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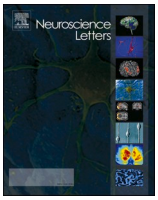

OPRM1 rs2075572 has potential to affect plasma buprenorphine level in opioid users, but not *OPRM1* rs562859☆

Dilek Kaya-Akyüzlü [a], Selin Özkan-Kotiloğlu [b,*], Mustafa Danışman [c], Ceylan Bal [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 Yıldırım Beyazıt University, Department of Medical Biochemistry, Ankara, Turkey*

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ABSTRACT

OPRM1 gene encoding mu-opioid receptor (MOR) is the primary candidate gene for buprenorphine (BUP) pharmacogenetics. *OPRM1* undergoes alternative splicing leading to multiple MOR subtypes. Thus, in the current study 2 SNPs (rs1799972 and rs562859) were selected due to evidence for their contribution to alternative splicing of *OPRM1*.

The effects of 2 SNPs of *OPRM1* gene on plasma buprenorphine and norbuprenorphine levels in a sample of 233 OUD patients receiving BUP/naloxone were examined. Polymorphisms were analyzed by PCR and RFLP. BUP and norbuprenorphine concentrations in plasma were measured by LC–MS/MS.

OPRM1 rs2075572 GC + CC (0.12 ng/ml) had significantly higher plasma BUP level compared to GG (0.084 ng/ml) ($p = 0.043$). Although there was not a statistically significant difference between *OPRM1* rs562859 genotypes ($p = 0.46$), patients with *OPRM1* rs562859 CT + TT had higher plasma BUP and BUP-related values as compared to those with CC.

In conclusion, the effect of *OPRM1* rs2075572 on BUP levels in opioid users' plasma was shown in a Caucasian population for the first time. On the other hand, OPRM1 rs562859 seems not to influence the BUP pharmacology.

1. Introduction

The consequences of the opioid epidemic that has affected human beings since nearly the 1950 s are devastating since more than fifty thousand people die due to overdose annually and there is a huge number of patients (approximately 7 million in 2014) with opioid use disorder (OUD) needed to receive medical care [1]. As OUD is a relapsing disorder, long-term treatment is needed and the best long-term medication-assisted treatment is provided by opioid substitution medications such as methadone and buprenorphine/naloxone combination currently [2]. The aim of the replacement treatment of OUD is to enable individuals to replace illicit opioid drugs with clinically controlled opioids [3,4]. Both methadone (full agonist) and buprenorphine (partial agonist) exert their effects by decreasing craving and drug seeking and reducing the intensity of depression and anxiety [2]. Although the efficacy of these pharmacotherapies is high, a significant percentage of individuals with OUD still sustain illicit opioid use since they could not complete the maintenance treatment [5,6]. For example, most patients with OUD can be treated with 16 mg/day buprenorphine sublingually daily (which is the FDA target dose), whereas others could require higher dosing (24–32 mg) [7], which shows that the bioavailability of medication-assisted treatment is highly intervariable [2]. This inter-individual variability in human responses to opioids could be attributed partially to gene variations in genes encoding receptors, transporters, or enzymes [8]. Pharmacogenomics could explain this individual variability [9], and thus, treatment outcomes can be improved, and various side-effects can be reduced by optimizing pharmacotherapies according to individuals' genetic make-up in the future [8,10]. Genetic testing can assist clinicians to drive medication selection decisions and to adjust the dose of medications, thus therapeutic efficacy can be maximized [9,11,12]. There are more than 300 drugs including some opioids such as codeine and tramadol that have

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* Corresponding author.
E-mail addresses: selin.ozkan@ahievran.edu.tr, selin.ozkan11@alumni.imperial.ac.uk (S. Özkan-Kotiloğlu).

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pharmacogenomics information in their FDA-approved drug labeling [13], whereas the labeling for buprenorphine does not still include pharmacogenomics information since most of the pharmacogenetic studies of buprenorphine have not been replicated and have a modest sample size [12]. Thus, as suggested by Meaden *et al.* [12] and Wiseman *et al.* [14], evidence in the field of buprenorphine pharmacogenetics are urgently needed for the clinical applicability of pharmacogenomics information.

Genes encoding opioid receptors found in neurons of the reward pathways are good candidates to demonstrate the effect of genetic variants on treatment responses since opioid drugs including buprenorphine mediates its analgesic or euphoric effects by means of opioid receptors including mu-opioid receptor (MOR), delta- (DOR) and kappa-opioid receptor (KOR) [8]. Amongst, it is considered that MOR is the main action site of buprenorphine. Norbuprenorphine, one of the metabolites of Phase I metabolism of buprenorphine, is a MOR and DOR agonist and KOR antagonist partially [15]. *OPRM1* gene encodes MOR, and the variations on this gene could have the potential to change the receptor function. The most studied variation in *OPRM1* gene is rs1799971 (Asn40Asp/A118G) in the coding region of exon 1 causing an alteration in the N-terminal of MOR [16]. Hitherto, it has been shown that the G allele has an effect on the mRNA levels, morphine-induced analgesia, binding capacity of MOR for agonists and receptor availability [17–20]. However, there is a growing interest in intronic sequence involving in alternative splicing of the single gene resulting in different isoforms of *OPRM1* gene [21,22], which results in different mu receptor subtypes with distinct binding affinity for the opioids [23]. To date, several human *OPRM1* splice variants (MOR-1*A*-*1F* and MOR-1*Y*, MOR-1*O* and MO-1X) have been identified [22–26]. Many splice variants contained alternative fourth exon such as exon 0 or exon X in their carboxyl terminus in addition to exons 1–3 found in the original *OPRM1* [22]. *OPRM1* rs562859 is in the alternative exon X [21]. The exact functions of rs2075572 are not still well understood, but existing evidence supports the role of *OPRM1* rs2075572 in affinity of transcriptional factors to the intronic sequences [27]. Furthermore, their associations with tramadol response have been reported in the ClinVar (RCV001029119.2 and RCV001029075.2). Based on this background, we explored the effect of two intronic *OPRM1* SNPs (rs2075572 and rs562859) on buprenorphine pharmacology in individuals with OUD in a Caucasian ancestry due to both their potential influence on gene expression and their population frequencies (>10 %) for the first time.

2. Patients and methods

Patients with OUD according to the DSM–5 criteria (n = 223), who had administered to AMATEM Clinic in Ankara as outpatients for the opioid treatment with sublingual BUP/naloxone combination (trade name: Suboxone). Subjects (i) who had been receiving at least 10 days of OUD treatment with BUP/naloxone combination for the steady-state plasma BUP concentrations, (ii) who were over 18 years of age, and (iii) without any acute health issues were included in the study by clinicians. Patients (i) with active drug addiction throughout treatment, which was confirmed by routine urine analysis in the AMATEM laboratory, (ii) with any psychotic disorders, mood disorders, or severe depression, and (iii) who had been receiving any other drugs inducing or inhibiting the metabolism of BUP were excluded in the current study.

