perez et al., 2009). Consistently, plasma BDNF level was found to be significantly correlated with the intensity of craving in the present study. Furthermore, due to the negative and significant correlation between the plasma BUP/D level and the intensity of craving, it is plausible to propose that BUP could decrease drug-seeking behavior. Previously, Ahmadi et al. (2018) and Johnson et al. (1992) also reported that BUP could decrease craving.

In the present study, beta-endorphin levels were also measured in OUD patients receiving BUP treatment and were compared with controls. Previously, one study by Kosten et al. (1992) examined the effect of BUP on beta-endorphin levels in only six individuals with OUD before and after being switched to sublingual BUP and found that beta-endorphin levels were not statistically different. In addition, they reported that the beta-endorphin level of unmedicated normals did not differ from those on BUP. Contrary to this previous study, we observed that the beta-endorphin levels of controls were significantly higher than that of OUD patients (n = 111) receiving BUP at least 10 days after controlling of age (p = 0.001). Beta-endorphin is an endogenous opioid

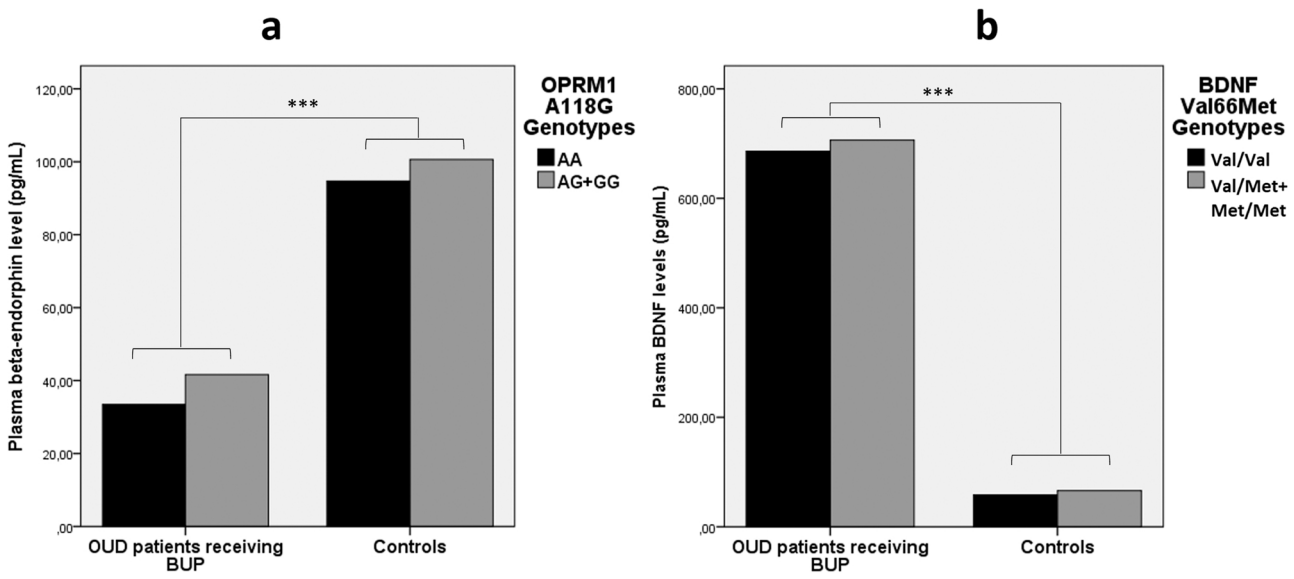

Fig. 2. (a) Effect of *OPRM1* A118G genotypes according to A-recessive model (AA versus AG+GG) on the plasma median beta-endorphin levels, (b) Effect of *BDNF* Val66Met genotypes according to Val-recessive model (Val/Val versus Val/Met+Met/Met) on the plasma median BDNF levels in OUD patients and the controls. Significance was set as $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***).

Table 6
Effect of the combination of the *OPRM1* A118G and *BDNF* Val66Met genotypes on daily BUP dosing, plasma dose-adjusted BUP concentrations, the scores of craving (SCS) and withdrawal (COWS) scales and plasma levels of beta-endorphin and BDNF in OUD patients.

