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The role of the *OPRM1* gene polymorphism and its methylation in people in dependence on substances and with different intensity of pain

Abstract

Opioid receptors belong to the group of Gi and Go coupled receptors, inhibiting the activity of the neuron. Opioid receptors regulate reward and aversion. The opioid system contributes to self and species survival by promoting reward elicited by natural stimuli (such as food, sex and social interaction), regulating mood states and facilitating efficient coping with pain and stress. It is suggested that *OPRM1* polymorphism is associated with alcohol consumption especially increased in the case of G alleles subjects than A-alleles homozygotes. In several studies, *OPRM1* methylation was suspected to be predictive factor of opioid dependence in pain treatment.

The relationship of postoperative or preoperative pain with methylation of some CpG sites in the *OPRM1* promoter has also been demonstrated. It is known that *OPRM1* SNPs provide changes in the structure of the MOR receptor, so by confirming the pharmacogenetic effects of *OPRM1* polymorphisms and using these results to guide therapeutic decisions, patients can be prescribed treatment options with the best efficacy and greatest tolerance. Pharmacogenomics of *OPRM1* can improve pain management by predicting individual response to pain medications before treatment and facilitate the development of new and more effective pain medications for post-operative pain.

Keywords: polymorphism *OPRM1*, opioid receptor, alcohol dependence, analgesia, methylation.

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INTRODUCTION

The Mu opioid receptor (also known as OP3 receptor, MOP, MOR) it has high affinity for enkephalins and beta-endorphins, mainly in neurons (central nervous system) and in some types of smooth muscles. Mu1,2,3 receptors (MOR) is member of the family of G protein-coupled opioid receptors (GPCRs). To the MOR bind endogenous ligands – beta-endorphin, endomorphin 1 and 2 with proopiomelanocortin (POMC) being the precursor. The mu-1 receptor is responsible for analgesia and dependence. The mu-2 receptor is vital for euphoria, dependence, respiratory depression, miosis, decreased digestive tract motility/constipation. Mu-3 receptor causes vasodilation. Kappa receptors (KOR) bind to dynorphin A and B (Prodynorphin as the precursor). They provide analgesia, diuresis, and dysphoria [1,2]. Opioid receptors belong to the group of Gi and Go coupled receptors, inhibiting the activity of the neuron. The Mu receptor consists of 3 domains: extracellular, intracellular and intracellular. The intracellular domain is related to the G1/G0 heterotrimeric proteins. Heterotrimeric G proteins comprise three proteins, one Gα subunit, and a heterodimer of β and γ subunits [3,4]. Activation of MOR by agonists leads to dissociation of GDP from the Gα subunit, which is replaced by GTP, and separation of the Gα-GTP from the βγ heterodimer. The now active Gα-GTP and βγ subunit complex interact with intracellular signaling proteins, including inwardly rectifying potassium channels, calcium channels, phospholipase C and the mitogen-activated protein kinase (MAPK) pathway as well as a variety of adenylate cyclase isoforms, to generate physiological responses. The intracellular signal is terminated by endogenous GTPase activity of the Gα subunit which hydrolyses the Gα bound GTP to GDP. Gα-GDP can no longer activate effector proteins and moreover, it re-associates with the βγ heterodimer to terminate βγ signaling and reform the GDP-bound heterotrimer [4,5].

One common thread between the opioid receptor subtypes is the interesting observation that receptor trafficking and regulation vary depending upon the agonist. For example, morphine is unable to promote receptor internalization, in contrast to DAMGO, which causes robust internalization [6-8]. Following activation, MORs undergo rapid phosphorylation, which triggers a decline in their G protein signaling as well as the recruitment of proteins that will result in receptor internalization. Phosphorylation of the MOR and its impact on receptor desensitisation and internalisation has been widely studied [9,10]. While this phosphorylation is mostly mediated by G protein

