research article

Association of *OPRM1*, *MIR23B*, and *MIR107* genetic variability with acute pain, chronic pain and adverse effects after postoperative tramadol and paracetamol treatment in breast cancer

Zala Vidic[1], Katja Goricar[1], Branka Strazisar[2], Nikola Besic[2], Vita Dolzan[1]

[1] Pharmacogenetics Laboratory, Institute of Biochemistry and Molecular Genetics, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia
[2] Institute of Oncology, Ljubljana, Slovenia

Radiol Oncol 2023; 57(1): 111-120.

Received 14 September 2022
Accepted 28 September 2022

Correspondence to: Prof. Vita Dolžan, M.D., Ph.D., Pharmacogenetics Laboratory, Institute of Biochemistry and Molecular Genetics, Faculty of Medicine, University of Ljubljana, Vrazov trg 2, 1000 Ljubljana, Slovenia. E-mail: vita.dolzan@mf.uni-lj.si

Disclosure: No potential conflicts of interest were disclosed.

Background. Tramadol is an opioid analgesic often used for pain management after breast cancer surgery. Its analgesic activity is due to the activation of the μ-opioid receptor, encoded by the *OPRM1* gene. This study investigated the association of genetic variability in *OPRM1* and its regulatory miRNA genes with outcomes of tramadol/paracetamol treatment after breast cancer surgery with axillary lymphadenectomy.

Patients and methods. The study included 113 breast cancer patients after breast cancer surgery with axillary lymphadenectomy treated with either 75/650 mg or 37.5/325 mg of tramadol with paracetamol for pain relief within the randomized clinical trial KCT 04/2015-DORETAonko/si at the Institute of Oncology Ljubljana. All patients were genotyped for *OPRM1* rs1799971 and rs677830, *MIR23B* rs1011784, and *MIR107* rs2296616 using competitive allele-specific PCR. The association of genetic factors with acute and chronic pain as well as adverse effects of tramadol treatment was evaluated using logistic regression, Fisher's exact test, and Mann-Whitney test.

Results. The investigated *OPRM1* related polymorphisms were not associated with acute pain assessed with the VAS scale within four weeks after surgery (all $P > 0.05$). Carriers of at least one polymorphic *OPRM1* rs1799971 allele had a higher risk of constipation in the first four weeks after surgery compared to non-carriers (OR = 4.5, 95% CI = 1.6–12.64, P = 0.004). Carriers of at least one polymorphic *OPRM1* rs677830 allele had a higher risk of constipation after third week of tramadol treatment (OR = 3.11, 95% CI = 1.08–8.89, P = 0.035). Furthermore, carriers of two polymorphic *MIR23B* rs1011784 alleles had a higher risk of nausea after 28 days of tramadol treatment (OR = 7.35, 95% CI = 1.27–42.6, P = 0.026), while heterozygotes for *MIR107* rs2296616 allele had a lower risk of nausea after 21 days of tramadol treatment (OR = 0.21, 95% CI = 0.05–0.87, P = 0.031). In carriers of two polymorphic *MIR107* rs2296616 alleles, chronic pain was significantly more common than in carriers of two wild-type alleles (P = 0.004). Carriers of at least one polymorphic *MIR23B* rs1011784 allele experienced more neuropathic pain after adjustment for tramadol dose (OR = 2.85, 95% CI = 1.07–7.59, P = 0.036), while carriers of at least one polymorphic *OPRM1* rs677830 allele experienced less neuropathic pain compared to carriers of two wild-type alleles (OR = 0.38, 95% CI = 0.15–0.99, P = 0.047).

Conclusions. Genetic variability of *OPRM1* and genes coding for miRNAs that could affect *OPRM1* expression may be associated with adverse effects of tramadol/paracetamol treatment as well as with chronic and neuropathic pain after breast cancer surgery with axillary lymphadenectomy.

Key words: breast cancer surgery; miRNA; OPRM1; pain treatment; tramadol

Introduction

Tramadol is a synthetic centrally acting opioid drug that exhibits its analgesic activity by binding to μ-opioid receptor (MOR).[1] It is widely used for the treatment of moderate to severe acute pain after breast cancer surgery with axillary lymphadenectomy.[2] Tramadol is considered a relatively safe opioid drug since it does not cause respiratory depression at therapeutic doses and is less likely to cause addiction compared to many other opioids. However, its analgesic efficiency is lesser compared to morphine.[1-3]

MORs, encoded by the *OPRM1* gene, are members of the Rhodopsin family of G-protein coupled receptors.[4] MORs are expressed in the cerebral cortex, thalamus, periaqueductal gray, nucleus accumbens, and basolateral amygdala, and are therefore responsible for the occurrence of euphoria, dependence, respiratory depression, and activation of the reward system.[5] They are crucial for the analgesic effects of the majority of opioid drugs.[6]

It has been shown that polymorphisms in the *OPRM1* gene can affect the efficacy of analgesic action of several opioid drugs. One of the most studied single nucleotide polymorphisms (SNPs) of the *OPRM1* gene is rs1799971 (c.118A>G; p.Asn40Asp).[4] A study performed in European subjects on long-term treatment for chronic pain with different opioids, including tramadol, reported a higher frequency of adverse effects in wild-type subjects compared to individuals with the AG or GG genotype.[7] A study in Chinese population showed greater analgesic action of tramadol combined with paracetamol (acetaminophen) for the treatment of peripheral neuropathy in wild-type patients compared to carriers of polymorphic G allele.[8] Numerous studies focused on investigating the impact of this SNP on response to morphine treatment in different populations. Many of them confirmed the association between rs1799971 and pain relief or morphine dose requirements.[9-12] Furthermore, a haplotype of seven *OPRM1* SNPs, that included rs1799971 and another non-synonymous SNP, rs677830 (c.1231C>T, p.Gln411Ter) was associated with postoperative morphine response in Caucasians.[12]