Combined genotypes		BUP dosing (mg/day)	BUP/D ng/ml per mg/day	SCS score	COWS score	Beta-endorphin levels (pg/ml)	BDNF levels (pg/ml)
***OPRM1* A118G**	***BDNF* Val66Met**	Median (IQR)					
AA (G^-) n = 63	Val/Val (Met^-)	6.0 (4.0–8.0)	0.042 (0.023–0.096)	5.0 (0.0–12.0)	0.0 (0.0–2.0)	33.47 (20.75–73.39)	686.3 (488.4–1004.0)
AG+GG (G^+) n = 12	Val/Met+Met/Met (Met^+)	4.0 (4.0–7.5)	0.057 (0.019–0.11)	4.5 (0.25–8.75)	1.0 (0.0–3.0)	38.29 (24.16–65.99)	695.0 (518.2–927.1)
AA (G^-) n = 16	Val/Met+Met/Met (Met^+)	4.0 (4.0–6.0)	0.042 (0.021–0.12)	1.5 (0.0–16.75)	0.5 (0.0–0.75)	60.13 (19.87–92.69)	741.8 (547.9–972.2)
AG+GG (G^+) n = 20	Val/Val (Met^-)	7.0 (4.0–8.0)	0.031 (0.017–0.08)	7.0 (3.25–14.00)	1.0 (0.0–2.0)	41.65 (21.15–71.64)	681.5 (455.6–852.9)
Kruskal-Wallis test		$\chi2 = 3.642$ $p = 0.303$	$\chi2 = 1.435$ $p = 0.697$	$\chi2 = 3.666$ $p = 0.30$	$\chi2 = 1.201$ $p = 0.753$	$\chi2 = 1.574$ $p = 0.665$	$\chi2 = 0.924$ $p = 0.820$

peptide with the highest affinity for the mu-opioid receptor (Pilozzi et al., 2020). However, animal studies suggested that some pharmacological responses induced by beta-endorphin in supraspinal sites seem to be mediated by both mu-opioid receptors and partially non-mu and non-delta opioid receptors (the so-called putative epsilon-opioid receptor) (Goodman et al., 1983; Houghten et al., 1984; Mizoguchi et al., 2002). Nock et al., (1990, 1993) and Nock (1995) found that buprenorphine also shows a higher affinity for this putative epsilon-opioid receptor in their reports. Mizoguchi et al. (2002) measured the occupation of opioid receptors by respective receptor agonists using a guanosine-5'-O-(3-[^{35}S]thio)triphosphate([^{35}S]GTPQS)-binding assay and reported an increase in [^{35}S]GTPQS binding induced by the selective mu-opioid receptor agonist. Furthermore, Mizoguchi et al. (2002) observed that buprenorphine acts as an antagonist for putative epsilon-opioid receptors in mu-opioid receptor knockout mice. Based on these previous studies and our findings, it is feasible to suggest that the binding of beta-endorphin to mu-opioid receptors could be increased in the presence of BUP as an agonist. For this reason, the plasma beta-endorphin levels of OUD patients receiving sublingual BUP may be decreased compared to that of controls.

Beta-endorphin levels were found to be significantly correlated with plasma BDNF levels in controls, but not in OUD group. Both endogenous opioids and BDNF are involved in neurocircuits regulating emotions. Zhang et al. (2006) reported that centrally administered beta-endorphin increased BDNF mRNA expression in various brain regions of rats in a dose-dependent manner, which explained the positive correlation between beta-endorphin and BDNF levels in the present study. This correlation could not be seen in OUD group receiving BUP most probably due to the different effects of BUP on plasma beta-endorphin and BDNF levels, as mentioned above. Nevertheless, additional studies are warranted to confirm our suggestions.

Since BDNF contributes the neuroplastic modifications in reward circuits, its dysfunction may relate to behavioral changes seen in drug use disorders. Previous genetic studies have demonstrated that single nucleotide polymorphisms (SNPs) on *BDNF* gene could cause this dysfunction by different mechanisms (Greenwald et al., 2013; Roviš et al., 2018). Hence, the effect of *BDNF* Val66Met polymorphism, the most frequently studied SNP in the promoter of the *BDNF* gene, on plasma BDNF level as well as various parameters related to drug abuse was investigated in the present study. This polymorphism results in a

substitution of valine (Val) to methionine (Met) at codon 66 in the prodomain of BDNF. Although it does not affect mature protein function, this *BDNF* polymorphism seemed to have a functional significance by altering intracellular trafficking and packaging of pro-BDNF and activity dependent secretion of BDNF. Studies that have examined the effect of *BDNF* Val66Met polymorphism on substance use disorder as well as plasma BDNF level have shown contradicting results. Chen et al. (2015), Yoshimura et al. (2011) and Zhou et al. (2011) found no significant differences in BDNF levels between *BDNF* Val66Met genotypes. Rovis et al. (2018) showed that slightly higher serum BDNF levels in homozygotes Val/Val carriers compared to those with Met allele, whereas Bus et al. (2012), Lang et al. (2009) and Minelli et al. (2011) found higher BDNF levels in Met allele carriers. In the present study, individuals with at least one Met allele had higher plasma BDNF levels than those with *BDNF* Val/Val genotype in both OUD and control groups, which indicated that the *BDNF* Val66Met polymorphism could affect plasma BDNF levels. However, there was not a statistical difference between *BDNF* Val66Met genotypes in view of plasma BDNF levels ($p > 0.05$). According to these conflicting results, more studies seemed to be needed in order to determine the effect of *BDNF* Val66Met polymorphism on plasma BDNF levels.

