AIMS Medical Science, 4(2): 233–240
DOI: 10.3934/medsci.2017.2.233
Received 17 March 2017,
Accepted 14 May 2017,
Published 24 June 2017

http://www.aimspress.com/journal/medicalScience

Research article

Lack of Association between Human μ-Opioid Receptor (*OPRM1*) Gene Polymorphisms and Heroin Addiction in A Sample of Southeast Iranian Population

Mansour Shakiba [1, 2], Mohammad Hashemi [3, 4, *], Zahra Rahbari [2], Salah Mahdar [2], Hiva Danesh [4], Fatemeh Bizhani [4] and Gholamreza Bahari [4]

[1] Health Promotion Research Center, Zahedan University of Medical Sciences, Zahedan, Iran;
[2] Department of Psychiatry, School of Medicine, Zahedan University of Medical Sciences, Zahedan, Iran;
[3] Cellular and Molecular Research Center, Zahedan University of Medical Sciences, Zahedan, Iran;
[4] Department of Clinical Biochemistry, School of Medicine, Zahedan University of Medical Sciences, Zahedan, Iran

*** Correspondence:** Email: mhd.hashemi@gmail.com; hashemim@zaums.ac.ir; Tel: +98-543 3235122

Abstract: It has been proposed that genetic factors account for 30%–50% of the risk for cocaine and heroin addiction. The present study was aimed to find out the impact of μ-opioid receptor gene (*OPRM1*) rs1799971 A > G and rs9479757 polymorphisms on heroin dependence in a sample of southeast Iranian population. This case-control study was done on 123 heroin addicts and 140 non-addicts Iranian male. Genomic DNA was extracted from peripheral blood cells using salting out method. Genotyping of *OPRM1* rs1799971 and rs9479757 polymorphisms were performed using PCR-RFLP method. Overall, our results did not support an association between *OPRM1* variants and risk of heroin dependence in a sample of southeast Iranian population. Further studies with larges sample sizes and different ethnicities are required to validate our findings.

Keywords: OPRM1; polymorphism; addiction; heroin

1. Introduction

Opioid abuse is an important major social and economic problem worldwide. The annual global prevalence of opioid abuse was estimated at between 28 and 38 million users [1]. Physiological dependence is one of the key features that maintain repeated opioid use, contributing significantly to the cycle of chronic use/abuse [2]. It has been proposed that genetic and environmental factors contribution to individual differences in vulnerability to drug addictions. Heroin addiction is a chronic disease characterized by compulsive drug seeking, drug abuse, tolerance and physical dependence. Twin studies have proposed that genetic factors account for nearly 30%–60% of the overall variance in the risk of developing drug addiction [3–5].

μ-opioid receptor gene (OPRM1; OMIM# 600018) located on chromosome 6 (6q25.2) is a primary target of opioids. The OPRM1 receptor, a membrane of the G protein-coupled receptor (GPCR) family [6], interacts with multiple endogenous opioid peptides such as beta-endorphin endomorphins [7,8] as well as of numerous opioid analgesic agents and drugs including morphine, methadone, fentanyl and heroin [9–11].

A common functional polymorphism in exon 1 of OPRM1 (rs1799971 or 118A > G) results in an Asn40Asp amino-acid change increase the affinity of the receptor to its ligands [6]. Several studies evaluated the impact of *OPRM1* polymorphisms on heroin dependence but the findings were inconsistent [12–17].

To the best of our knowledge, there is no data regarding the impact of *OPRM1* polymorphisms on heroin addiction in Iranian population. Thus, in the current study we focus on the association between *OPRM1* rs1799971 (A118G, Asn40ASp) and rs9479757 gene polymorphisms and the risk of heroin addiction in a sample of southeast Iranian population.

2. Materials and Methods

2.1. Patients

This case-control study was done in Zahedan (southeast Iran) on 123 heroin addicts men who referred to Baharan Hospital (Psychiatric hospital of Zahedan University of Medical Sciences) for methadone maintenance therapy and 140 healthy men who declared that they did not suffer substance abuse. The local Ethics Committee of the Zahedan University of Medical Sciences approved the project, and written informed consent was taken from all individuals. Two milliliter of venous blood was drawn from each participant and genomic DNA was extracted by using salting out method [18].