The institutional ethics committee approved the study design (Approval No: I6-385–21 in 2021). Each subject signed the written informed consent. Samplings complied with the principles of "The Declaration of Helsinki". Sociodemographic information including age, education, martial and employment status, and doses and times of BUP therapy was gathered by the help of a small questionnaire completed by all participants.

Blood sample (4 ml) was taken into tubes with ethylenediaminetetraacetic acid (EDTA) from each individual with OUD for the determination of the *OPRM1* rs2075572 and rs562859 polymorphisms. Simultaneously, in another tube with EDTA, 4 ml whole blood sample was taken to measure the concentrations of plasma buprenorphine (BUP) and norbuprenorphine (norBUP). All blood samples were collected before taking the daily BUP dose. To detect the steady-state BUP level, continuous sublingual BUP (suboxone) for at least 10 days was waited. Blood samples were kept at −20 °C till genetic and analytical analysis. BUP and norBUP levels in human plasma were measured by LC–MS/MS (Liquid Chromatography Tandem Mass Spectrometry), as described by Kaya-Akyüzlü *et al.* [28].

DNA was isolated via Thermo GeneJET Genomic DNA Purification Kit (USA), as described in the handbook of the kit and isolated DNA was kept at −20 °C until genotyping. Genotypes of *OPRM1* rs2075572 and rs562859 SNPs were determined via PCR-RFLP method, as previously optimized by Ding *et al.* [29] and Smith *et al.* [30], respectively. PCR mixture contained genomic DNA (50 ng), primers (forward and reverse) (10 pmol), Taq DNA polymerase (1,25U; Ampliqon, Denmark), 10 × PCR buffer (Ampliqon, Denmark), dNTPs (0.16 mM). The cycling protocol was (i) 94 °C, 5 min, (ii) 35 cycles of 94 °C, 60 °C, and 72 °C (1 min each), (iii) 72 °C, 10 min. After PCR amplification, 241-bp PCR product for rs2075572 and a 362-bp fragment PCR product for rs562859 was digested with 5 U of restriction enzymes (*HinfI* and *DdeI*, respectively) in 1 h at 37 °C. The PCR amplicons and the digested PCR products were also identified in agarose gel (3 %) with EtBr.

The SPSS software (version 25.0) was utilized to perform analyses statistically. Genotype and allele frequencies of the *OPRM1* rs2075572 and rs562859 were calculated by direct count. The chi-square test was used to assess the departure from the Hardy-Weinberg equilibrium ($p2 + 2pq + q2 = 1$). Plasma BUP and norBUP levels were given as ng/ml. BUP and norBUP values were adjusted with BUP daily dose and individuals' weight, as previously described by Kaya-Akyüzlü *et al.* [28]. Dose-normalized BUP and norBUP values were given as ng/mL per mg/day and dose/kg-normalized BUP and norBUP values were given as ng/mL per mg/kg/day. The Kolmogorov-Smirnov test showed whether numerical variables were in normal distribution. All non-normal distributed numerical data were shown as median (interquartile range). For categorical data, numbers with percentages and 95 % confidence intervals were given. Genotypes were compared according to three different genetic models (co-dominant, dominant, and recessive). Mann Whitney test or Kruskal-Wallis test, as appropriate, was used to compare the genotypes in terms of BUP and norBUP levels in plasma, values of BUP/D (dose-normalized BUP concentration), BUP/D.kg^{-1} (dose/kg-normalized BUP concentration), norBUP/D (dose-normalized norBUP concentration), norBUP/D.kg^{-1} (dose/kg-normalized norBUP concentration) and MR (metabolic ratio, norBUP/BUP). $p < 0.05$ was accepted as significant.

3. Results

A total of 233 OUD patients on BUP maintenance treatment was included in the current study, and of them, 18 were females (7.7 %) and 125 were males (92.3 %). The characteristics of patients were given on Table 1. Daily doses of buprenorphine/naloxone combination were in the range of 1.0–10.0 mg/day. The median daily dose of BUP was found as 6.0 mg/day (IQR:4.0–8.0 mg/day). The median steady-state plasma concentrations of BUP and norBUP were 0.106 ng/mL (IQR: 0.05–0.23 ng/mL) and 0.72 ng/mL (IQR: 0.37–1.59 ng/mL), respectively. After normalized by dose and dose/body weight, values of BUP/D and BUP/D.kg^{-1} were 0.022 ng/mL per mg/day and 0.136 ng/mL per mg/kg/day, respectively. Median values of norBUP/D and norBUP/D.kg^{-1} 1.45 ng/mL per mg/day and 9.65 ng/mL per mg/kg/day, respectively. The norBUP/BUP value (metabolite-to-parent drug ratio) was calculated as 7.57 (IQR: 1.62–24.67).

The allele and genotype distributions with frequencies and 95 % confidence interval was presented in Table 2. Hardy–Weinberg equilibrium (HWE) of the rs2075572 and rs562859 SNPs of *OPRM1* in 223 Caucasian individuals was also given in Table 2. The frequencies of

Table 1
The characteristics of the individuals with OUD.

Characteristics	**OUD (*n* = 233)**	
Age (years) Median (IQR)	28.00 (25.00–33.00)	
Weight (kg) Median (IQR)	70.0 (60.00–76.5)	
Buprenorphine dosing(mg/day) Median (IQR)	6.0 (4.0–8.0)	
First age of opioid use (years) Median (IQR)	21.6 (19.0–26.0)	
Education	***n***	**% Frequency (95 % *CI*)**
Primary	42	18.0 (13.01–22.9)
Secondary	107	45.9 (39.5–52.3)
High School	72	30.9(25.0–36.8)
Under-graduate	12	5.2 (2.35–8.05)
Occupation		
Not working	93	39.9 (33.6–46.2)
Working	140	60.1 (53.8–66.4)
Living		
Alone	11	4.7 (2.0–7.4)
With a partner	5	2.1 (0.3–3.9)
With husband/wife	58	24.9 (19.3–30.5)
With a family	159	68.3 (62.1–74.5)
Marital status		
Single	145	62.2 (55.9–68.4)
Married	68	7.7 (4.28–11.1)
Widow/divorced	12	5.2 (2.35–8.05)
Gender		
Female	18	7.7 (4.3–11.1)
Male	215	92.3 (88.9–95.7)

n: sample size, OUD: Opioid use disorder, IQR: Inter Quartile Range, CI: Confidence interval.

Table 2
Genotype and allele frequencies of rs2075572 and rs562859 polymorphisms in *OPRM1* in OUD patients.