Similar to *BDNF* Val66Met polymorphism, *OPRM1* A118G SNP (rs1799971, Asn40Asp) is a functional polymorphism; thus, we investigated the relationship between variations within the human *OPRM1*, the main site of action for many clinically important opioids, and the plasma beta-endorphin level, plasma BUP/D concentration as well as some dependence-related traits at the genetic level. To our knowledge, no other clinical studies have analyzed the mu-opioid receptor A118G polymorphism in combination with its agonists, beta-endorphin and buprenorphine at the same time. *OPRM1* A118G polymorphism arises from an A to G substitution at nucleotide 118 and changes the asparagine to aspartate amino acid at position 40 in the N-terminal domain of mu-opioid receptor. This amino acid exchange results in removing a putative glycosylation site (Singh et al., 1997). Although the main effects of this polymorphism have not been fully understood, data from human and animal studies have suggested that the minor G allele is associated with decreased mRNA and protein levels (Zhang et al., 2005), less morphine-induced analgesia (Janicki et al., 2006), lower cell-surface MOR agonist binding capacity (Kroslak et al., 2007) and diminished MOR receptor availability (Ray et al., 2011). Ahmed et al. (2018) suggested that *OPRM1* A118G polymorphism may repress *OPRM1* transcription and number of available receptors to interact with drugs by chromatin condensation and allele-specific transcription factor binding using in *silico* analysis, but not altered binding affinity to beta-endorphin for both wild-type and mutated mu-opioid receptors. Consistently, Beyer et al. (2004) and Befort et al. (2001) reported similar beta-endorphin binding affinities between both receptors in HEK293 and COS-7 cells, respectively. On the other hand, Bond et al. (1998) observed a three-fold increase in beta-endorphin affinity for the A118G variant receptors in AV-12 cells and *Xenopus* oocytes. There have also been discrepancies between studies examining the effect of *OPRM1* A118G SNP on different mu-opioid receptor agonist and antagonist opioids. Lötsch et al. (2002) described a decreased potency of morphine-6-glucuronide at the A118G receptor in humans, whereas Bond et al. (1998) reported an increased potency of beta-endorphin. Therefore, this SNP in *OPRM1* gene was suggested to be associated with heterogeneous response various ligands including heroin, cocaine, morphine-6-glucuronide and beta-endorphin (Ahmed et al., 2018). Furthermore, Knapman et al. (2014) suggested that the A118G variant may affect mu-opioid receptor signaling in a ligand-dependent manner. In consistent with this suggestion, in the present study, the effect of this polymorphism on plasma beta-endorphin level and dose-normalized BUP concentration was found to be different. While there was a statistically significant difference between *OPRM1* A118G genotypes, OUD patients with AG+GG genotypes had higher plasma beta-endorphin level, but lower dose-normalized BUP concentration even though both of them are mu-opioid receptor agonists. Our results supported the Knapman's suggestion. However, more detailed studies are needed to confirm it.

For the effect of *OPRM1* A118G polymorphism on beta-endorphin levels, it was found that individuals with AA had lower plasma beta-endorphin levels that those with AG+GG genotype in both OUD (33.47 vs. 41.65 pg/ml) and control (94.5 vs. 100.7 pg/ml) groups. Although these differences between genotypes in controls and OUD patients were not statistically significant, there was a tendency towards higher beta-endorphin levels among G-carriers. It could be speculated that this finding is consistent with previous positron emission tomography (PET) studies showing G118 allele is associated with lower mu-opioid receptor binding potential at the basal scan across multiple brain regions including nucleus accumbens and amygdale (Ray et al., 2011; Weerts et al., 2013). In individuals having at least one G allele, the plasma beta-endorphin levels could be increased due to the lower binding of beta-endorphin to mu-opioid receptors. Lower MOR availability seemed to increase plasma beta-endorphin concentration. As for BUP/D concentration, the same increase could not be detected in the plasma samples of OUD patients having G allele. On the contrary, plasma BUP/D levels were lower in patients having AG+GG genotype than those having AA genotype (0.039 vs 0.042 ng/ml per mg/day). This result may indicate that *OPRM1* A118G polymorphism may have an effect on the plasma BUP concentration and that the efficacy of BUP was lower in G-allele carriers due to the lower BUP/D concentration. Consistent with our finding, Knapman et al. (2014) observed over 50% reduction in BUP efficacy at N40D variant for AC inhibition and ERK1/2 phosphorylation. Increased the plasma levels of BUP with larger daily BUP doses resulted in higher clinical efficacy due to the higher agonistic substitution of MORs (Santiago et al., 2020). Clinical studies have also demonstrated the relationship between MOR saturation with higher BUP plasma levels and the suppression of withdrawal symptoms (Greenwald et al., 2013). Contrary to the previous studies, patients having AG+GG genotype had also higher intensity of craving and withdrawal symptoms, and they consumed larger daily BUP doses. However, it seemed that their mu-opioid receptors could not be occupied enough most probably due to the alteration of receptor sensitivity. Based on existing studies and our findings, we suggested that OUD patients with G allele may have greater vulnerability for poorer treatment responses to BUP treatment.