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receptor kinases (GRKs) [10,11], there is also evidence that other intracellular kinases such as $Ca2^{+}$/calmodulin-dependent protein kinase II, proto-oncogene tyrosine-protein kinase, and protein kinase C can phosphorylate the receptor [10,12]. Importantly, the phosphorylation barcode, as well as its signaling consequences, are highly dependent on the ligand bound to the MOR. Sequential and hierarchical phosphorylation of MORs results in the recruitment of thecytosolic protein β-arrestin. MOR phosphorylation and β-arrestin recruitment result in MORs desensitisation, namely the uncoupling of the G protein signaling cascades, which has been proposed to be the initial step leading to opioid tolerance [10,13]. MOR can recruit β-arrestin 1 and 2 isoforms, although this seems to be agonist dependent. While β-arrestins are essential to initiate MORs endocytosis via clathrin-coated pits, not all ligands that recruit arrestins induce robust receptor internalisation. This differential ability of agonists to induce receptor internalisation, has been linked to the phosphorylation barcodes mentioned earlier [9,10]. Moreover, β-arrestin1 can promote MOR ubiquitination by acting as E3-ubiquitin ligase adaptors [10,12].

This ubiquitination is also ligand dependent (occurs with high efficacy ligands such as DAMGO, but not with morphine) and results in degradation of the MOR within the lysosomes. However, MOR is not always degraded after endocytosis. Instead, and as opposed to the DOR, internalised MORs are usually recycled back to the plasma membrane, in a process known as resensitisation [10,14]. It is clear that MOR internalisation, recycling, and degradation are processes related to the development of tolerance [10,15].

The MOR receptor is encoded by the *OPRM1* gene, which is located on chromosome 6 (6q25.2) and contains 19 exons [10,16]. Most studied SNP of *OPRM1* is SNP A118G, which was reported as clinically significant. A118G *OPRM1* has changed adenine (A) to a guanine (G). Interestingly, the G-containing allele is present in 15-30% of Europeans, 40-50% of Asians, and 1-3% of Latinos and African Americans [10,17]. At a protein level, this SNP results in a change of amino acid at position 40, located in the N terminus of the receptor; from an asparagine (Asn, N) to an aspartate (Asp, D). This N40D change removes a potential site for asparagine-linked glycosylation, which has been suggested to alter MOR affinity for different ligands, its transduction cascade [10,18]. This N40D change removes a potential site for asparagine-linked glycosylation, which has been suggested to alter MOR affinity for different ligands, its transduction cascade [10,18], as well as the half-life of the receptor at the membrane [10,19]. Moreover, G118 adds a methylation site, which has been reported to result in a reduction of the levels of MOR messenger RNA (mRNA) [10,20].

OPRM1, alcohol dependence treatment

Opioid receptors regulate reward and aversion. The opioid system contributes to self and species survival by promoting reward elicited by natural stimuli (such as food, sex and social interaction), regulating mood states and facilitating efficient coping with pain and stress. Animal data indicate that MORs drive rewarding properties of opioid drugs (via direct, on-target effects) and other drugs of abuse (via indirect, opioid peptide-mediated effects) in both recreational consumption and binge or intoxication, and that repeated MOR activation leads to reduced drug reward (tolerance) and compensatory adaptations (dependence or withdrawal symptoms) [21].

The μ-opioid receptor is a key mediator of the effects of many opioid agonists [22,23]. μ-opioid receptors in the ventral tegmental area (VTA) regulate the activity of dopaminergic neurons in the nucleus accumbens (NAc). Infusion of a μ receptor agonist into the VTA increases dopamine release in the NAc, while infusion of a μ receptor antagonist decreases dopamine release [23,24]. Mice lacking the μ-opioid receptor [23,25,26] show a loss of morphine-induced analgesia, reward, and withdrawal symptoms. The μ-opioid receptor also plays a role in the rewarding properties of ethanol and other drugs of abuse, effects that may be mediated by these drugs' capacity to increase dopamine release in medial forebrain structures [23,27]. It was confirmed that mice with inhibited MOR in brain areas responsible for reward, consumed less alcohol [28]. Some studies found that young adolescents with *OPMR1* G-allele variants are more likely to consume alcohol than non-carriers [29]. Among male social drinkers with the *OPRM1* A118G have bigger propensity to abuse alcohol [30]. However, some studies reported no association between *OPRM1* A118G polymorphism and severity of alcohol dependence [31,32]. It is strongly possible that the G allele described in numerous studies can be associated with a response to treatment, but not with typology or the very predisposition toward alcoholism. It is necessary to carry out further research which would embrace a larger group of patients; it should be divided into other homogeneous subgroups, including, e.g., how the patients respond to naltrexone pharmacotherapy [33,34].