OPRM1 gene expression may be regulated by non-coding RNAs such as microRNAs (miRNAs) that bind to target mRNAs molecules and prevent protein translation or promote mRNA degradation.[13,14] It was shown that several miRNAs can affect MOR expression, or lead to opioid dependence or tolerance.[15,16,17] Furthermore, opioids can regulate the expression of several miRNAs.[15]

miRNA-23b was the first identified miRNA molecule that regulates *OPRM1* expression[17], however miRNA-107 was shown to influence *OPRM1* expression as well.[15] Both miR-23b and miR107 were experimentally proven to reduce the expression level of *OPRM1* splice variants in mouse or human neuronal cell lines, human transfected embryonic kidney cell lines, or mouse animal models. The binding of these miRNAs to 3' UTR of *OPRM1* mRNAs prevented them from binding to ribosomes without altering the respective mRNAs levels. Furthermore, these studies also reported that long-term morphine treatment increased miRNAs expression.[15,17,18]

The aim of this study was to evaluate the association of *OPRM1* rs1799971 and rs677830, *MIR23B* 1011784, and *MIR107* rs2296616 polymorphisms with the severity of acute pain and the presence of adverse effects in the first four weeks after breast cancer surgery with axillary lymphadenectomy as well as with chronic and neuropathic pain at one year after surgery.

Patients and methods

Patients

Our study included breast cancer patients that participated in a prospective double-blinded randomized clinical trial at the Institute of Oncology Ljubljana from 2015 to 2018 (Trial KCT 04/2015-DORETAonko/si).[2,19] Written informed consent was obtained from all the patients before the inclusion in the clinical trial. The study was approved by the Institutional Review Board of the Institute of Oncology Ljubljana and by The National Medical Ethics Committee of the Republic of Slovenia (Approval number 32/03/15).

The main inclusion criteria was treatment with tramadol and paracetamol after breast cancer surgery with axillary lymphadenectomy, while exclusion criteria were: concomitant breast reconstruction with a tissue expander or free-flap, hypersensitivity to the drugs used in the study, male gender, pregnancy, high risk of anesthesia according to the criteria of the American Society of Anaesthesiology (ASA over 3), patient's age under 18 and over 70 years, severe liver or kidney disease, regular use of analgesics or antidepressants, history of opioid abuse or presence of psychiatric illness, such as dementia, schizophrenia, or manic depressive illness.

Before the surgery, all patients were examined according to the standard protocol of the Institute of Oncology Ljubljana. Data was collected about the body characteristics, associated diseases or allergies, the presence of pain, the use of other medications, and the anaesthesia risk was assessed according to the criteria of the American Society of Anaesthesiology.[20]

During surgery, patients received standard postoperative local analgesia. On the first postoperative day, all patients received 37.5/325 mg of tramadol with paracetamol every 8 h, 550 mg of naproxen sodium every 12 h, and 10 mg of metoclopramide every 8 h. From the second to the 29th postoperative day inclusive, tramadol and paracetamol dose depended on the group into which a patient was randomized at inclusion in the study. The group with lower analgesia received 37.5/325 mg of tramadol with paracetamol every 8 hours, while the group with higher analgesia received 75/650 mg every 8 hours. The tramadol and paracetamol doses for each patient were unblinded 15 months after the inclusion of the last patient. For four weeks, all patients also received 550 mg of naproxen sodium twice a day and 20 mg of pantoprazole once a day. In the case of severe pain despite taking tramadol/paracetamol and naproxen sodium, patients received 500 mg of metamizole up to 4 g per day.

In the first four weeks after surgery, patients recorded the severity of the acute pain in the area of the breast and shoulder using the standard visual analog scale (VAS) three times a day. Every day, patients recorded the consumption of medications and the frequency of shoulder exercises. Patients also recorded adverse effects and reported the collected data to the healthcare professionals every seven days when they came for the weekly check-ups at the outpatients' clinic.

Before surgery, and again at the final follow-up examination at 12 to 15 months after the surgery, the patients filled in the standardized questionnaire of the Institute of Oncology on the presence of chronic pain and the DN4 questionnaire on the presence of neuropathic pain.

Methods

Bioinformatics analysis

First, we searched for potentially functional SNPs in the *OPRM1* gene by performing a literature search in PubMed (https://pubmed.ncbi.nlm.nih.gov/), Scholar (https://scholar.google.com/), and SNPedia (https://www.snpedia.com).[21] Their minor allele frequency (MAF) was obtained from the dbSNP database from The National Center for Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov/snp/) and linkage disequilibrium between polymorphisms was evaluated by the LDlink Tool (https://analysistools.cancer.gov/LDlink/?tab=ldmatrix).[22] We identified four missense or nonsense SNPs in the coding regions of the *OPRM1* gene with potential function in tramadol treatment outcome. Among them, only three met the MAF criteria of at least 5% in Caucasians: rs1799971, rs677830 and rs540825. As *OPRM1* rs540825 and rs677830 were in high linkage disequilibrium ($R^2 = 0.98$), only rs1799971 and rs677830 were included in our study.

Next, we searched PubMed, Scholar, and SNPedia for miRNAs with experimentally proven impact on MOR expression or activity. Moreover, we searched the following online databases that can predict the targets of miRNAs or report known associations between a specific gene and miRNA molecules: miRTarBase (http://mirtarbase.cuhk.edu.cn/php/index.php)[23], miRDB (http://www.mirdb.org)[24], and online tool miRNet (https://www.mirnet.ca)[25], to identify miRNAs that can regulate *OPRM1*. After narrowing down the list of potential miRNAs with impact on MOR function, we identified polymorphisms in genes coding for selected miRNAs with MAF of at least 5% in Caucasians. After the literature search for established roles of these polymorphisms in the expression of *OPRM1* or other genes we have selected two polymorphisms in two miRNA genes for the analysis.