Substance use disorder is generally initiated in early adulthood before the age of 20 and results in a variety of long-term negative outcomes such as academic and employment problems, reduced engagement in social and relational impairments and delinquent behavior (Poudel and Gautam, 2017). Moreover, earlier studies implicated that individuals with early onset experience more and longer episodes of relapse (Hingson et al., 2006). Therefore, an understanding of the genetic basis of the age onset of first heroin use should be crucial for prevention and treatment researches. In the present study, the effects of *OPRM1* A118G and *BDNF* Val66Met polymorphisms on early age onset of first heroin use were demonstrated. OUD patient receiving BUP treatment with AA and AG genotypes (21.0 years) had significantly later age of heroin first use compared to those with the GG genotype (15.5 years), which was consistent with Woodcock et al. (2015).

A limitation of our study is that a group of OUD patients not receiving BUP treatment was not included. Thus, although the negative correlation between BUP concentration and BDNF plasma levels in OUD patients was found, we could not suggest that BUP has a causal effect on plasma BDNF levels. In order to determine whether this effect are due to BUP, a history of heroin use, or the combination of them, a study with a group of OUD patients not receiving BUP treatment or a within-subject design investigating levels before and after starting BUP treatment is needed to be conducted in the future. Despite this limitation, our study brings to attention an effect of BUP treatment on BDNF and beta-endorphin levels. In the near future, we hope that our findings will contribute to improve outcomes in maintaining the treatment of heroin

addiction.

In conclusion, the present study demonstrated that sublingual BUP/naloxone increased plasma BDNF levels, but decreased beta-endorphin levels in individuals with OUD for the first time. A negative and significant correlation between the plasma BUP/D and BDNF levels suggested that plasma BDNF level could be decreased in a BUP/D concentration-dependent manner in OUD group.

Funding

This study was supported by the Ankara University Scientific Research Projects Coordination Unit (Grant number: 20B0217001 awarded to Dilek Kaya-Akyüzlü).

CRediT authorship contribution statement

Dilek Kaya-Akyüzlü: Conceptualization, Methodology, Formal analysis, Investigation, Writing – review & editing. **Selin Ozkan-Kotiloglu:** Conceptualization, Methodology, Investigation; Writing – review & editing. **Ceylan Bal:** Methodology. **Gamze Avcıoglu:** Methodology. **Safak Yalçın-Sahiner:** Resources. **İsmail Volkan Sahiner:** Resources.

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.

Data Availability

Data will be made available on request.

Acknowledgements

The authors would also like to thank the staff of Blood Donation Center of Ankara University and Rabia Yurdakul and Gizem Düzgün for collecting venous blood samples and obtaining SCS and COWS from the controls, respectively.

Author contributions

DKA designed and directed the study. DKA and SOK conducted the genetic analysis and prepared the manuscript. CB performed measurement of plasma buprenorphine and norbuprenorphine levels with LC-MS/MS and plasma beta-endorphin and BDNF levels with ELISA kits. GA contributed to laboratory analysis under the supervision of CB. SYS and İVS collected venous blood samples and demographic data of all subjects.

References

Ahmadi, J., Jahromi, M.S., Ghahremani, D., London, E.D., 2018. Single high-dose buprenorphine for opioid craving during withdrawal. Trials 19, 675. https://doi.org/10.1186/s13063-018-3055-z.

Ahmed, M., Haq, I.U., Faisal, M., Waseem, D., Taqi, M.M., 2018. Implication of *OPRM1* A118G polymorphism in opioids addicts in Pakistan: in vitro and in silico analysis. J. Mol. Neurosci. 65 (4), 472–479. https://doi.org/10.1007/s12031-018-1123-1.

Anders, Q.S., Ferreira, L.V.B., Rodrigues, L.C.M., Nakamura-Palacios, E.M., 2020. BDNF mRNA expression in leukocytes and frontal cortex function in drug use disorder. Front. Psychiatry 11, 469. https://doi.org/10.3389/fpsyt.2020.00469.

Bawor, M., Dennis, B.B., Tan, C., Pare, G., Varenbut, M., Daiter, J., Plater, C., Worster, A., Marsh, D.C., Steiner, M., Anglin, R., Desai, D., Thabane, L., Samaan, Z., 2015. Contribution of BDNF and DRD2 genetic polymorphisms to continued opioid use in patients receiving methadone treatment for opioid use disorder: an observational study. Addict. Sci. Clin. Pr. 10, 19. https://doi.org/10.1186/s13722-015-0040-7.

Befort, K., Filliol, D., Decaillot, F.M., Gavériaux-Ruff, C., Hoehe, M.R., Kieffer, B.L., 2001. A single nucleotide polymorphic mutation in the human mu-opioid receptor severely impairs receptor signaling. J. Biol. Chem. 0, 3130–3137. https://doi.org/10.1074/jbc.M006352200.

Beyer, A., Koch, T., Schröder, H., Schulz, S., Höllt, V., 2004. Effect of the A118G polymorphism on binding affinity, potency and agonist-mediated endocytosis, desensitization, and resensitization of the human mu-opioid receptor. J. Neurochem 89, 553–560. https://doi.org/10.1111/j.1471-4159.2004.02340.x.