Allele					
rs2075572	N (466)	% Frequency (95 % CI)	**rs562859**	N (466)	% Frequency (95 % CI)
G	201	43.1 (38.6–47.6)	C	151	32 (27.8–36.2)
C	265	56.9 (52.4–61.4)	T	315	68 (63.8–72.2)
Genotype					
rs2075572	N (233)	% Frequency (95 % CI)	**rs562859**	N (223)	% Frequency (95 % CI)
GG	50	21.5 (16.2–26.8)	CC	27	11.6 (7.4–15.8)
GC	101	43.3 (36.9–49.7)	CT	97	41.6 (35.1–48.1)
CC	82	35.2 (29.1–41.3)	TT	109	46.8 (40.3–53.3)
HWE p-value	$\chi2 = 3.16$ $p = 0.08$		HWE p-value	$\chi2 = 0.58$ $p = 0.44$	

HWE: Hardy-Weinberg Equilibrium, n: sample size, CI: Confidence Interval,

rs2075572 genotypes were 21.5 % for GG (homozygous wild type), 43.3 % for GC and 35.2 % for CC (homozygous variant). The frequency of the G allele was 43.1 % (n = 201) and of C allele was 56.9 % (n = 265). For *OPRM1* rs562859 SNP, the genotype frequencies were 11.6 % for homozygous wild type (CC; n = 27), 41.6 % for heterozygote (CT; n = 97) and 46.8 % for homozygous variant (TT; n = 109). The frequencies rs562859 alleles were 32.0 % for C and 68.0 % for T. For each polymorphism, the distribution for the genotypes were in HWE ($p > 0.05$). In addition, no statistically significant differences between neither *OPRM1* rs2075572 nor rs562859 genotype subgroups in view of gender distribution, age, body height and weight, daily dose of BUP (mg/day), except gender for rs562859, were found (Table 3).

Table 4 showed the comparison of *OPRM1* rs2075572 genotype subgroups in view of plasma buprenorphine and norbuprenorphine-related concentrations in the codominant, G-dominant and G-recessive models. The effect of rs2075572 on plasma BUP concentration did not reach a statistically significant level but showed a trend towards association ($p = 0.08$). A statistically significant difference in buprenorphine concentration between rs2075572 genotype subgroups was found according to the G-dominant model comparing GC and CC vs GG (reference), with a median plasma buprenorphine concentration of 0.08 ng/mL for GG genotype and 0.12 ng/mL for GC + CC genotypes ($p = 0.04$). There was not a statistically significant difference between these genotypes in view of plasma norbuprenorphine concentration, and dose- and dose/kg-normalized BUP and norBUP values ($p > 0.05$). Additionally, in the codominant and G-recessive models no statistically significant association was found. However, as seen in Table 4, the median values of dose- and dose/kg-normalized norBUP and norBUP/BUP were higher in GG compared with the GC + CC (0.17 vs 0.13 ng/mL per mg/day, 11.4 vs. 9.96 ng/mL per mg/kg/day and 9.31 vs 6.53, respectively).

The comparison of *OPRM1* rs562859 genotype subgroups in terms of buprenorphine and norbuprenorphine-related concentrations in the codominant, G-dominant and G-recessive models were also shown in Table 5. No significant differences between genotype subgroups in terms of the plasma BUP ($p = 0.46$) and norBUP ($p = 0.24$) concentrations, dose- or dose/kg-normalized BUP ($p = 0.81$, $p = 0.84$) and norBUP ($p = 0.16$, $p = 0.11$) values, and norBUP/BUP ($p = 0.34$) were found. Nevertheless, patients with CT + TT had higher plasma BUP and BUP-related values as compared to those with CC. However, patients having CT + TT had lower plasma norBUP and norBUP-related values as compared to those having CC.

4. Discussion

The association of *OPRM1* rs2075572 and rs562859 genotypes with BUP pharmacology in OUD patients receiving BUP/naloxone sublingually was investigated for the first time in literature. MOR is the main action site of opioids. The gene variations in *OPRM1* gene that encodes MOR can affect structure and density of the receptor, which contributes to inter-individual variability in development of tolerance throughout the long-term maintenance treatment [31]. Among SNPs in the *OPRM1* gene, the most analyzed variation is rs1799971 since it reduces the affinity for opioids by changing the receptor glycosylation [29]. However, increasing evidence has suggested that the distinct actions of various mu-opioid receptors are also mediated by splice variants created during alternative splicing [23]. Hitherto, several *OPRM1* variants due to alternative splicing have been identified and all could affect the MOR activity by changing in the C-terminal of this receptor in terms of amino acid sequence [22–26]. Furthermore, the number of studies showing the functional significance of the SNPs in intronic sequences involving alternative splicing have been increased [27,32–38]. But none of them examined the effect of SNPs in intron involving in alternative DNA splicing on BUP pharmacology. Thus, in the current study, *OPRM1* rs2075572 and rs562859 SNPs located in intron 2 were selected. The other reason is that both SNPs are associated with tramadol, a synthetic MOR agonist used for the management of pain response in the ClinVar of dbSNP.

OPRM1 rs2075572 is located within intron 2. It is located at 691 bp downstream of exon 2 [39]. It has been suggested that this genetic variation could alter the regulation of the expression of *OPRM1* gene [31,39]. In literature, the exact role of this SNP on *OPRM1* expression levels has not been delineated. *OPRM1* rs2075572 is in high linkage disequilibrium with rs9479757 [29,35]. Xu *et al.* [33] suggested that *OPRM1* rs9479757, at 31 bases downstream of exon 2, could modulate exon 2 splicing through heterogeneous nuclear ribonucleoprotein H (hnRNPH)-mediated splicing regulation. This modulation may cause alterations in expressions of hMOR-1 proteins. Thus, it is plausible to suggest that *OPRM1* rs2075572 could be functional due to the nearly complete LD with rs9479757. In the current study, we showed that patients with rs2075572 GC + CC genotype (0.12 ng/ml) had significantly higher plasma BUP concentration compared to those with GG

Table 3
Characteristics of participants according to *OPRM1* rs2075572 and rs562859 genotypes.

Characteris-tics	*OPRM1* rs2075572			P value	*OPRM1* rs562859			p value
	GG (n = 50)	GC (n = 101)	CC (n = 82)		CC (n = 27)	CT (n = 97)	TT (n = 109)	
Age (years) Median (IQR)	28 (23–31.5)	28 (26–34)	28 (25–32)	0.393 $\chi2 = 1.870$	31.3 (26.0–35.0)	29.8 (26.0–33.0)	29.7 (25.0–32.0)	0.699 $\chi2 = 0.717$
Gender (males/ females)	48/2	93/8	74/8	0.484	27/0	93/4	95/14	0.018
Body height (cm) Median (IQR)	174 (170–180)	174 (168.5–179.5)	176 (170–180)	0.183 $\chi2 = 3.397$	175.2 (170.0–181.0)	173.5 (170.0–180.0)	174.6 (170.0–180.0)	0.593 $\chi2 = 1.047$
Body weight (kg) Median (IQR)	70 (62.75–80)	67 (60.5–74.5)	70 (60–80)	0.283 $\chi2 = 2.522$	71.6 (63.0–80.0)	67.1 (60.5–74.5)	70.2 (60.0–80.0)	0.217 $\chi2 = 3.053$
Drug dosage (mg/day) Median (IQR)	4.82 (3.5–8)	6 (4–8)	5.82 (4–8)	0.239 $\chi2 = 2.865$	5.22 (2.0–8.0)	5.91 (4.0–8.0)	5.52 (4.0–8.0)	0.238 $\chi2 = 2.867$

n: sample size; IQR: Inter Quartile Range.