In total, four polymorphisms in three genes were selected for inclusion in our study: *OPRM1* rs1799971 (NP_000905.3: p.Asn40Asp), *OPRM1* rs677830 (NP_001138758.1: p.Gln411Ter), *MIR23B* rs1011784 (NR_029664.1: n.525G>C), and *MIR107* rs2296616 (NR_029524.1: n.-382C>T).

Molecular analysis

DNA samples were isolated from peripheral blood samples using Qiagen FlexiGene Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Genotyping of selected polymorphisms was performed retrospectively using custom made validated competitive allele-specific polymerase chain reaction (KASP) assays following the instructions of the manufacturer (LGC Biosearch Technologies, UK). The analysis was repeated for 10% of samples and the results were concordant.

TABLE 1. Patients' clinical characteristics

		All subjects	Stronger postoperative analgesia with tramadol/paracetamol	Weaker postoperative analgesia with tramadol/paracetamol
Sample size		**N = 113**	**N = 55**	**N = 58**
Characteristic		**N (%)**	**N (%)**	**N (%)**
Age (years)	Median (25–75 %)	55 (48–63)	57 (49–64)	53,5 (47.8–61)
Weight (kg)	Median (25–75 %)	72 (63.5–82)	72 (64–82)	72 (63–82.3)
Body Mass Index (kg/m²)	Median (25–75 %)	27.1 (23.4–31.0)	26.7 (23.0–30.8)	27.6 (23.5–31.2)
Smoking	No	87 (77.7) [1]	41 (75.9) [1]	46 (79.3)
	Yes	25 (22.3)	13 (24.1)	12 (20.7)
ASA score	1	22 (19.6) [1]	10 (18.2)	12 (21.1) [1]
	2	81 (72.3)	39 (70.9)	42 (73.7)
	3	9 (8.0)	6 (10.9)	3 (5.3)
Side of the operation	Left	62 (54.9)	30 (54.5)	32 (55.2)
	Right	50 (44.2)	24 (43.6)	26 (44.8)
	Bilateral	1 (0.9)	1 (1.8)	0 (0.0)
Tumor grade	1	4 (3.6) [1]	1 (1.9) [1]	3 (5.2)
	1–2	3 (2.7)	2 (3.7)	1 (1.7)
	2	39 (34.8)	17 (31.5)	22 (37.9)
	2–3	8 (7.1)	2 (3.7)	6 (10.3)
	3	58 (51.3)	32 (59.3)	26 (44.8)
VAS after 7 days	Median (25–75 %)	2 (1–3) [5]	1.3 (1–2) [3]	2 (1–3) [2]
VAS after 14 days	Median (25–75 %)	1.5 (0–2) [11]	1 (0–2) [9]	2 (1–2) [2]
VAS after 21 days	Median (25–75 %)	1 (0.4–2) [11]	1 (0–2) [9]	1 (1–2) [2]
VAS after 28 days	Median (25–75 %)	1 (0–2) [14]	1 (0–1) [10]	1 (1–2) [4]

ASA = American Society of Anaesthesiology; N = sample size; [n] = number of missing data; VAS = visual analog scale

Genotype frequencies, MAF, and Hardy-Weinberg equilibrium analysis (P_{HWE}) of investigated polymorphisms in all patients enrolled in our study are presented in Table 2.

Statistical analysis

Statistical analysis was performed using IBM SPSS v27.0 (IBM Corporation, Armonk, NY, USA). Continuous data were presented with median and interquartile range, while categorical data were presented with frequencies. For each polymorphism, a standard χ^2-test was used to evaluate whether genotype distribution followed Hardy-Weinberg equilibrium (HWE). Only the dominant genetic model was used in all statistical analyses for *OPRM1* polymorphisms due to the small numbers of carriers of two polymorphic alleles, while both dominant and additive genetic model was used in the analyses for polymorphisms in miRNA genes. The association of genetic factors with adverse effects, chronic and neuropathic pain was evaluated using binary logistic regression to obtain odds ratios (OR) with corresponding 95-% confidence intervals (95% CI). Fisher's exact test was used if there were no subjects within one of the groups. Mann-Whitney test was used to evaluate the association of polymorphisms with the severity of acute pain. All tests were two-sided and the level of statistical significance was set at 0.05.

Results

In total, 113 patients were included in the study, 55 (48.7%) received stronger postoperative analgesia and 58 (51.3%) received weaker postoperative analgesia. Patients' clinical characteristics are shown in Table 1. Among all patients, 14 (12.4%) patients discontinued tramadol treatment due to severe adverse events before the end of four weeks. Adverse

TABLE 2. The investigated polymorphism's characteristics and frequencies

Gene	SNP	Genotype	N (%)	PAF (%)	MAF HapMap CEU expected (%)	P_{HWE}
OPRM1	rs1799971	AA	84 (74.3)	14.2	15.6–16.7	0.570
		AG	26 (23.0)			
		GG	3 (2.7)			
	rs677830	CC	56 (49.6)	27.4	20.7	0.098
		CT	52 (46.0)			
		TT	5 (4.4)			
MIR23B	rs1011784*	CC	63 (56.3) [1]	26.8	23.3	0.153
		CG	38 (33.9)			
		GG	11 (9.8)			
MIR107	rs2296616*	GG	21 (18.6)	57.1	54.2–55.3	0.944
		GA	55 (48.7)			
		AA	37 (32.7)			

ASA = American Society of Anaesthesiology; CEU = Northern Europeans from Utah; HGVS = Human Genome Variation Society; HWE = Hardy-Weinberg equilibrium; N = sample size; PAF = polymorphic allele frequency; SNP = single nucleotide polymorphism; *KASP assays were designed on the reverse strand

events were therefore evaluated in 99 patients. The vast majority of the patients who discontinued tramadol were from the higher dose group (P = 0.004). A total of 101 patients completed the follow-up visit one year after surgery and were included in the analysis of genetic factors associated with the chronic and neuropathic pain.