Bond, C., LaForge, K.S., Tian, M., Melia, D., Zhang, S., Borg, L., Gong, J., Schluger, J., Strong, J.A., Leal, S.M., Tischfield, J.A., Kreek, M.J., Yu, L., 1998. Single-nucleotide polymorphism in the human mu opioid receptor gene alters beta-endorphin binding and activity: possible implications for opiate addiction. Proc. Natl. Acad. Sci. USA 95, 9608–9613. https://doi.org/10.1073/pnas.95.16.9608.

Buresh, M., Ratner, J., Zgierska, A., Gordin, V., Alvanzo, A., 2020. Treating perioperative and acute pain in patients on buprenorphine: narrative literature review and practice recommendations. J. Gen. Intern Med. 35, 3635–3643. https://doi.org/10.1007/s11606-020-06115-3.

Bus, B.A.A., Arias-Vasquez, A., Franke, B., Prickaerts, J., de Graaf, J., Voshaar, R.C.O., 2012. Increase in serum brain-derived neurotrophic factor in met allele carriers of the BDNF Val66Met polymorphism is specific to males. Neuropsychobiology 65, 183–187. https://doi.org/10.1159/000336997.

Canan, F., Kuloglu, M., Guven, M., Gecici, O., 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.

Chawarski, M.C., Schottenfeld, R.S., O'Connor, P.G., Pakes, J., 1999. Plasma concentrations of buprenorphine 24 to 72 h after dosing. Drug Alcohol. Depend. 55, 157–163. https://doi.org/10.1016/s0376-8716(98)00192-6.

Chen, S.L., Lee, S.Y., Chang, Y.H., Wang, T.Z., Chen, S.H., Chu, C.H., Chen, P.S., Yang, Y.K., Hong, J.S., Lu, R.B., 2015. The BDNF Val66Met polymorphism and plasma brain-derived neurotrophic factor levels in Han Chinese heroin-dependent patients. Sci. Rep. 5, 8148. https://doi.org/10.1038/srep08148.

Cheng, X.R., Xia, P.G., Shi, Z.Y., Xu, Q.Y., Luo, C.H., Lei, M.Y., Zhang, Q., 2019. Increased risk of intracranial hemorrhage in preterm infants with OPRM1 gene A118G polymorphism. Ann. Transl. Med. 7, 478. https://doi.org/10.21037/atm.2019.08.53.

Coe, M.A., Lofwall, M.R., Walsh, S.L., 2019. Buprenorphine pharmacology review: update on transmucosal and long-acting formulations. J. Addict. Med. 13, 93–103. https://doi.org/10.1097/ADM.0000000000000457.

Crist, R.C., Li, J., Doyle, G.A., Gilbert, A., Dechairo, B.M., Berrettini, W.H., 2018. Pharmacogenetic analysis of opioid dependence treatment dose and dropout rate. Am. J. Drug Alcohol Abus. 44, 431–440. https://doi.org/10.1080/00952990.2017.1420795.

Ettienne, E.B., Ofoegbu, A., Maneno, M.K., Briggs, J., Ezeude, G., Williams, S., Walker, C., Chapman, E., 2019. Pharmacogenomics and opioid use disorder: clinical decision support in an African American Cohort. J. Natl. Med. Assoc. 111, 674–681. https://doi.org/10.1016/j.jnma.2019.09.006.

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. Psychiatry 22, 70–85.

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).

Goodman, R.R., Houghten, R.A., Pasternak, G.W., 1983. Autoradiography of [3H]L-endorphin binding in brain. Brain Res. 288, 334–337. https://doi.org/10.1016/0006-8993(83)90113-0.

Graham, D.L., Edwards, S., Bachtell, R.K., DiLeone, R.J., Rios, M., Self, D.W., 2007. Dynamic BDNF activity in nucleus accumbens with cocaine use increases self-administration and relapse. Nat. Neurosci. 10, 1029–1037. https://doi.org/10.1038/nn1929.

Greenwald, M.K., Steinmiller, C.L., Sliwerska, E., Lundahl, L., Burmeister, M., 2013. BDNF Val(66)Met genotype is associated with drug-seeking phenotypes in heroin-dependent individuals: a pilot study. Addict. Biol. 18, 836–845. https://doi.org/10.1111/j.1369-1600.2011.00431.x.

Han, B., Zhang, X.Y., Wang, D.Y., Ren, W.W., Gu, Y.Y., Zhu, L., Chang, Y.L., Wang, L.P., Wu, C.W., Jin, Q.Q., Chen, C., Lyu, D.Z., Zhao, J.Y., Shao, B., He, J.C, B., 2015. Serum brain-derived neurotrophic factor levels and psychotic symptoms in heroin dependence. Compr. Psychiatry 62, 80–85. https://doi.org/10.1016/j.comppsych.2015.06.008.

Heberlein, A., Dürsteler-MacFarland, K.M., Lenz, B., Frieling, H., Grösch, M., Bönsch, D., Kornhuber, J., Wiesbeck, G.A., Bleich, S., Hillemacher, T., 2011. Serum levels of BDNF are associated with craving in opiate-dependent patients. J. Psychopharmacol. 25, 1480–4148. https://doi.org/10.1177/0269881111411332.