Table 4
BUP and norBUP concentrations according to the *OPRM1* rs2075572 genotypes using different models in OUD patients.

***OPRM1* rs2075572 / Co-dominant model**							
Geno-types	BUP (ng/ml)	norBUP (ng/ml)	Dose-normalized BUP	Dose/kg normalized BUP	Dose-normalized norBUP	Dose/kg normalized norBUP	Metabolic ratio
GG (n = 50)	0.08 (0.04–0.17)	0.72 (0.44–1.57)	0.017 (0.01–0.05)	1.1 (0.6–3.5)	0.17 (0.07–0.36)	11.4 (5.3–25.3)	9.96 (2.0–25.8)
GC (n = 101)	0.12 (0.06–0.26)	0.71 (0.35–1.63)	0.03 (0.01–0.06)	1.7 (0.7–3.4)	0.13 (0.06–0.33)	8.71 (4.1–21.3)	7.57 (1.22–21.7)
CC (n = 82)	0.11 (0.05–0.24)	0.75 (0.32–1.57)	0.017 (0.01–0.08)	1.28 (0.6–5.1)	0.14 (0.08–0.32)	9.7 (4.8–21.0)	5.48 (1.66–27.1)
Kruskal-Wallis test	$\chi2 = 4.981$ **p = 0.08**	$\chi2 = 0.098$ p = 0.952	$\chi2 = 1.574$ p = 0.455	$\chi2 = 1.288$ p = 0.525	$\chi2 = 0.852$ p = 0.653	$\chi2 = 1.299$ p = 0.522	$\chi2 = 1.776$ p = 0.441
G-recessive model							
GC + CC (n = 183)	0.12 (0.06–0.26)	0.72 (0.34–1.61)	0.024 (0.01–0.07)	1.55 (0.7–4.3)	0.13 (0.07–0.32)	9.31 (4.25–20.8)	6.53 (1.4–24.5)
GG (n = 50)	0.08 (0.04–0.17)	0.72 (0.44–1.57)	0.017 (0.01–0.05)	1.1 (0.6–3.5)	0.17 (0.07–0.36)	11.4 (5.3–25.3)	9.96 (2.0–25.8)
Mann-Whitney *U* test	U = 3722.0 **p = 0.04** Z = -2. 02	U = 4449.0 p = 0.765 Z = -0.298	U = 4112.0 p = 0.27 Z = -1.096	U = 4175.5 p = 0.34 Z = -0.95	U = 4222.0 p = 0.403 Z = -0.836	U = 4147.5 p = 0.312 Z = -1.012	U = 4071.0 p = 0.233 Z = -1.193
G-dominant model							
GG + GC (n = 151)	0.106 (0.05–0.23)	0.71 (0.37–1.61)	0.025 (0.01–0.06)	1.55 (0.68–3.4)	0.14 (0.07–0.35)	9.65 (4.8–22.9)	8.20 (1.5–24.5)
CC (n = 82)	0.11 (0.05–0.24)	0.75 (0.32–1.57)	0.017 (0.01–0.08)	1.28 (0.6–5.1)	0.14 (0.08–0.32)	9.7 (4.8–21.0)	5.48 (1.66–27.1)
Mann-Whitney *U* test	U = 6142.5 p = 0.92 Z = -0.09	U = 6177.5 p = 0.98 Z = -0.027	U = 6122.0 p = 0.88 Z = -0.14	U = 6085.5 p = 0.83 Z = -0.215	U = 6171.5 p = 0.968 Z = -0.04	U = 6145.0 p = 0.925 Z = -0.094	U = 6147.5 p = 0.929 Z = -0.089

(0.08 ng/ml), which indicated that this polymorphism could cause a decreased binding of BUP to MORs in individuals with at least one variant allele as compared to those with wild type allele. Beneficial clinical outcomes of BUP depends on the occupancy of MORs through its both agonist and antagonist effects. Opioid craving and withdrawal is decreased owing to its agonist effect, whereas it blocks the reinforcing effects of exogenous opioids due to its antagonist effect. According to Nasser *et al.* [40] 68–75 % MOR occupancy is required for opioid craving and abstinence. On the other hand, if the affinity of MORs to BUP could decrease due to *OPRM1* rs2075572, the efficacy of BUP treatment would reduce even if enough BUP is present in the plasma. Based on this knowledge, it is plausible to suggest that the decreased binding of BUP to MORs due to the decreased expression levels of mu-opioid receptors in OUD patients with minor C allele as compared to major G allele could result in increased circulating BUP level of OUD patients with GG and they could require higher BUP doses to engage more mu-opioid receptors. In consistent with this hypothesis, patients with rs2075572 GC + CC took higher daily doses of BUP related to those with GG (6 mg/day vs. 4.8 mg/day). Similarly, Wang *et al.* [41] reported that patients carrying rs2075572 G allele required a lower methadone dose as compared to C allele. Altogether, our findings supported the previous studies regarding the relationship between buprenorphine plasma concentrations, brain MOR occupancy [42]. In addition, patients with rs2075572 GC + CC took higher doses of BUP daily related to those with GG (6 mg/day vs. 4.8 mg/day). It is plausible to suggest that the decreased binding of BUP to MORs due to the decreased expression levels of mu-opioid receptors in OUD patients with minor C allele as compared to major G allele could result in increased circulating BUP level of OUD patients with GG and they could require higher BUP doses to engage more mu-opioid receptors. Consistent with Wang *et al.* [41] reporting that patients carrying rs2075572 G allele required a lower methadone dose as compared to C allele.

The results of previous studies analyzing the effect of *OPRM1* rs2075572 on substance use disorder or maintenance treatment

Table 5
BUP and norBUP levels according to the *OPRM1* rs562859 genotypes in OUD patients.