Within the first four weeks of tramadol/paracetamol treatment, the severity of the self-perceived acute pain assessed with the VAS scale was not associated with the patient's genotypes for any of the investigated polymorphisms in none of the time points (all P > 0.05) (Supplementary Table 1).

Several patients experienced the side effects that could be related to tramadol treatment at least once within the first four weeks: 36 (36.7%) patients experienced nausea, 9 (9.1%) patients experienced vomiting, 35 (35.4%) patients experienced dizziness, and 48 (48.5%) patients experienced constipation.

TABLE 3. The impact of investigated polymorphisms on constipation anytime during the first four weeks of tramadol treatment (N = 99)

SNP	Genotype	Constipation anytime N (%)	OR (95% CI)	P	$OR\ (95\%\ CI)_{adj}$	P_{adj}
OPRM1 rs1799971	AA	30 (40)	Ref.		Ref.	
	AG+GG	18 (75)	4.5 (1.6–12.64)	**0.004**	4.31 (1.52–12.17)	**0.006**
OPRM1 rs677830	CC	24 (48)	Ref.		Ref.	
	CT+TT	24 (49)	1.04 (0.47–2.29)	0.922	0.98 (0.44–2.19)	0.964
MIR23B rs1011784	CC	29 (50)	Ref.		Ref.	
	CG	16 (50)	1 (0.42–2.37)	1.000	0.96 (0.4–2.31)	0.930
	GG	3 (37.5)	0.6 (0.13–2.75)	0.510	0.46 (0.1–2.23)	0.338
	CG+GG	19 (47.5)	0.9 (0.4–2.03)	0.808	0.84 (0.37–1.91)	0.676
MIR107 rs2296616	GG	11 (57.9)	Ref.		Ref.	
	GA	23 (48.9)	0.7 (0.24–2.04)	0.511	0.73 (0.25–2.16)	0.566
	AA	14 (42.4)	0.54 (0.17–1.68)	0.285	0.53 (0.17–1.67)	0.276
	GA+AA	37 (46.3)	0.63 (0.23–1.72)	0.364	0.64 (0.23–1.77)	0.386

adj = adjustment for tramadol dose; N = sample size; OR = odds ratio; SNP = single nucleotide polymorphism; 95% CI = 95% confidence interval

TABLE 4. The impact of investigated miRNA polymorphisms on nausea after 21 and 28 days of tramadol treatment (N = 99)

SNP	Genotype	Nausea 21 days N (%)	OR (95% CI)	P	Nausea 28 days N (%)	OR (95% CI)	P
MIR23B rs1011784	CC	9 (16.4)	Ref.		4 (7.5)	Ref.	
	CG	4 (12.9)	0.76 (0.21–2.7)	0.668	5 (16.1)	2.36 (0.58–9.54)	0.230
	GG	2 (25)	1.7 (0.3–9.83)	0.551	3 (37.5)	7.35 (1.27–42.6)	**0.026**
	CG+GG	6 (15.4)	0.93 (0.3–2.86)	0.898	8 (20.5)	3.16 (0.88–11.39)	0.078
MIR107 rs2296616	GG	6 (31.6)	Ref.		2 (10.5)	Ref.	
	GA	4 (8.9)	0.21 (0.05–0.87)	**0.031**	4 (8.7)	0.81 (0.14–4.84)	0.817
	AA	5 (16.7)	0.43 (0.11–1.69)	0.229	6 (21.4)	2.32 (0.41–12.96)	0.338
	GA+AA	9 (12)	0.30 (0.09–0.97)	**0.045**	10 (13.5)	1.33 (0.27–6.64)	0.730

N = sample size; OR = odds ratio; SNP = single nucleotide polymorphism; 95% CI = 95% confidence interval

Carriers of at least one polymorphic *OPRM1* rs1799971 allele had a higher risk of constipation in the first four weeks compared to carriers of two wild-type alleles (OR = 4.5, 95% CI = 1.6–12.64, P = 0.004). This association remained significant after the adjustment for the tramadol dose (OR = 4.31, 95% CI = 1.52–12.17, P = 0.006). None of the other polymorphisms were associated with the overall risk for constipation during the first month after surgery (Table 3). Data for constipation for each week separately are shown in Supplementary Table 2. Carriers of at least one polymorphic *OPRM1* rs677830 allele had a higher risk of constipation after 21 days of tramadol treatment when compared to carriers of two wild-type alleles (OR = 3.11, 95% CI = 1.08–8.89, P = 0.035).

The risk of nausea after 28 days of tramadol treatment was significantly higher in carriers of two polymorphic *MIR23B* rs1011784 alleles compared to carriers of two wild-type alleles (OR = 7.35, 95% CI = 1.27–42.6, P = 0.026). Heterozygotes for *MIR107* rs2296616 allele had a lower risk of nausea after the third week of tramadol treatment compared to carriers of two wild-type alleles (OR = 0.21, 95% CI = 0.05–0.87, P = 0.031). Similarly, carriers of at least one polymorphic *MIR107* rs2296616 had a lower risk of nausea after the third week of tramadol treatment compared to carriers of two wild-type alleles (OR = 0.30, 95% CI = 0.09–0.97, P = 0.045) (Table 4). None of the investigated polymorphisms were associated with the risk for nausea within the first two weeks after surgery (Supplementary Table 3).