Hilburn, C., Nejtek, V.A., Underwood, W.A., Singh, M., Patel, G., Gangwani, P., Forster, M.J., 2011. Is serum brain-derived neurotrophic factor related to craving for or use of alcohol, cocaine, or methamphetamine? Neuropsychiatr. Dis. Treat. 7, 357–364. https://doi.org/10.2147/NDT.S18953.

Hingson, R.W., Heeren, T., Winter, M.R., 2006. Age of alcohol-dependence onset: associations with severity of dependence and seeking treatment. Pediatrics 118 (3), e755–e763. https://doi.org/10.1542/peds.2006-0223.

Hjelmström, P., Nordbeck, E.B., Tiberg, F., 2020. Optimal dose of buprenorphine in opioid use disorder treatment: a review of pharmacodynamic and efficacy data. Drug Dev. Ind. Pharm. 46, 1–7. https://doi.org/10.1080/03639045.2019.1706552.

Houghten, R.A., Johnson, N., Pasternak, G.W., 1984. [3H]-L-Endorphin binding in rat brain. J. Neurosci. 4, 2460–2465. https://doi.org/10.1523/JNEUROSCI.04-10-02460.1984.

Hyman, C., Hofer, M., Barde, Y.A., Melissa Juhasz, M., Yancopoulos, G.D., Stephen, P., Squinto, S.P., Lindsay, R.M., 1991. BDNF is a neurotrophic factor for dopaminergic neurons of the substantia nigra. Nature 350, 230–232. https://doi.org/10.1038/350230a0.

Janicki, P.K., Schuler, G., Francis, D., Bohr, A., Gordin, V., Jarzembowski, T., Ruiz-Velasco, V., Mets, B., 2006. A genetic association study of the functional A118G polymorphism of the human Mu-opioid receptor gene in patients with acute and chronic pain. Anesth. Analg. 103, 1011–1017. https://doi.org/10.1213/01.ane.0000231634.20341.88.

Johnson, R.E., Jaffe, J.H., Fudala, P.J., 1992. A controlled trial of buprenorphine treatment for opioid dependence. JAMA 267, 2750–2755.

Kim, D.J., Roh, S., Kim, Y., Yoon, S.J., Lee, H.K., Han, C.S., Kim, Y.K., 2005. High concentrations of plasma brain-derived neurotrophic factor in methamphetamine users. Neurosci. Lett. 388, 112–115. https://doi.org/10.1016/j.neulet.2005.06.042.

Knapman, A., Santiago, M., Connor, M., 2014. Buprenorphine signalling is compromised at the N40D polymorphism of the human μ opioid receptor in vitro. Br. J. Pharmacol. 171, 4273–4288. https://doi.org/10.1111/bph.12785.

Kosten, T.R., Morgan, C., Kreek, M.J., 1992. Beta endorphin levels during heroin, methadone, buprenorphine, and naloxone challenges: preliminary findings. Biol. Psychiatry 32, 523–528. https://doi.org/10.1016/0006-3223(92)90220-t.

Kranzler, H.R., Lynch, K.G., Crist, R.C., Hartwell, E., Le Moigne, A., Laffont, C.M., Andorn, A.C., 2021. A delta-opioid receptor gene polymorphism moderates the therapeutic response to extended-release buprenorphine in opioid use disorder. Int. J. Neuropsychopharmacol. 24, 89–96. https://doi.org/10.1093/ijnp/pyaa069.

Kroslak, T., Laforge, K.S., Gianotti, R.J., Ho, A., Nielsen, D.A., Kreek, M.J., 2007. The single nucleotide polymorphism A118G alters functional properties of the human mu opioid receptor. J. Neurochem 103, 77–87. https://doi.org/10.1111/j.1471-4159.2007.04738.x.

Kumar, R., Viswanath, O., Saadabadi, A. 2021. Buprenorphine. [Updated 2021 Aug 6]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2021 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK459126/.

Laib, A.K., Böttcher, M., Hiemke, C., Havemann-Reinecke, U., 2013. Therapeutic drug-monitoring for opiate-dependent patients receiving buprenorphine for substitution. Pharmacopsychiatry 46, A32.

Lang, U.E., Hellweg, R., Sander, T., Gallinat, J., 2009. The Met allele of the BDNF Val66Met polymorphism is associated with increased BDNF serum concentrations. Mol. Psychiatry 14, 120–122. https://doi.org/10.1038/mp.2008.80.

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.

Lötsch, J., Zimmermann, M., Darimont, J., Marx, C., Dudziak, R., Skarke, C., Geisslinger, G., 2002. Does the A118G polymorphism at the mu-opioid receptor gene protect against morphine-6-glucuronide toxicity? Anesthesiology 97, 814–819. https://doi.org/10.1097/00000542-200210000-00011.