Naltrexone is an agent that blocks opioid receptors, particularly the μ-opioid receptor. Use of this agent in animal models leads to a reduction of dopamine levels in the nucleus accumbens [35-38]. Meta-analyses of alcohol dependence treatment [23,29] show clearly that naltrexone is superior to placebo on a number of drinking outcomes, there is considerable variability in efficacy among studies. Even in studies, in which the naltrexone group shows better outcomes than the placebo group, the medication is not efficacious for all patients who receive it. The variable treatment response underscores the need to identify which individuals respond best to naltrexone therapy and the processes by which the medication exerts its therapeutic effects. Efforts to identify clinical features that moderate the naltrexone response have shown that a family history of alcohol dependence is the most consistent predictor, such that individuals having a greater percentage of alcoholic family members show a more robust treatment response [23.40-42]. Thus, it may be possible to identify genetic variation that can be used to identify which alcohol-dependent individuals are most likely to benefit from opioid antagonist treatment [23].

A number of studies were conducted in order to find association between *OPRM1* polymorphism and effect of naltrexone therapy. Schacht et al. studied potential factors of NTX response in 152 AUDs and measured alcohol induced reward brain areas after NTX treatment. NTX significantly reduced heavy drinking and reward from alcohol, however there was no difference in drinking and brain activation between *OPRM1* A118 genotype group and control group. *OPRM1* A118 did not have significant influence on VS activation. Results stated that *OPRM1* A118 genotype did not constitute a valuable predictor factor in NTX treatment [43].

In the study by Ziaudden et al. likewise no significant interaction of *OPRM1* A118G genotype with naltrexone treatment was found, which questions role of SNP A118G as predictor factor [44]. However, more recent study stated that success

of NTX therapy is based on interaction of *OPRM1* with other genotypes. In the study with 152 subjects, NTX were more effective for patients with *OPRM1* G variants and *DAT1* 10/10 VNTR. Regarding *OPRM1* A/A genotype subjects, those with additionally *DAT1* 9 VNTR responded more effectively to treatment. Results showed that response to naltrexone can be affected by many genetic factors associated with dopamine regulation. Therefore, it needs to be studied more, in order to find more genetic interactions regarding NTX treatment and develop more personalized AUDs treatment [45].

Interestingly, it has been suggested, that *OPRM1* polymorphism contributes to alcohol drinking during NTX treatment. More alcohol in drinks per drinking day during NTX therapy was consumed by G allele *OPRM1* subjects than A-allele homozygotes. However, no difference between genotypes wasobserved in decrease of alcohol after treatment. *OPMR1* heterozygotes underwent treatment more severely, thus by concentrating studies on responsiveness of G-allele subjects, new personalized therapy strategy can be developed [46].

OPRM1 and analgesia

Activation of the primary afferent nociceptor (PAN) is induced by potentially damaging stimuli. Then the information is sent from receptor via pain pathways to the dorsal horn of the spinal cord. The terminals of the PAN contact neurons in specific laminae of the dorsal horn where they release glutamate and peptides to activate the second order neurons. The axons of nociceptive dorsal horn neurons cross to the contralateral anterolateral quadrant to form an ascending tract, which terminates in the brainstem and several distinct areas of the thalamus, which contain higher order neurons that project to various cortical regions that mediate different aspects of the pain experience [47].