***OPRM1* rs562859 (Co-dominant model)**							
Geno-types	BUP (ng/ml)	norBUP (ng/ml)	Dose-normalized BUP	Dose/kg normalized BUP	Dose-normalized norBUP	Dose/kg normalized norBUP	Metabolic ratio
CC	0.07	1.22	0.015	1.05	0.21	13.3	17.5
(n = 27)	(0.04–0.17)	(0.44–2.59)	(0.01–0.06)	(0.6–4.85)	(0.07–0.39)	(5.09–35.4)	(1.6–41.7)
CT	0.12	0.61	0.025	1.55	0.11	7.94	6.07
(n = 97)	(0.06–0.23)	(0.35–1.38)	(0.01–0.06)	(0.68–3.43)	(0.06–0.28)	(4.1–18.8)	(1.2–21.9)
TT	0.105	0.77	0.023	1.51	0.15	10.3	6.61
(n = 109)	(0.05–0.26)	(0.39–1.63)	(0.01–0.07)	(0.7–4.43)	(0.08–0.35)	(5.5–23.4)	(1.8–26.2)
Kruskal-Wallis test	$\chi2 = 1.54$	$\chi2 = 2.88$	$\chi2 = 0.43$	$\chi2 = 0.35$	$\chi2 = 3.65$	$\chi2 = 4.47$	$\chi2 = 2.17$
	p = 0.46	p = 0.24	p = 0.81	p = 0.84	p = 0.16	p = 0.11	p = 0.34
C-recessive model							
CT + TT	0.11	0.7	0.023	1.53	0.13	9.32	6.57
(n = 206)	(0.05–0.23)	(0.36–1.56)	(0.01–0.06)	(0.67–3.9)	(0.07–0.31)	(4.68–21.00)	(1.57–23.9)
CC	0.07	1.22	0.015	1.05	0.21	13.3	17.5
(n = 27)	(0.04–0.17)	(0.44–2.59)	(0.01–0.06)	(0.6–4.85)	(0.07–0.39)	(5.09–35.4)	(1.6–41.7)
Mann-Whitney *U* test	U = 2381.0	U = 2373.5	U = 2572.0	U = 2603.5	U = 2353.5	U = 2315.5	U = 2392.0
	p = 0.224	p = 0.216	p = 0.526	p = 0.59	p = 0.194	p = 0.158	p = 0.238
	Z = -1.22	Z = -1.23	Z = -0.635	Z = -0.539	Z = -1.298	Z = -1.413	Z = -1.181
C-dominant model							
CC + CT	0.106	0.71	0.02	1.43	0.12	8.72	7.7
(n = 124)	(0.05–0.23)	(0.36–1.54)	(0.01–0.06)	(0.7–3.4)	(0.06–0.33)	(4.27–21.6)	(1.3–24.3)
TT	0.105	0.77	0.023	1.51	0.15	10.3	6.61
(n = 109)	(0.05–0.26)	(0.39–1.63)	(0.01–0.07)	(0.7–4.43)	(0.08–0.35)	(5.5–23.4)	(1.8–26.2)
Mann-Whitney *U* test	U = 6667.5	U = 6412.5	U = 6573.0	U = 6 543.5	U = 6308.0	U = 6245.0	U = 6358.5
	p = 0.86	p = 0.501	p = 0.719	p = 0.676	p = 0.381	p = 0.318	p = 0.67
	Z = -0.176	Z = -0.673	Z = -0.360	Z = -0.418	Z = -0.877	Z = -0.99	Z = -0.428

responses have been conflicting. Zahari *et al.* [27] reported that *OPRM1* rs2075572 CC genotype had an opposing role in pain tolerance in Malay opioid user males during methadone maintenance treatment (MMT). Zahari *et al.* [37] showed that *OPRM1* rs2075572 was not associated with better sleep quality alone although patients with GG genotype had lower the Pittsburgh Sleep Quality Index score. However, diplotype analysis of the two *OPRM1* SNP (rs1799971 and rs2075572) showed the significant effect of AC/AG diplotype on sleep quality in opioid users receiving MMT. Zahari *et al.* [38] also analyzed the effect of *OPRM1* SNP rs1799971 and rs2075572 heterozygous genotype on sleep quality in opioid-naïve individuals and found that GC/AG diplotype was associated with poorer sleep quality. Wang *et al.* [41] suggested that *OPRM1* rs2075572 may affect the side effects such as change-in-libido in a MMT cohort from Taiwan. Xie *et al.* [43] found that rs2075572 has an effect on maximal pain intensity and patients with GG complained more serious pain. On the other hand, there have been studies that could not show the functional effect of *OPRM1* rs2075572 on respiratory or cardiac arrest [44], on methadone level in plasma [41], on methadone-induced fatigue or withdrawal [40], on levels of morphine and glucuronide-conjugates in plasma [45], on opioid dose requirements [46]. These inconsistent findings indicated that more studies in larger sample sizes are required with other populations.

Human *OPRM1* gene is located on chromosome 6 (6q24–25) [47]. It was identified initially as having four coding exons (1–4) separated by 3 introns. Although there is only a single-copy *OPRM1* gene, divergent responses to mu opioids among individuals due to the various MOR subtypes can be explained by the formation of splice variants with different mechanisms such as alternative 5′ or 3′ splicing, exon skipping, alternative promoters and poly(A) sites [48]. Among the genes coding opioid receptors, only *OPRM1* undergoes extensive alternative splicing that is important functionally. Three major types of splice variants in humans are as follows: (i) the full-length 7- transmembrane variants having exons 1–3 encoding the N-terminus. Exon 4 is formed by replacing of alternative exons at the C-terminus), (ii) truncated 6-TM variants (involving exon-11 associated variants with no exon 1 because of exon skipping), (iii) truncated variants with a single TM [49]. The first identified *OPRM1* splice variants are MOR-1A (human) and the MOR-1B (rat) [25,26]. Among the identified *OPRM1* splice variants so far, MOR-1X contained exon as the alternative fourth exon as the other all full-length 7-TM variants in addition to exons 1–3 found in the original *OPRM1* gene of human [22]. Their intracellular C-terminal tails with various numbers of amino acids are different from the original mu-opioid receptor. The functional significance of full-length 7-TM variants in MORs has been analyzed using various cell models. It was found that these receptors differ in their desensitization, internalization and agonist-selective receptor phosphorylation, [50–52], G protein coupling [22–24,33,53], and post-endocytic sorting [54]. *OPRM1* rs562859, the second SNP examined in the current study, is on the alternative exon X of the *OPRM1*. This polymorphism arises from a T to C substitution that leaves a Leucine in the C-terminus of the hMOR-1X receptor. Although it does not lead to a modification of the MOR amino acid sequence [21], there have been evidences showing the association of the rs562859 polymorphism and OUD, MMT, treatment response for OUD with diazepam and naltrexone and for citalopram in major depressive disorder [30,55–58]. Some of these priori studies indicated that rs562859 could be functionally effective. For example, Peng *et al.* [57] reported that individuals receiving MMT with T allele would have a better compliance rate compared to C allele. In the current study, the difference between genotypes in view of BUP and norBUP levels in plasma cannot reach the significance level. *OPRM1* rs562859 CC genotype subgroups had lower plasma BUP concentration and, BUP/D, BUP/D.kg^{-1} values, but higher plasma norBUP concentration and, norBUP/D, norBUP/D.kg^{-1}, values as compared to CT + TT genotype. This finding could indicate that the effect of *OPRM1* rs562859 may be substrate-specific. It seems that further studies are needed to understand the influence of *OPRM1* rs562859 on BUP pharmacology.

The limitation of the present study is that the effects of *CYP3A4* polymorphisms on buprenorphine pharmacology was not examined due to the fact that CYP3A4 is the main metabolizing enzyme for buprenorphine. Second, it cannot be established a causality with the results of the current study since they are descriptive and correlational. Even though it has limitations, this study contributes additional evidence suggesting the effects of *OPRM1* polymorphisms on BUP pharmacology. Our results warrant to repeat with a larger sample in different ethnic

populations and with other genetic factors influencing BUP pharmacology. If they could be confirmed, clinicians would decide the right drugs at right dose according to pharmacogenetics testing rather than the trial-and-error method, which will increase the efficacy of BUP treatment.