Lu, L., Dempsey, J., Liu, S.Y., Bossert, J.M., Shaham, Y., 2004. A single infusion of brain-derived neurotrophic factor into the ventral tegmental area induces long-lasting potentiation of cocaine seeking after withdrawal. J. Neurosci. 24, 1604–1611. https://doi.org/10.1523/JNEUROSCI.5124-03.2004.

Madison, C.A., Eitan, S, Shoshana, 2020. Buprenorphine: prospective novel therapy for depression and PTSD. Psychol. Med. 50, 881–893. https://doi.org/10.1017/S0033291720000525.

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.

Minelli, A., Zanardini, R., Bonvicini, C., Sartori, R., Pedrini, L., Gennarelli, M., Bocchio-Chiavetto, L., 2011. BDNF serum levels, but not BDNF Val66Met genotype, are correlated with personality traits in healthy subjects. Eur. Arch. Psychiatry Clin. Neurosci. 261, 323–329. https://doi.org/10.1007/s00406-011-0189-3.

Mizoguchi, H., Wu, H.-E., Narita, M., Hall, F.S., Sora, I., Uhl, G.R., Nagase, H., Tseng, L. F., 2002. Antagonistic property of buprenorphine for putative epsilon-opioid receptor-mediated G-protein activation by beta-endorphin in pons/medulla of the mu-opioid receptor knockout mouse. Neuroscience 115, 715–721. https://doi.org/10.1016/s0306-4522(02)00486-4.

Negus, S.S., Bidlack, J.M., Mello, N.K., Furness, M.S., Rice, K.C., Brandt, M.R., 2002. Delta opioid antagonist efects of buprenorphine in rhesus monkeys. Behav. Pharmacol. 13, 557–570. https://doi.org/10.1097/00008877-200211000-00005.

Nock, B., Giordano, A.L., Cicero, T.J., O'Connor, L.H., 1990. Affinity of drugs and peptides for U-69, 593-sensitive and -insensitive kappa opiate binding sites: the U-69, 593-insensitive site appears to be the beta-endorphin-specific epsilon receptor. J. Pharmacol. Exp. Ther. 254, 412–419.

Nock, B., Giordano, A.L., Moore, B.W., Cicero, T.J., 1993. Properties of the putative epsilon opioid receptor: identication in rat, guinea pig, cow, pig and chicken brain. J. Pharmacol. Exp. . R. 264, 349–359.

Nock, B.U., 1995. Kappa and epsilon receptor binding. In: Tseng, L.F. (Ed.), The Pharmacology of Opioid Peptides. Harwood Academic Publishers, Singapore, pp. 29–56.

Pilozzi, A., Carro, C., Huang, X., 2020. Roles of β-endorphin in stress, behavior, neuroinflammation, and brain energy metabolism. Int. J. Mol. Sci. 22, 338. https://doi.org/10.3390/ijms22010338.

Popova, N.K., Ilchibaeva, T.V., Naumenko, V.S., 2017. Neurotrophic Factors (BDNF and GDNF) and the serotonergic system of the brain. Biochem. (Mosc.) 82, 308–317. https://doi.org/10.1134/S0006297917030099.

Poudel, A., Gautam, S., 2017. Age of onset of substance use and psychosocial problems among individuals with substance use disorders. BMC Psychiatry 17, 10. https://doi.org/10.1186/s12888-016-1191-0.

Randesi, M., Rotrosen, J., Nunes, E.V., Lee, J.D., Novo, P., Levran, O., Ott, J., Pavlicova, M., Scodes, J., Kreek, M.J., 2020. Variants of opioid genes and response to treatment of opioid use disorder with buprenorphine-naloxone versus extended-release naltrexone in Caucasians. Am. J. Drug Alcohol Abus. 46, 761–768. https://doi.org/10.1080/00952990.2020.1797064.

Ray, R., Ruparel, K., Newberg, A., Wileyto, E.P., Loughead, J.W., Divgi, C., Blendy, J.A., Logan, J., Zubieta, J.K., Lerman, C., 2011. Human Mu opioid receptor (OPRM1 A118G) polymorphism is associated with brain mu-opioid receptor binding potential in smokers. Proc. Natl. Acad. Sci. USA 108, 9268–9273. https://doi.org/10.1073/pnas.1018699108.

Roviš, D., Bizjak, M.C., Marchesi, V.V., Petelin, A., Jenuš, T., Vidic, S., Drevenšek, G., Pražnikar, Z.J., 2018. Increased risk-taking behaviour and brain-derived neurotrophic factor Val66Met polymorphism correlates to decreased serum brain-derived neurotrophic factor level in heroin users. Eur. Addict. Res. 24, 189–200. https://doi.org/10.1159/000492582.

Santiago, D., Mangas-Sanjuan, V., Melin, K., Duconge, J., Zhao, W., Raman Venkataramanan, R., 2020. Pharmacokinetic characterization and external evaluation of a quantitative framework of sublingual buprenorphine in patients with an opioid disorder in puerto rico. Pharmaceutics 12, 1226. https://doi.org/10.3390/pharmaceutics12121226.