In the dorsal horn, when MORs are activated, there is an inhibition of neuropeptide release (Substance P and CGRP), which promotes analgesia. However, the major sites for the analgesic properties are the periaqueductal grey and the rostral ventromedial medulla. These two areas of the descending modulatory pain pathway are characterised by two different populations of neurons that enhance (on-cells) or attenuate (off-cells) pain sensation by modulating dorsal horn nociceptive neurons activity. Because of the different locations of the MOR, in the on-cells or in inhibitory neurons controlling off-neurons activity, in both cases, the activation of MOR leads to analgesia [10,47].

Human *OPRM1* polymorphisms were studied in order to explore mechanisms of pain tolerance and analgesia. It is known that SNPs of *OPRM1* provide alterations in construction of the MOR receptor, so by confirming the pharmacogenetic effects of *OPRM1* polymorphisms and using those findings to guide treatment decisions, patients can be prescribed the therapeutic options with the best efficacy and the greatest tolerability [48].

Pharmacogenomics of *OPRM1* can improve pain management by predicting the individual response to analgesics prior to therapy and facilitate the development of novel and more effective pain medication for postoperative pain [49].

OPRM1 expression was analyzed in number of studies, which concentrated in different kinds of pain: postsurgical, in combat sport, in hip-osteoarthritis and in low back pain.

Coexistence OPMR1 and *COMT* genotype were investigated in postoperative pain and opioid usage in 153 subjects. Patients with combined Met158Met of *COMT* rs4680 and AG/GG of *OPRM1* A118G consumed more opioid compared to patients with other combinations. Interaction of *OPRM1* and the low pain sensitivity haplotype (LPS) of *COMT* was found, in which subjects with no LPS haplotype and homozygotes A of *OPRM1* A118G had higher pain experience than the subjects with genotypes AG/GG. However, patients with at least one LPS haplotype, AA of *OPRM1* A118G had lower pain score than variants AG/GG [50].

Olesem et al. studied polymorphism of *OPRM1* and pain severity in patients with hip osteoarthritis. The evaluation was performed for 175 patients who had planned operation of the hip. Higher pain severity was observed in the group with the *OPRM1* rs589046T allele in comparison to non-carriers [51].

Regarding postsurgical pain, some studies showed no significant association between *OPRM1* and pain. Matić et al. addressed the lack of the role of *OPRM1* polymorphisms in postoperative acute, chronic and thermal experimental pain in 126 subjects after cardiac surgery. However, different to prior mentioned studies the influence of *OPRM1* A118G on postoperative could not be confirmed. No genetic association between *COMT* and *OPRM1* and the development of chronic pain was found [52].

Furthermore, Wang et al. analyzed the association *OPRM1* rs1799971 polymorphisms on chronic postsurgical pain (CPSP), acute pain and analgesic consumption after elective caesarean delivery in 266 patients. There were no statistically significant differences in the distribution of CPSP across the genotypic groups ($p=0.684$ for rs4680 and $p=0.227$ for rs1799971, respectively) [53]. Karatas et al. studied association between polymorphisms in opioid receptor Mu 1 gene with postoperative pain after root canal treatment in 95 patients. From the *OPRM1* gene rs1799971 and rs1799973 were investigated. There was found no connection between these polymorphisms and differences in postsurgical pain level [54]. Lie et al. investigated the effect of *OPRM1* rs1799971 A118 and other genetic variants on the experimental pain by pressure and heat in group of 232 patients with low back pain. Genotyped data showed no significance association between *OPRM1* A118G and severity of experimental pain [55]. Leznicka et al. studied the potential interaction between polymorphism *COMT* rs4680:G>A and μ-opioid receptor *OPRM1* rs1799971:A >G on pain perception (cold and pressure pain) in group of 214 combat athletes. From genotyped DNA it was reported, that there was no difference in pain sensitivity between control group and group with *OPRM1* polymorphism [56].

Methylation of *OPRM1*

Epigenetic regulations are reversible changes to the expression of genes that have inheritable phenotypic effects. In the mammalian genome, DNA methylation is an epigenetic mechanism involving the transfer of a methyl group onto the C5 position of the cytosine to form 5-methylcytosine. DNA methylation regulates gene expression by recruiting proteins involved in gene repression or by inhibiting the binding of transcription factors (TFs) to DNA. DNA methylation in the mammalian genome predominantly occurs on cytosine in the context of the 50-CpG-30 dinucleotides. Stretches of GC-rich sequences in the genome called CpG islands (CGIs) that are associated with open transcriptionally competent chromatin structures were discovered in gene promoters [57,58].