One year after surgery 21 (20.8%) patients experienced chronic pain and 25 (24.8%) experienced neuropathic pain. In carriers of two polymorphic *MIR107* rs2296616 alleles, chronic pain was significantly more common compared to carriers of two wild-type alleles (35.3% compared to 0% of patients, P = 0.004). Carriers of at least one polymorphic *OPRM1* rs677830 allele experienced less neuropathic pain compared to carriers of two wild-type alleles (OR = 0.38, 95% CI = 0.15–0.99, P = 0.047), but the difference was no longer significant after adjustment for tramadol dose (P = 0.060). Carriers of at least one polymorphic *MIR23B* rs1011784 allele experienced more neuropathic pain compared to carriers of two wild-type alleles, but the difference was only significant after adjustment for tramadol dose (OR = 2.85, 95% CI = 1.07–7.59, P = 0.036). The other investigated miRNA polymorphism was not associated with the persistence of chronic or neuropathic pain (Table 5).

Discussion

Our study investigated the association of genetic variability of *OPRM1* and genes coding for miRNAs regulating *OPRM1* expression with acute and long-term pain management as well as adverse effects of tramadol treatment after surgical treatment of breast cancer and axillary lymphadenectomy.

None of the investigated polymorphisms were associated with the intensity of acute pain after the surgery. However, we confirmed the association between some of the investigated polymorphisms and the presence of adverse effects as well as chronic and neuropathic pain. In particular, *OPRM1* rs1799971 polymorphism increased the risk of constipation in the first month of tramadol treatment, while *OPRM1* rs677830 polymorphism

TABLE 5. The impact of investigated polymorphisms on chronic and neuropathic pain (N = 101)

SNP	Genotype	Chronic pain N (%)	OR (95% CI)	P	OR (95% CI)$_{adj}$	P$_{adj}$
OPRM1 rs1799971	AA	15 (20.3)	Ref.		Ref.	
	AG+GG	6 (22.2)	1.12 (0.39–3.28)	0.831	1.2 (0.4–3.57)	0.739
OPRM1 rs677830	CC	14 (27.5)	Ref.		Ref.	
	CT+TT	7 (14)	0.43 (0.16–1.18)	0.101	0.44 (0.16–1.21)	0.112
MIR23B rs1011784	CC	9 (15.3)	Ref.		Ref.	
	CG	10 (32.3)	2.65 (0.94–7.45)	0.065	2.85 (0.99–8.19)	0.052
	GG	2 (20)	1.39 (0.25–7.64)	0.706	1.68 (0.29–9.83)	0.563
	CG+GG	12 (29.3)	2.3 (0.86–6.11)	0.095	2.58 (0.94–7.1)	0.067
MIR107 rs2296616	GG	0 (0)	Ref.		Ref.	
	GA	9 (18.4)	/	0.099*		
	AA	12 (35.3)	/	**0.004***		
	GA+AA	21 (25.3)	/	**0.021***		
SNP	**Genotype**	**Neuropathic pain N (%)**	**OR (95% CI)**	**P**	**OR (95% CI)$_{adj}$**	**P$_{adj}$**
OPRM1 rs1799971	AA	19 (25.7)	Ref.		Ref.	
	AG+GG	6 (22.2)	0.83 (0.29–2.36)	0.722	0.94 (0.32–2.75)	0.915
OPRM1 rs677830	CC	17 (33.3)	Ref.		Ref.	
	CT+TT	8 (16)	0.38 (0.15–0.99)	**0.047**	0.4 (0.15–1.04)	0.060
MIR23B rs1011784	CC	11 (18.6)	Ref.		Ref.	
	CG	12 (38.7)	2.76 (1.04–7.31)	**0.042**	3.25 (1.17–9.02)	**0.023**
	GG	2 (20)	1.09 (0.2–5.87)	0.919	1.59 (0.27–9.24)	0.607
	CG+GG	14 (34.1)	2.26 (0.9–5.68)	0.082	2.85 (1.07–7.59)	**0.036**
MIR107 rs2296616	GG	3 (16.7)	Ref.		Ref.	
	GA	11 (22.4)	1.45 (0.35–5.93)	0.607	1.37 (0.33–5.69)	0.666
	AA	11 (32.4)	2.39 (0.57–10.02)	0.233	2.34 (0.55–9.97)	0.249
	GA+AA	22 (26.5)	1.8 (0.48–6.83)	0.386	1.73 (0.45–6.65)	0.424

adj = adjustment for tramadol dose, N = sample size, OR = odds ratio, SNP = single nucleotide polymorphism, 95% CI = 95% confidence interval; *calculated using Fisher's exact test

increased the risk of constipation in the third week of tramadol treatment. *OPRM1* rs677830 polymorphism also reduced the risk of neuropathic pain one year after the surgery. The presence of *MIR23B* rs1011784 polymorphism increased the risk of nausea in the fourth week of tramadol treatment and increased the risk of neuropathic pain one year after the surgery. Lastly, the presence of *MIR107* rs2296616 polymorphism reduced the risk of nausea in the third week of treatment and increased the risk of chronic pain one year after the surgery.