In conclusion, this study indicated for the first time a prominent influence of the *OPRM1* rs2075572 gene variation on plasma BUP concentrations in individuals on maintenance treatment with buprenorphine. However, *OPRM1* rs562859 had no significant effect on plasma BUP and norBUP levels. If our results are adequately replicated in an increased number of individuals with different ethnic backgrounds, the functional effects of *OPRM1* rs2075572 could be clinically actionable.

Author contributions

DKA designed and directed the study. DKA and SOK conducted the genetic analysis and wrote and edited the manuscript. MD collected venous blood samples and demographic data of all subjects. CB performed measurement of plasma buprenorphine and norbuprenorphine levels with LC-MS/MS.

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CRediT authorship contribution statement

Dilek Kaya-Akyüzlü: Writing – review & editing, Writing – original draft, Supervision, Methodology, Funding acquisition, Formal analysis, Conceptualization. **Selin Özkan-Kotiloğlu:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis. **Mustafa Danışman:** Resources. **Ceylan Bal:** Methodology.

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.

References

[1] Y.L. Hurd, C.P. O'Brien, Molecular Genetics and New Medication Strategies for Opioid Addiction, Am. J. Psychiatry. 175 (2018) 935–942, https://doi.org/10.1176/appi.ajp.2018.18030352.
[2] H. Ellerbroek, S.A.S. van den Heuvel, A. Dahan, H. Timmerman, C. Kramers, A.F. A. Schellekens, Buprenorphine/naloxone versus methadone opioid rotation in patients with prescription opioid use disorder and chronic pain: study protocol for a randomized controlled trial, Addict. Sci. Clin. Pract. 17 (2022) 47, https://doi.org/10.1186/s13722-022-00326-1.
[3] R.C. Crist, T.K. Clarke, W.H. Berrettini, Pharmacogenetics of Opioid Use Disorder Treatment, CNS Drugs. 32 (2018) 305–320, https://doi.org/10.1007/s40263-018-0513-9.
[4] R.C. Crist, K.A. Phillips, M.A. Furnari, M.M. Landhing, G.A. Doyle, L.F. McNicholas, J.W. Cornish, K.M. Kampman, K.L. Preston, W.H. Berrettini, Replication of the pharmacogenetic effect of rs678849 on buprenorphine efficacy in African-Americans with opioid use disorder, Pharmacogenomics J. 19 (2019) 260–268, https://doi.org/10.1038/s41397-018-0065-x.
[5] A.M. Neumann, R.D. Blondell, U. Jaanimägi, A.K. Giambrone, G.G. Homish, J. R. Lozano, U. Kowalik, M. Azadfard, A preliminary study comparing methadone and buprenorphine in patients with chronic pain and coexistent opioid addiction, J. Addict. Dis. 32 (2013) 68–78, https://doi.org/10.1080/10550887.2012.759872.
[6] A.M. Neumann, R.D. Blondell, R.A. Hoopsick, G.G. Homish, Randomized clinical trial comparing buprenorphine/naloxone and methadone for the treatment of patients with failed back surgery syndrome and opioid addiction, J. Addict. Dis. 38 (2020) 33–41, https://doi.org/10.1080/10550887.2019.1690929.
[7] H.E. Jones, Practical considerations for the clinical use of buprenorphine, Sci. Pract. Perspect. 2 (2004) 4–20, https://doi.org/10.1151/spp04224.
[8] D. Eapen-John, A.G. Mohiuddin, J.L. Kennedy, A potential paradigm shift in opioid crisis management: The role of pharmacogenomics, World, J Biol. Psychiatry. 23 (2022) 411–423, https://doi.org/10.1080/15622975.2021.2012397.
[9] C.W. Meaden, A. Mozeika, R. Asri, C.D. Santos, A review of the existing literature on buprenorphine pharmacogenomics, Pharmacogenomics J. 21 (2021) 128–139, https://doi.org/10.1038/s41397-020-00198-1.
[10] J.L. Rutter, Symbiotic relationship of pharmacogenetics and drugs of abuse, AAPS J. 8 (2006) E174–E184, https://doi.org/10.1208/aapsj080121.
[11] L. Dean, Thioridazine Therapy and CYP2D6 Genotypes. In: Pratt VM, Scott SA, Pirmohamed M, et al., editors. Medical Genetics Summaries [Internet]. Bethesda (MD): National Center for Biotechnology Information (US); 2012, 2017.
[12] E. Brandl, Z. Halford, M.D. Clark, C. Herndon, Pharmacogenomics in Pain Management: A Review of Relevant Gene-Drug Associations and Clinical Considerations, Ann. Pharmacother. 55 (2021) 1486–1501, https://doi.org/10.1177/10600280211003875.
[13] U.S. Food and Drug Administration [Internet]. 2020. Center for Drug Evaluation and Research. [cited 2021 Dec 15]. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/label/2011/020733s007s008lbl.pdf.