Promoters play an essential role in understanding the transcriptional mechanisms of genes. CpG islands located within

promoter regions appear to create a more conducive chromatin state that favors transcription or allows gene expression silencing through intensive CpG methylation [58,59]. The *OPRM* gene promoter is heavily methylated in the undifferentiated P19 cells where the MORngene is silenced [60-62]. The silenced *OPRM* gene can be activated by decreasing the expression of methyl-CpG-binding protein 2 (MeCP2), an important player in gene silencing [60,62], or by the addition of a pan-histone acetylation inducer such as trichostatin A (TSA) [62,63]. Furthermore, DNA methylation on the *OPRM* promoter can be reduced by the addition of an artificial demethylation agent, 5-Aza-2′-deoxycytidine (5-Aza-C) [61,62].

Correlation between methylation of *OPRM1* and pain severity was intensively studied in past couple years. Its goal was to find out how methylation of *OPRM1* influences pain sensitization, so more specific pain therapy could be applied for patients, to ensure analgesia.

Sun et al., conducted a study, in which mice were split into two groups – dorsal root ganglion injury (DRG) group after chronic constriction injury (CCI) and control group after sham operation. After surgery, the changes in the expression of *MeCP2*, *HDAC1* and in methylation of *OPRM1* promotor region were measured. Mice under chronic neuropathic pain due to injury had increased level of *MeCP2*, *HDAC1* expression in the promotor region of *OPRM1*, in comparison to control group. The injured DRG presented lowered level of MOR in the ipsilateral spinal dorsal horn than in sham dorsal horn. Pyrosequencing of promotor region in CCI group showed three of eight CpG regions in promotor were more methylated than in control group. Blocking *MeCP2* showed increasing levels of protein and mRNA of MOR and caused greater morphine analgesia. Moreover, administrating inhibitor of *HDAC1*, similarly as in *MeCP2* blockade, caused overexpression of MOR and better analgesia. In conclusion, results shed light on mechanism of analgesia blockade by postsurgical pain. Future medical pain therapies could use methylation inhibitors in order to treat chronic pain, by ensuring better analgesic response. Additionally, measuring levels of *HDAC1*, *MeCP2*, and *OPRM1* promotor methylation could be beneficial as diagnostic predictor in postsurgical pain [64].

The linkage of postoperative or preoperative pain to methylation of the certain CpG sites in the promoter of *OPRM1* was confirmed in the study by Chidambaran et al. in a group of 121 subjects with chronic pain and 127 witch preoperative pain. High methylation of CpG sites in *OPRM1* gene were reported to be associated with preoperative and postoperative pain [65]. Sensitization of the wounds can be also affected by methylation of *OPRM1*. After skin incision on paw in a group of mice, the level of its methylated DNA increased. By providing methylation inhibitor 5-Aza-2′-deoxycytidine, the swelling effect and pain of incision was reduced. Additionally, results indicate he role of DNA methylation as sensitization inductor by measuring level of DNA methyltransferases, which were higher in injured group than in control group.

Expression of MOR in incision site and after injection of DNMT inhibitor 5-Aza-2′-deoxycytidine increased in mice, which results in hypersensitization of the wound. By providing naloxone (opioid receptor antagonist), focal analgesia increased, which confirmed participation of MOR in the whole process. Therefore, it is possible in the future to stop inflammation and pain in a wound by providing demethylation factors [66].

In several studies, *OPRM1* methylation was suspected to be predictive factor of opioid dependence in pain treatment. There was also suggestion that by initial intake of opioids, DNA methylation level increases. It was confirmed that among 33 patients with prescribed opioid drugs, there was a significant higher DNA methylation at *OPRM1* promotor, which was result of opioid exposure [67]. Methylation of *OPRM1* could be used also as a status indicator, whose subject is more likely to be opium dependent [68].