So far, many studies confirmed the impact of *OPRM1* rs1799971 polymorphism on pain perception, opioid response, the presence of adverse effects, and susceptibility to alcohol or drug dependence, but the majority of studies of *OPRM1* rs1799971 polymorphisms were conducted on Asian population. According to systematic review and metaanalysis, carriers of at least one polymorphic *OPRM1* rs1799971 allele have higher opioid dose requirements to manage postoperative pain.[26] However, after adjustment for different ethnic groups and different opioids, this effect remained significant only for the Asian population and in the group receiving morphine. In the Caucasian population, the association had not been confirmed. The different ethnic origin of patients is, according to the authors of the review, one of the

main reasons for mentioned differences in opioid response among different populations, since MAF for polymorphic *OPRM1* rs1799971 allele in the Caucasian population is lower compared to the Asian population, and thus limiting the recognition of the recessive effect of the polymorphism. Besides, it is possible that other gene variants with an impact on MOR activity, that are in linkage disequilibrium with *OPRM1* rs1799971 polymorphism, are the reason for differences in opioid response.[26] Other authors also state that ethnic origin is an important cause for conflicting research results on the impact of *OPRM1* rs1799971 polymorphism on subjects' response to opioid drugs.[4] According to the 1000Genome project, MAF in the Asian population is between 39 and 42%, while MAF in the European population is 16%.[27] In our group of patients, MAF for *OPRM1* rs1799971 was 14.2%, which coincides with the expected frequency for Europeans. Another study conducted on the Caucasian population, investigating the impact of *OPRM1* and *COMT* polymorphisms on postoperative acute and chronic pain could not confirm the impact of *OPRM1* rs1799971 polymorphism on any of these types of pain[28], which is consistent with the results of our study. The combination of tramadol and paracetamol was more effective for the treatment of neuropathic pain in wild-type homozygotes compared to carriers of *OPRM1* rs1799971 polymorphic allele in a group of the Asian patients with oxaliplatin-induced neuropathy.[8] Furthermore, wild-type homozygotes had lower opioid dose requirements for the treatment of chronic pain compared to carriers of *OPRM1* rs1799971 polymorphic allele in European patients.[29]

Adverse effects, especially nausea and gastrointestinal events, were more common in a group of people with wild-type genotype compared to the carriers of polymorphic *OPRM1* rs1799971 allele.[7,30] A possible reason for this could be a loss of the N-glycosylation site of MOR in the carriers of polymorphic allele[29], since the N-glycosylation site of MOR may be involved in the trafficking into the cell membrane, binding of ligands, and signal transduction.[31] On the other hand, *OPRM1* rs1799971 polymorphism could impact the expression levels of MOR directly and consequently reduce the risk of opioid toxicity.[29] Contrary to expectations, our results showed an increased risk of constipation in the carriers of the polymorphic *OPRM1* rs1799971 allele. Since the expression of MOR is tissue-specific, the impact on constipation cannot be explained by the central action of opioids. Instead, we should investigate the expression patterns and mechanisms of action of MOR on the periphery.

A study of the association between *OPRM1* rs677830 polymorphism and postoperative pain treatment with morphine in the Caucasian population showed no significant influence of polymorphism, although an impact of the haplotype of seven polymorphisms of the *OPRM1* gene, including rs1799971 and rs677830, on morphine requirement has been observed.[12] Due to the presence of a stop codon that leads to reduced expression of MOR caused by *OPRM1* rs677830 polymorphism[32], it was expected that greater severity of acute pain will be reported from the carriers of at least one polymorphic *OPRM1* rs677830 allele. As the greater severity of acute pain is one of the main risk factors for the presence of persistent pain[33], we expected increased risk of chronic or neuropathic pain in carriers of at least one polymorphic allele, yet our results showed no impact on severity of acute pain and even indicated on reduced risk of neuropathic pain in carriers of polymorphic rs677830 allele. A literature search showed no report on the impact of mentioned polymorphism on adverse effects after opioid pain treatment or the presence of chronic or neuropathic pain so far.

A study conducted on the mouse and human neuronal cells showed that long-term morphine treatment cannot influence the transcription of *OPRM1*. On the other hand, it can lead to increased miR-23b levels, which can bind to mRNA for MOR1 and thus prevent the translation of mRNA to the receptor, which causes a drug tolerance.[17,34] Tramadol and morphine have a similar molecular structure, thus there is a possibility that MOR expression may be affected by tramadol in the same way. An altered sequence of miR-23b, caused by *MIR23B* rs1011784 polymorphism, could change its target sequence and therefore prevent binding of miR-23b to mRNA for MOR1. In that case, receptor activity would remain unchanged, but we were unable to confirm these assumptions.

Similar to miR-23b, long-term morphine exposure led to up-regulated miR-107 levels, leading to decreased levels of MOR1A in human neuroblastoma cells and striatum of morphine-tolerant mouse.[15] It would be interesting to evaluate the possible impact of tramadol on miR-107 expression as well. In addition, even though *MIR107* rs2296616 polymorphism does not lead to decreased transcription of DNA to pre-miRNA, it could affect the processing of pre-miR-107 to mature transcript and consequently levels of miR-107.[35] As a result, it

could affect MOR expression, but to confirm this we would have to do the quantification of miR-107 and MOR levels.

Despite the confirmed association between polymorphisms in miRNA genes and tramadol treatment outcome, it is not sure that these miRNA acts through the altered activity of MOR. miR-23b played a crucial role in neuropathic pain relief in a mouse model after spinal cord injury. When a higher level of pain was present, decreased expression level of miR-23b was measured and therefore increased levels of its target gene *NOX4*, coding for NADPH oxidase 4, that contributes to reactive oxygen species formation. After infusion of ectopic miR-23b molecules, an expression level of NOX4 decreased, and the symptoms improved.[36] From the results of the mentioned study, we can conclude that miR-23b can influence the perception of pain through its influence on various genes, not only *OPRM1*.

Additionally, miR-107 paralogue miR-103 that differs in only one nucleotide in 3'-end, therefore regulating overlapping target molecules[15], including mRNA for MOR in mice and humans, was shown to be regulating pain perception in rats and was down-regulated in neuropathic animals. It is responsible for the altered expression level of three subunits of Cav1.2 L-type calcium channels that play a crucial role in the sensation in chronic neuropathic pain.[37] It is reasonable to assume that the miR-107 molecule could have the same effect. If polymorphism in *MIR107* alters the target sequence of a mentioned molecule, it would consequently lead to the same effect as downregulation of the miR-107, therefore increased neuropathic pain. Yet our results only suggested the impact of miR-107 on chronic pain perception.