Singh, V.K., Bajpai, K., Biswas, S., Haq, W., Khan, M.Y., Mathur, K.B., 1997. Molecular biology of opioid receptors. Neuroimmunomodulation 4, 285–297. https://doi.org/10.1159/000097349.

Skibinska, M., Groszewska, A., Kapelski, P., Rajewska-Rager, A., Pawlak, J., Dmitrzak-Weglarz, M., Szczepankiewicz, A., Twarowska-Hauser, J., 2018. Val66Met functional polymorphism and serum protein level of brain-derived neurotrophic factor (BDNF) in acute episode of schizophrenia and depression. Pharmacol. Rep. 70, 55–59. https://doi.org/10.1016/j.pharep.2017.08.002.

Taqi, M.M., Faisal, M., Zaman, H., 2019. *OPRM1* A118G polymorphisms and its role in opioid addiction: implication on severity and treatment approaches. Pharmgenom. Pers. Med 12, 361–368. https://doi.org/10.2147/PGPM.S198654.

Tsai, M.C., Huang, T.L., 2017. Brain-derived neurotrophic factor (BDNF) and oxidative stress in heroin-dependent male patients undergoing methadone maintenance treatment. Psychiatry Res. 249, 46–50. https://doi.org/10.1016/j.psychres.2016.12.042.

Vargas-Perez, H., Kee, Ting-A., Walton, R., Hansen, C.H., Razavi, D.M., Clarke, R., Bufalino, L., Allison, M.R., Steffensen, D.W., van der Kooy, D, S.C., 2009. Ventral tegmental area BDNF induces an opiate-dependent-like reward state in naive rats. Science 324, 1732–1734. https://doi.org/10.1126/science.1168501.

Wang, S., 2019. Historical review: opiate addiction and opioid receptors. Cell Transpl. 28, 233–238. https://doi.org/10.1177/0963689718811060.

Weerts, E.M., McCaul, M.E., Kuwabara, H., Yang, X., Xu, X., Dannals, R.F., Frost, J.J., Wong, D.F., Wand, G.S., 2013. Influence of OPRM1 Asn40Asp variant (A118G) on [11C]carfentanil binding potential: preliminary findings in human subjects. Int. J. Neuropsychopharmacol. 16, 47–53. https://doi.org/10.1017/S146114571200017X.

Woodcock, E.A., Lundahl, L.H., Burmeister, M., Greenwald, M.K., 2015. Functional mu opioid receptor polymorphism (OPRM1 A(118) G) associated with heroin use outcomes in Caucasian males: a pilot study. Am. J. Addict. 24, 329–335. https://doi.org/10.1111/ajad.12187.

Xhakaza, S.P., Khoza, L.J., Haripershad, A.M., Ghazi, T., Dhani, S., Mutsimhu, C., Molopa, M.J., Madurai, N.P., Madurai, L., Singh, S.D., Gopal, N.D., Kruger, H.G., Govender, T., Chuturgoon, A., Naicker, T., Baijnath, S., 2021. Alterations in neurotransmitter levels and transcription factor expression following intranasal buprenorphine administration. Biomed. Pharmacother. 138, 111515 https://doi.org/10.1016/j.biopha.2021.111515.

Yoshimura, R., Kishi, T., Suzuki, A., Umene-Nakano, W., Ikenouchi-Sugita, A., Hori, H., Otani, K., Iwata, N., Nakamura, J., 2011. The brain-derived neurotrophic factor (BDNF) polymorphism Val66Met is associated with neither serum BDNF level nor response to selective serotonin reuptake inhibitors in depressed Japanese patients. Prog. Neuropsychopharmacol. Biol. Psychiatry 35, 1022–1025. https://doi.org/10.1016/j.pnpbp.2011.02.009.

Zhang, H., Torregrossa, M.M., Jutkiewicz, E.M., Shi, Y.G., Rice, K.C., Woods, J.H., Watson, S.J., Ko, M.C., 2006. Endogenous opioids upregulate brain-derived neurotrophic factor mRNA through delta- and micro-opioid receptors independent of antidepressant-like effects. Eur. J. Neurosci. 23, 984–994. https://doi.org/10.1111/j.1460-9568.2006.04621.x.

Zhang, Y., Wang, D., Johnson, A.D., Papp, A.C., Sadée, W., 2005. Allelic expression imbalance of human mu opioid receptor (OPRM1) caused by variant A118G. J. Biol. Chem. 280, 32618–32624. https://doi.org/10.1074/jbc.M504942200.

Zhou, Z., Lu, T., Xu, G., Yue, X., Zhu, W., Ma, M., Liu, W., Zhu, S., Liu, X., 2011. Decreased serum brain-derived neurotrophic factor (BDNF) is associated with post-stroke depression but not with BDNF gene Val66Met polymorphism. Clin. Chem. Lab. Med. 49, 185–189. https://doi.org/10.1515/CCLM.2011.039.

Zoorob, R., Kowalchuk, A., de Grubb, M.M., 2018. Buprenorphine therapy for opioid use disorder. Am. Fam. Physician 97, 313–320.