[14] H.C. Wiseman, R.D. Bruce, K.M. Cammack, The Opioid Crisis: A Review of Genetic Predispositions, Current Policy and Future Directions, Impulse 18 (2021).
[15] P. Huang, G.B. Kehner, A. Cowan, L.Y. Liu-Chen, Comparison of pharmacological activities of buprenorphine and norbuprenorphine: norbuprenorphine is a potent opioid agonist, J. Pharmacol. Exp. Ther. 297 (2001) 688–695.
[16] V.K. Singh, K. Bajpai, S. Biswas, W. Haq, M.Y. Khan, K.B. Mathur, Molecular biology of opioid receptors, Neuroimmunomodulation. 4 (1997) 285–297, https://doi.org/10.1159/000097349.
[17] Y. Zhang, D. Wang, A.D. Johnson, A.C. Papp, W. Sadee, Allelic expression imbalance of human mu opioid receptor (OPRM1) caused by variant A118G, J. Biol. Chem. 280 (2005) 32618–32624, https://doi.org/10.1074/jbc.M504942200.
[18] P.K. Janicki, G. Schuler, D. Francis, A. Bohr, V. Gordin, T. Jarzembowski, V. Ruiz-Velasco, B. Mets, 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 (2006) 1011–1017, https://doi.org/10.1213/01.ane.0000231634.20341.88.
[19] T. Kroslak, K.S. Laforge, R.J. Gianotti, A. Ho, D.A. Nielsen, M.J. Kreek, The single nucleotide polymorphism A118G alters functional properties of the human mu opioid receptor, J. Neurochem. 103 (2007) 77–87, https://doi.org/10.1111/j.1471-4159.2007.04738.x.
[20] R. Ray, K. Ruparel, A. Newberg, E.P. Wileyto, J.W. Loughead, C. Divgi, J.A. Blendy, J. Logan, J.-K. Zubieta, C. Lerman, Human Mu Opioid Receptor (OPRM1 A118G) polymorphism is associated with brain mu-opioid receptor binding potential in smokers, Proc. Natl. Acad. Sci. u.s.a. 108 (2011) 9268–9273, https://doi.org/10.1073/pnas.1018699108.
[21] L. Xu, F. Zhang, D.-D. Zhang, X.-D. Chen, M. Lu, R.-Y. Lin, H. Wen, L. Jin, X.-F. Wang, OPRM1 gene is associated with BMI in Uyghur population, Obesity (silver Spring). 17 (2009) 121–125, https://doi.org/10.1038/oby.2008.504.
[22] Y.-X. Pan, J. Xu, L. Mahurter, M. Xu, A.-K. Gilbert, G.W. Pasternak, Identification and characterization of two new human mu opioid receptor splice variants, hMOR-1O and hMOR-1X, Biochem. Biophys. Res. Commun. 301 (2003) 1057–1061, https://doi.org/10.1016/s0006-291x(03)00089-5.
[23] L. Pan, J. Xu, R. Yu, M.-M. Xu, Y.-X. Pan, G.W. Pasternak, Identification and characterization of six new alternatively spliced variants of the human mu opioid receptor gene, Oprm, Neuroscience. 133 (2005) 209–220, https://doi.org/10.1016/j.neuroscience.2004.12.033.
[24] Y.X. Pan, J. Xu, E. Bolan, A. Chang, L. Mahurter, G. Rossi, G.W. Pasternak, Isolation and expression of a novel alternatively spliced mu opioid receptor isoform, MOR-1F. FEBS Lett. 466 (2000) 337–340. Doi: 10.1016/s0014-5793(00)01095-4.
[25] A. Zimprich, T. Simon, V. Höllt, Cloning and expression of an isoform of the rat mu opioid receptor (rMOR1B) which differs in agonist induced desensitization from rMOR1, FEBS Lett. 359 (1995) 142–146, https://doi.org/10.1016/0014-5793(95)00028-8.
[26] L.A. Bare, E. Mansson, D. Yang, Expression of two variants of the human mu opioid receptor mRNA in SK-N-SH cells and human brain, FEBS Lett. 354 (1994) 213–216, https://doi.org/10.1016/0014-5793(94)01129-x.
[27] Z. Zahari, C.S. Lee, M.A. Ibrahim, N. Musa, M.A.M. Yasin, Y.Y. Lee, S.C. Tan, N. Mohamad, R. Ismail, The Opposing Roles of IVS2+691 CC Genotype and AC/AG Diplotype of 118A>G and IVS2+691G>C of OPRM1 Polymorphisms in Cold Pain Tolerance Among Opioid-Dependent Malay Males on Methadone Therapy, Pain Ther. 4 (2015) 179–196, https://doi.org/10.1007/s40122-015-0041-y.
[28] D. Kaya-Akyüzlü, S. Özkan-Kotiloğlu, C. Bal, Ş. Yalçın-Şahiner, G. Avcıoğlu, M. Danışman, Effects of UGT2B7 rs7662029 and rs7439366 polymorphisms on sublingual buprenorphine metabolism in heroin addicts: An improved PCR-RFLP assay for the detection of rs7662029 polymorphism, Environ. Toxicol. Pharmacol. 94 (2022) 103902, https://doi.org/10.1016/j.etap.2022.103902.
[29] S. Ding, B. Chen, Y. Zheng, Association study of OPRM1 polymorphisms with Schizophrenia in Han Chinese population, BMC Psychiatry. 13 (2013) 107, https://doi.org/10.1186/1471-244X-13-107.
[30] R.J. Smith, G.A. Doyle, A.M. Han, J.J. Crowley, D.W. Oslin, A.A. Patkar, P. Mannelli, P.A. Demaria Jr, C.P. O'Brien, W.H. Berrettini, Novel exonic mu-opioid receptor gene (OPRM1) polymorphisms not associated with opioid dependence, Am. J. Med. Genet. b. Neuropsychiatr. Genet. 133B (2005) 105–109, https://doi.org/10.1002/ajmg.b.30105.