Our study was one of the few studies inspecting the impact of polymorphisms of the *OPRM1* gene on the tramadol treatment outcome in Caucasians. Nevertheless, it has some limitations such as a relatively small sample size of the patients and that the genotyping analysis was performed retrospectively. Since only a few studies investigated the impact of miRNA on the expression and functionality of MOR, it was a challenge to narrow the selection of miRNA and potentially functional polymorphisms in genes coding for miRNA. Furthermore, we investigated the impact of polymorphisms on pain, which is always liable to subjective assessment, even though we used VAS and other standardized questionnaires to limit this impact. On the other hand, the advantage of our study was an ethnically homogenous group of patients, as well as the unified treatment and follow-up protocol of patients. Overall, it was a longitudinal, prospective, randomized double-blind clinical trial, where neither the healthcare professionals nor the patients knew the dose level of tramadol.

Conclusions

In this study, we observed for the first time to our knowledge, that the presence of *OPRM1* rs677830 polymorphism reduces the risk for the presence of neuropathic pain and increases the risk of constipation in response to tramadol pain treatment. Furthermore, we observed for the first time the association of investigated polymorphisms in miR-23b and miR-107 coding genes with tramadol treatment outcome. The results of our study are expanding the knowledge in the field of personalized medicine that could lead to improved pain management and reduced risk of adverse effects, therefore improving the quality of patient's life.[38]

Acknowledgments

This work was financially supported by the Slovenian Research Agency (ARRS Grants No. P1-0170 and P3-0289).

References

1. Subedi M, Bajaj S, Kumar MS, Yc M. An overview of tramadol and its usage in pain management and future perspective. *Biomed Pharmacother* 2019; **111:** 443-51. doi: 10.1016/j.biopha.2018.12.085
2. Besic N, Smrekar J, Strazisar B. Chronic adverse effects after an axillary lymphadenectomy in breast cancer patients after administering weaker and stronger postoperative analgesia: results of a prospective double-blind randomized study. *Breast Cancer Res Treat* 2020; **182:** 655-63. doi: 10.1007/s10549-020-05713-3
3. World Health Organisation. *Tramadol, update review report*. [Internet]. 2014. p. 1-39. [cited 2022 Aug 14]. Available at: http://www.who.int/medicines/areas/quality_safety/6_1_Update.pdf
4. Crist RC, Berrettini WH. Pharmacogenetics of OPRM1. *Pharmacol Biochem Behav* 2014; **123:** 25-33. doi: 10.1016/j.pbb.2013.10.018
5. Wang S. Historical review: opiate addiction and opioid receptors. *Cell Transplant* 2019; **28:** 233-8. doi: 10.1177/0963689718811060
6. Yoshida K, Nishizawa D, Ide S, Ichinohe T, Fukuda KI, Ikeda K. A pharmacogenetics approach to pain management. *Neuropsychopharmacol Rep* 2018; **38:** 2-8. doi: 10.1002/npr2.12003
7. Muriel J, Margarit C, Barrachina J, Ballester P, Flor A, Morales D, et al. Pharmacogenetics and prediction of adverse events in prescription opioid use disorder patients. *Basic Clin Pharmacol Toxicol* 2019; **124:** 439-48. doi: 10.1111/bcpt.13155
8. Liu YC, Wang WS. Human mu-opioid receptor gene A118G polymorphism predicts the efficacy of tramadol/acetaminophen combination tablets (ultracet) in oxaliplatin-induced painful neuropathy. *Cancer* 2012; **118:** 1718-25. doi: 10.1002/cncr.26430

9. Campa D, Gioia A, Tomei A, Poli P, Barale R. Association of ABCB1/MDR1 and OPRM1 gene polymorphisms with morphine pain relief. *Clin Pharmacol Ther* 2008; **83:** 559-66. doi: 10.1038/sj.clpt.6100385

10. Lötsch J, von Hentig N, Freynhagen R, Griessinger N, Zimmermann M, Doehring A, et al. Cross-sectional analysis of the influence of currently known pharmacogenetic modulators on opioid therapy in outpatient pain centers. *Pharmacogenet Genomics* 2009; **19:** 429-36. doi: 10.1097/fpc.0b013e32832b89da

11. Klepstad P, Rakvåg TT, Kaasa S, Holthe M, Dale O, Borchgrevink PC, et al. 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* 2004; **48:** 1232-9. doi: 10.1111/j.1399-6576.2004.00517.x

12. De Gregori M, Diatchenko L, Ingelmo PM, Napolioni V, Klepstad P, Belfer I, et al. Human genetic variability contributes to postoperative morphine consumption. *J Pain* 2016; **17:** 628-36. doi: 10.1016/j.jpain.2016.02.003

13. Riffo-Campos ÁL, Riquelme I, Brebi-Mieville P. Tools for sequence-based miRNA target prediction: what to choose? *Int J Mol Sci* 2016; **17:** 1987. doi: 10.3390/ijms17121987

14. Xu W, San Lucas A, Wang Z, Liu Y. Identifying microRNA targets in different gene regions. *BMC Bioinformatics* 2014; **15:** S4. doi: 10.1186/1471-2105-15-S7-S4

15. Lu Z, Xu J, Xu M, Pasternak GW, Pan YX. Morphine regulates expression of μ-opioid receptor MOR-1A, an intron-retention carboxyl terminal splice variant of the μ-opioid receptor (OPRM1) gene via miR-103/miR-107. *Mol Pharmacol* 2014; **85:** 368-80. doi: 10.1124/mol.113.089292

16. Zheng H, Law PY, Loh HH. Non-coding RNAs regulating morphine function: with emphasis on the in vivo and in vitro functions of miR-190. *Front Genet* 2012; **3:** 113. doi: 10.3389/fgene.2012.00113