2.2. Genotyping

Genotyping of *OPMR1* polymorphisms was done by polymerase chain reaction-restriction fragments polymorphism (PCR-RFLP). The primers are listed in Table 1. In each 0.20 mL PCR reaction tube, 1 µL of genomic DNA (~100 ng/mL), 1 µL of each primer (10 µM) and 10 µL of 2 × Prime Taq Premix (Genet Bio, Korea) and 7 µL ddH_2O were added. The PCR cycling conditions were set as follows: 95˚C for 5 min, 30 cycles of 95˚C for 30 s, annealing temperature (62˚C for rs1799971 and 66˚C for rs1799971) for 30 s, and 72˚C for 30 s and a final extension step of 72˚C for 10 min. Ten microliter of PCR product were digested by appropriate restriction enzyme (Table 1) and the fragments analyzed by electrophoresis on a 2.5% agarose gel containing 0.5 µg/mL of ethidium bromide and transilluminated with ultraviolet light and digitally imaged for genotyping (Figure 1).

Table 1. Primers sequences for detection of *OPRM1* polymorphisms using PCR-RFLP method.

OPRM1 Polymorphisms	Primer sequence (5'-3')	Restriction enzyme	Fragment (bp)
rs1799971 A>G	GTCTCGGTGCTCCTGGC TACCTCGC (F) TTCGGACCGCATGGGTC GGACCGGT (R)	Bsh1285I	A allele: 153 + 44 G allele: 129 + 44 + 24
rs9479757 G>A	AGCATATTCACCCTCTG CACC (F) CACCAACATATCAGGCT GTGAAGC (R)	AluI	G allele: 233 A allele: 210 + 23

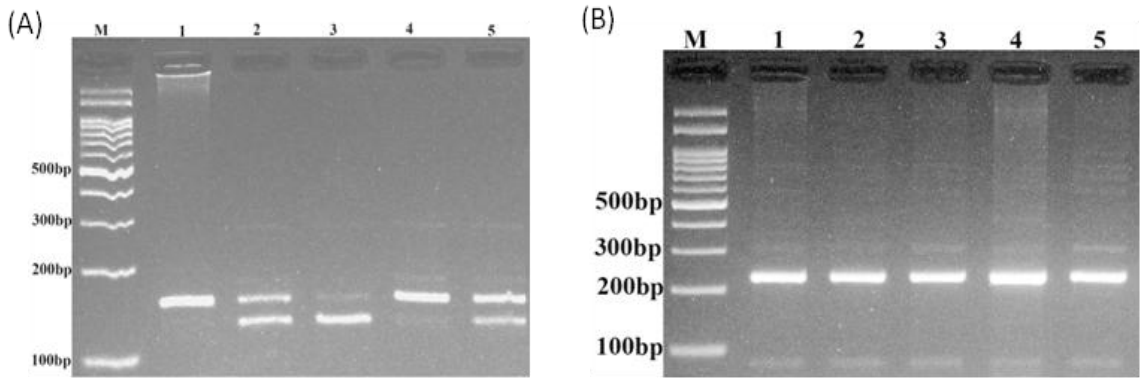

Figure 1. Photograph of the PCR product of the *OPRM1* polymorphisms by PCR-RFLP method. (A) For rs1799971 A>G variant, M: DNA marker; lanes 1 and 4: AA; lanes 2 and 5: AG; lane 3: GG. (B) For rs9479757 G>A polymorphism, all participants were GG genotype.

2.3. Statistical analysis

Statistical analysis was done by statistical package SPSS 22 software. Data were analyzed by independent sample *t*-test or χ^2 test according to the data. Odds ratio (OR) and 95% confidence intervals (95%CI) were calculated from unconditional logistic regression analyses to find out the possible association between the variants and heroin dependence. A *P*-value less than 0.05 were considered statistically significant.

3. Results

Totally 263 subject including 123 heroin addicts men with an age average 38.50 ± 11.7 years and 140 healthy men with an average age of 35.7 ± 11.1 years were included in the study. No significant difference was found between the groups regarding age ($P = 0.212$).