[31] B. Wendel, M.R. Hoehe, The human mu opioid receptor gene: 5' regulatory and intronic sequences, J. Mol. Med. (berl) 76 (1998) 525–532, https://doi.org/10.1007/s001090050246.
[32] V. Yuferov, E.R. Butelman, M. Randesi, Analyses of polymorphisms of intron 2 of OPRK1 (kappa-opioid receptor gene) in association with opioid and cocaine dependence diagnoses in an African-American population, Neurosci. Lett. 768 (2022) 136364, https://doi.org/10.1016/j.neulet.2021.136364.
[33] J. Xu, M. Xu, E. Bolan, A.-K. Gilbert, G.W. Pasternak, Y.-X. Pan, Isolating and characterizing three alternatively spliced mu opioid receptor variants: mMOR-1A, mMOR-1O, and mMOR-1P, Synapse 68 (2014) 144–152, https://doi.org/10.1002/syn.21727.
[34] J. Shi, L. Hui, Y. Xu, F. Wang, W. Huang, G. Hu, Sequence variations in the mu-opioid receptor gene (OPRM1) associated with human addiction to heroin, Hum. Mutat. 19 (2002) 459–460, https://doi.org/10.1002/humu.9026.
[35] L. Zhang, K.S. Kendler, X. Chen, The mu-opioid receptor gene and smoking initiation and nicotine dependence, Behav. Brain. Funct. 2 (2006), https://doi.org/10.1186/1744-9081-2-28.
[36] C.-J. Huang, H.-F. Liu, N.-Y. Su, Y.-W. Hsu, C.-H. Yang, C.-C. Chen, P.-S. Tsai, Association between human opioid receptor genes polymorphisms and pressure pain sensitivity in females, Anaesthesia 63 (2008) 1288–1295, https://doi.org/10.1111/j.1365-2044.2008.05760.x.
[37] Z. Zahari, C.S. Lee, M.A. Ibrahim, N. Musa, M.A.M. Yasin, Y.Y. Lee, S.C. Tan, N. Mohamad, R. Ismail, The AC/AG Diplotype for the 118A>G and IVS2 + 691G>C Polymorphisms of OPRM1 Gene is Associated with Sleep Quality Among Opioid-Dependent Patients on Methadone Maintenance Therapy, Pain Ther. 5 (2016) 43–54, https://doi.org/10.1007/s40122-016-0044-3.
[38] Z. Zahari, M.A. Ibrahim, N. Musa, S.C. Tan, N. Mohamad, R. Ismail, Sleep quality and OPRM1 polymorphisms: a cross-sectional study among opioid-naive individuals Braz, J. Pharm. Sci. 54 (2018) e17217.
[39] M.R. Hoehe, K. Köpke, B. Wendel, K. Rohde, C. Flachmeier, K.K. Kidd, W. H. Berrettini, G.M. Church, Sequence variability and candidate gene analysis in complex disease: association of mu opioid receptor gene variation with substance dependence, Hum. Mol. Genet. 9 (2000) 2895–2908, https://doi.org/10.1093/hmg/9.19.2895.
[40] A.F. Nasser, C. Heidbreder, R. Gomeni, P.J. Fudala, B. Zheng, M.K. Greenwald, A population pharmacokinetic and pharmacodynamic modelling approach to support the clinical development of RBP-6000, a new, subcutaneously injectable, long-acting, sustained-release formulation of buprenorphine, for the treatment of opioid dependence, Clin. Pharmacokinet. 53 (2014) 813–824, https://doi.org/10.1007/s40262-014-0155-0.
[41] S.C. Wang, H.H. Tsou, C.H. Chen, Y.T. Chen, I.K. Ho, C.F. Hsiao, S.Y. Chou, Y. F. Lin, K.C. Fang, C.L. Huang, L.W. Su, Y.C. Fang, M.L. Liu, H.Y. Wu, K.M. Lin, S. C. Liu, H.W. Kuo, I.C. Chiang, A.C. Chen, J.N. Tian, Y.L. Liu, Genetic polymorphisms in the opioid receptor mu1 gene are associated with changes in libido and insomnia in methadone maintenance patients, Eur. Neuropsychopharmacol. 22 (2012) 695–703, https://doi.org/10.1016/j.euroneuro.2012.02.002.
[42] C.M. Laffont, E. Ngaimisi, M. Gopalakrishnan, V. Ivaturi, M. Young, M.K. Greenwald, C. Heidbreder, Buprenorphine exposure levels to optimize treatment outcomes in opioid use disorder. Front Pharmacol. 13 (2022) 1052113. Doi: 10.3389/fphar.2022.1052113.
[43] H. Xie, Q. Fan, Z. Ma, Z. Chen, Q. Shu, D. Li, W. Ge, Resistin Gene Polymorphism Is an Influencing Factor of Postoperative Pain for Chinese Patients, Pain Physician. 23 (2020) E695–E702.
[44] A.F. Manini, M.M. Jacobs, D. Vlahov, Y.L. Hurd, Opioid receptor polymorphism A118G associated with clinical severity in a drug overdose population, J. Med. Toxicol. 9 (2013) 148–154, https://doi.org/10.1007/s13181-012-0286-3.
[45] P. Klepstad, T.T. Rakvåg, S. Kaasa, M. Holthe, O. Dale, P.C. Borchgrevink, C. Baar, T. Vikan, H.E. Krokan, F. Skorpen, The 118 A > G polymorphism in the human mu-opioid receptor gene may increase morphine requirements in patients with pain caused by malignant disease, Acta Anaesthesiol. Scand. 48 (2004) 1232–1239, https://doi.org/10.1111/j.1399-6576.2004.00517.x.
[46] M. Hayashida, M. Nagashima, Y. Satoh, R. Katoh, M. Tagami, S. Ide, S. Kasai, D. Nishizawa, Y. Ogai, J. Hasegawa, H. Komatsu, I. Sora, K. Fukuda, H. Koga, K. Hanaoka, K. Ikeda, Analgesic requirements after major abdominal surgery are associated with OPRM1 gene polymorphism genotype and haplotype, Pharmacogenomics. 9 (2008) 1605–1616, https://doi.org/10.2217/14622416.9.11.1605.
[47] J.B. Wang, P.S. Johnson, A.M. Persico, A.L. Hawkins, C.A. Griffin, G.R. Uhl, Human μ opiate receptor, FEBS Lett. 338 (1994) 217–222, https://doi.org/10.1016/0014-5793(94)80368-4.
[48] S. Liu, W.J. Kang, A. Abrimian, J. Xu, L. Cartegni, S. Majumdar, P. Hesketh, A. Bekker, Y.-X. Pan, Alternative Pre-mRNA Splicing of the Mu Opioid Receptor Gene, OPRM1: Insight into Complex Mu Opioid Actions, Biomolecules. 11 (2021) 1525, https://doi.org/10.3390/biom11101525.
[49] G.W. Pasternak, Y.X. Pan, Mu opioids and their receptors: evolution of a concept, Pharmacol. Rev. 65 (2013) 1257–1317, https://doi.org/10.1124/pr.112.007138.
[50] C. Abbadie, G.W. Pasternak, Differential in vivo internalization of MOR-1 and MOR-1C by morphine, Neuroreport. 12 (2021) 3069–3072, https://doi.org/10.1097/00001756-200110080-00017.
[51] T. Koch, S. Schulz, H. Schröder, R. Wolf, E. Raulf, V. Höllt, Carboxyl-terminal splicing of the rat mu opioid receptor modulates agonist-mediated internalization and receptor resensitization, J. Biol. Chem. 273 (1998) 13652–13657, https://doi.org/10.1074/jbc.273.22.13652.
[52] T. Koch, S. Schulz, M. Pfeiffer, M. Klutzny, H. Schröder, E. Kahl, V. Höllt, C-terminal splice variants of the mouse mu-opioid receptor differ in morphine-induced internalization and receptor resensitization, J. Biol. Chem. 276 (2001) 31408–31414, https://doi.org/10.1074/jbc.M100305200.
[53] E.A. Bolan, Y.X. Pan, G.W. Pasternak, Functional analysis of MOR-1 splice variants of the mouse mu opioid receptor gene Oprm, Synapse. 51 (2004) 11–18, https://doi.org/10.1002/syn.10277.
[54] M. Tanowitz, J.N. Hislop, M. von Zastrow, Alternative splicing determines the post-endocytic sorting fate of G-protein-coupled receptors, J. Biol. Chem. 283 (2008) 35614–35621, https://doi.org/10.1074/jbc.M806588200.
[55] L.N. Al-Eitan, S.A. Jaradat, S.Y. Su, G.K. Tay, G.K. Hulse, Mu opioid receptor (OPRM1) as a predictor of treatment outcome in opiate-dependent individuals of Arab descent, Pharmgenomics. Pers. Med. 5 (2012) 99–111, https://doi.org/10.2147/PGPM.S33351.
[56] D.B. Hancock, J.L. Levy, N.C. Gaddis, C. Glasheen, N.L. Saccone, G.P. Page, G. K. Hulse, D. Wildenauer, E.A. Kelty, S.G. Schwab, L. Degenhardt, N.G. Martin, G. W. Montgomery, J. Attia, E.G. Holliday, M. McEvoy, R.J. Scott, L.J. Bierut, E. C. Nelson, A.H. Kral, E.O. Johnson, Cis-Expression Quantitative Trait Loci Mapping Reveals Replicable Associations with Heroin Addiction in *OPRM1*, Biol. Psychiatry. 78 (2015) 474–484, https://doi.org/10.1016/j.biopsych.2015.01.003.
[57] S. Peng, H. Jiang, J. Du, S. Lin, S. Pan, S. Yu, M. Zhao, Methadone Dosage and Plasma Levels, SNPs of OPRM1 Gene and Age of First Drug Use Were Associated With Outcomes of Methadone Maintenance Treatment, Front. Genet. 9 (2018) 450, https://doi.org/10.3389/fgene.2018.00450.
[58] H.A. Garriock, M. Tanowitz, J.B. Kraft, V.C. Dang, E.J. Peters, G.D. Jenkins, M. S. Reinalda, P.J. McGrath, M. von Zastrow, S.L. Slager, S.P. Hamilton, Association of mu-opioid receptor variants and response to citalopram treatment in major depressive disorder, Am. J. Psychiatry. 167 (2010) 565–573, https://doi.org/10.1176/appi.ajp.2009.08081167.