17. Wu Q, Law PY, Wei LN, Loh HH. Post-transcriptional regulation of mouse mu opioid receptor (MOR1) via its 3′ untranslated region: a role for microRNA23b. *FASEB J* 2008; **22:** 4085-95. doi: 10.1096/fj.08-108175

18. Rodríguez RE. Morphine and microRNA activity: is there a relation with addiction? *Front Genet* 2012; **3:** 223. doi: 10.3389/fgene.2012.00223

19. Besic N, Smrekar J, Strazisar B. Acute pain and side effects after tramadol in breast cancer patients: results of a prospective double-blind randomized study. *Sci Rep* 2020; **10:** 18766. doi: 10.1038/s41598-020-75961-2

20. Fitz-Henry J. The ASA classification and peri-operative risk. *Ann R Coll Surg Engl* 2011; **93:** 185-7. doi: 10.1308/rcsann.2011.93.3.185a

21. Cariaso M, Lennon G. SNPedia: a wiki supporting personal genome annotation, interpretation and analysis. *Nucleic Acids Res* 2012; **40:** D1308-12. doi: 10.1093/nar/gkr798

22. Machiela MJ, Chanock SJ. LDlink: a web-based application for exploring population-specific haplotype structure and linking correlated alleles of possible functional variants. *Bioinformatics* 2015; **31:** 3555-7. doi: 10.1093/bioinformatics/btv402

23. Huang H-Y, Lin Y-C-D, Li J, Huang K-Y, Shrestha S, Hong H-C, et al. miRTarBase 2020: updates to the experimentally validated microRNA–target interaction database. *Nucleic Acids Research* 2020; **48:** D148-54. doi: 10.1093/nar/gkz896

24. Chen Y, Wang X. miRDB: an online database for prediction of functional microRNA targets. *Nucleic Acids Research* 2020; **48:** D127-31, doi: 10.1093/nar/gkz757

25. Chang L, Zhou G, Soufan O, Xia J. miRNet 2.0: network-based visual analytics for miRNA functional analysis and systems biology. *Nucleic Acids Research* 2020; **48:** W244-51. 10.1093/nar/gkaa467

26. Hwang IC, Park JY, Myung SK, Ahn HY, Fukuda K, Liao Q. OPRM1 A118G gene variant and postoperative opioid requirement: a systematic review and meta-analysis. *Anesthesiology* 2014; **121:** 825-34. doi: 10.1097/ALN.0000000000000405

27. National Library of Medicine (US), National Center for Biotechnology Information. db SNP: rs1799971. [cited 2022 Oct 30]. Available at: https://www.ncbi.nlm.nih.gov/snp/rs1799971

28. Matic M, de Hoogd S, de Wildt SN, Tibboel D, Knibbe CA, van Schaik RH. OPRM1 and COMT polymorphisms: implications on postoperative acute, chronic and experimental pain after cardiac surgery. *Pharmacogenomics* 2020; **21:** 181-93. doi: 10.2217/pgs-2019-0141

29. Muriel J, Margarit C, Planelles B, Serralta MJ, Puga C, Inda MD, et al. OPRM1 influence on and effectiveness of an individualized treatment plan for prescription opioid use disorder patients. *Ann N Y Acad Sci* 2018; **1425:** 82-93. doi: 10.1111/nyas.13735

30. The Pharmacogenomics Knowledge Base (PharmGKB). Variant annotations: rs1799971. [cited 2022 Oct 30]. Avalable at: https://www.pharmgkb.org/variant/PA166156991/variantAnnotation

31. Kroslak T, Laforge KS, Gianotti RJ, Ho A, Nielsen DA, Kreek MJ. The single nucleotide polymorphism A118G alters functional properties of the human mu opioid receptor. *J Neurochem* 2007; **103:** 77-87. doi: 10.1111/j.1471-4159.2007.04738.x

32. National Library of Medicine (US), National Center for Biotechnology Information. db SNP: rs677830. [cited 2022 Oct 30]. Available at: https://www.ncbi.nlm.nih.gov/snp/rs677830

33. Wang L, Guyatt GH, Kennedy SA, Romerosa B, Kwon HY, Kaushal A, et al. Predictors of persistent pain after breast cancer surgery: a systematic review and meta-analysis of observational studies. *CMAJ* 2016; **188:** E352-61. doi: 10.1503/cmaj.151276

34. Wu Q, Zhang L, Law PY, Na Wei LN, Loh HH. Long-term morphine treatment decreases the association of μ-opioid receptor (MOR1) mRNA with polysomes through miRNA23b. *Mol Pharmacol* 2009; **75:** 744-50. doi: 10.1124/mol.108.053462

35. Wang S, Lv C, Jin H, Xu M, Kang M, Chu H, et al. A common genetic variation in the promoter of miR-107 is associated with gastric adenocarcinoma susceptibility and survival. *Mutat Res* 2014; **769:** 35-41. doi: 10.1016/j.mrfmmm.2014.07.002

36. Im YB, Jee MK, Choi JI, Cho HT, Kwon OH, Kang SK. Molecular targeting of NOX4 for neuropathic pain after traumatic injury of the spinal cord. *Cell Death Dis* 2012; **3:** e426. doi: 10.1038/cddis.2012.168

37. Favereaux A, Thoumine O, Bouali-Benazzouz R, Roques V, Papon MA, Salam SA, et al. Bidirectional integrative regulation of Cav1.2 calcium channel by microRNA miR-103: role in pain. *EMBO J* 2011; **3018:** 3830-41. doi: 10.1038/emboj.2011.249

38. Stamer UM, Zhang L, Stüber F. Personalized therapy in pain management: where do we stand? *Pharmacogenomics* 2010; **11:** 843-64. doi: 10.2217/pgs.10.47