High level of methylation was observed in 84 cancer patients with high dose of opioid intake. Moreover, pain treatment to patients with high methylation was not as effective as to patients with lower promoter methylation. In the mouse model of the study, adenoviruses were used to re-express either *OPRM1* or a control plasmid with GFP (hereafter referred to as Ad-OPRM1 or Ad-GFP groups). The re-expressed *OPRM1* (Ad-OPRM1 group) had less thermal and mechanical allodynia in comparison to control group. Re-expression of *OPRM1* reduced opioid tolerance by measuring ever lower doses of morphine needed to reduce pain until achieving the pain threshold. In comparison, higher morphine doses were needed to return to threshold, which signalizes morphine tolerance. In fact, the Ad-OPRM1 morphine group only required 24% of the morphine dose used by the Ad-OPRM1 vehicle group to achieve complete antinociception. Additionally, it was confirmed, that morphine tolerance was connected with high *OPRM1* methylation [69]. These findings suggest that measuring methylation of CpGs can be predictive for opioid dosage regulation and identify patients more exposed to abuse opioids. By inhibiting methylation and increasing *OPRM1* expression, process of achieving opioid dependence could be slowed down.

Methylation of *OPRM1* was studied for its significant association with alcohol dependence [70]. Regarding association of alcohol therapy success and DNA methylation, in study by Lin et al., alcoholism dependent subjects treated with naltrexone had similar methylation level in promoter region of *OPRM1* in comparison to placebo treated group. The number of days to relapse, and the percent drinking days in the first 13 weeks after the initiation of NTX or placebo treatment did not differ significantly between NTX and placebo treatment groups. It was suggested that methylation levels of individual *OPRM1* promoter CpG units do not contribute significantly to inter-individual variation in NTX response. Methylation of CpG units, NTX treatment, and treatment-by-methylation interactions did not significantly affect the probability of relapse in either AAs or EAs. However, the age of subjects in combination with a cluster of specific *OPRM1* promoter CpG units may affect NTX treatment outcome. Results suggest that by estimation of methylation level of *OPRM1* we could in the future individually treat alcohol dependent patients to reduce the risk of alcohol relapse. By using methylation data we could select specific group of AAs that would benefit the most form naltrexone treatment [71].

Neonatal abstinence syndrome was widely studied for its association with *OPRM1* methylation. Increased methylation of *OPRM1* at the -14, -10, and +84 CpG sites was observed in study with 86 infants with Neonatal abstinence syndrome (NAS), whose mothers were treated with opioids. Infants with methylated DNA had lowered expression of *OPRM*, which led to low number of MOR. These infants needed higher doses of opioids in order to treat NAS [72].

Similar results were presented by Wachman et al., 2018, in which link was found between the maternal methylation status and 58 infant with NAS. An association was found of infant methylation level and need for pharmacotherapy at the −14 CpG site within the *OPRM1* promoter. There were additional associations with the −18 CpG site within the infants, and the +84 CpG site within the mothers with more NAS pharmacotherapy [73].

In their most recent study, placental tissues subjects were collected from 64 opioid- using mothers in second or third trimester. In contrast to their prior study, *OPRM1* methylation level in placental samples from subjects was not correlated with neonatal opioid withdrawal syndrome (NOWS) measures in infants. Moreover, there was no significant difference in methylation between opioid-treated subjects and control group [74].

Neonatal abstinence syndrome may not be affected by DNA methylation, however further studies need to be conducted to find direct link between mothers opioid exposure, genotype variants and NOWS.

CONCLUSIONS

It is known that *OPRM1* SNPs provide changes in the structure of the MOR receptor, so by confirming the pharmacogenetic effects of *OPRM1* polymorphisms and using these results to guide therapeutic decisions, patients can be prescribed treatment options with the best efficacy and greatest tolerance. Pharmacogenomics of *OPRM1* can improve pain management by predicting individual response to pain medications before treatment and facilitate the development of new and more effective pain medications for post-operative pain.

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