Table 2 shows the genotype and allele frequencies of *OPMR1* rs1799971 A>G polymorphism in heroin addiction and healthy subjects. The findings proposed that this variant was not associated with heroin dependence. Regarding rs9479757 G>A variant of *OPMR1* gene, our findings revealed that this variant was not polymorphic in our population and all cases and controls were GG genotype.

Table 2. Genotype and allele frequency of OPRM1 rs1799971 A>G polymorphism in heroin addiction and control subjects.

rs1799971 A>G Polymorphism	Case (%)	Control (%)	OR (95%CI)	*P*-value
AA	84 (68.30)	100 (71.40)	1.00	-
AG	34 (27.60)	30 (21.40)	1.35(0.73–2.48)	0.376
GG	5 (4.10)	10 (7.20)	0.60 (0.17–1.99)	0.514
AG + GG	39 (31.7)	40 (28.6)	1.16 (0.68–1.97)	0.592
Allele				
A	202 (82.11)	230 (82.14)	1.00	-
G	44 (17.89)	50 (17.86)	1.00 (0.63–1.60)	0.916

4. Discussion

Drug addiction continues to be a serious medical and social problem. Genes encoding the opioid receptors (OPRM1, OPRD1 and OPRK1) are potentially candidates for association with the risk of heroin addiction. In the present study, we examined the impact of *OPRM1* rs1799971 (A118G, Asn40ASp) and rs9479757 polymorphisms on heroin dependence in a sample of southeast Iranian population. The findings presented here propose that rs1799971 variant was not associated with heroin dependence in the population studied. We found that the *OPRM1* rs9479757 variant was not polymorphic and all participants were GG genotype. Beer et al [19] investigated the impact of *OPRM1* rs9479757 variant on opioid dependence in European population. In contrast to our findings, they found that the frequencies of GG, GA and AA genotypes of *OPRM1* rs9479757 variant in cases

and controls were 0.92, 0.8, 0.01 and 0.84, 0.16, 0.0, respectively. The GA genotype was associated with protection against opioid dependence.

OPRM1 rs9478495, rs3778150, rs9384169 and rs562859 polymorphisms of *OPRM1* gene have been shown to be associated with the risk of heroin addiction [20]. Genetic findings suggest that sequence variations *OPRK1* genes which encode the kappa-opioid receptors associated with the risk for alcohol dependence as well as opioid and cocaine dependence [21,22]. Glatt et al [23] have found no significant association between the Asn40Asp polymorphism and opioid dependence in the family-based association analysis as well as a meta-analysis of case-control studies. A meta-analysis performed by Haerian et al [12] revealed that the nonsynonymous *OPRM1* rs1799971 (A118G, Asp40Asp) might be a risk factor for addiction to opioids or heroin in an Asian population. Nikolov et al [24] have found no significant association between *OPRM1* rs1799971 and heroin addiction in Bulgarian population. No significant association was observed between *OPRM1* polymorphisms and severe opioid dependence [25].

It has been shown that rs1799971 (A118G, Asn40ASp) variant of *OPRM1* is associated with heroin addiction [13] as well as alcoholic addition [26,27]. While, rs16918875, rs963549 and rs702764 polymorphisms of *OPRK1* were not associated with heroin addiction or alcoholic addition [26]. Franke [14] have found no significant association between rs1799971 polymorphism of the *OPRM1* and risk of heroin and alcohol dependence. Xu et al [15] have found no significant association for a role of *OPRM1* 17 T > C and 24 G > A as well as *OPRD1* 921 T > C and heroin dependence. Clarke et al [16] findings showed an association between *OPRM1* rs62638690 and cocaine and heroin addiction in European Americans [16]. It has been shown that *OPRM1* rs1799971 contributes to individual differences in sensitivity to the rewarding effects of alcohol. The rs1799971 variant is thought to increase receptor binding affinity for β-endorphin by threefold [6]. The functional rs1799971 (A118G, Asn40Asp) polymorphism of *OPRM1* gene has been shown to be associated with heroin use outcomes in Caucasian males [28].

Epigenetics refers to heritable and possibly reversible modifications in gene expression that do not involve changes to the underlying DNA sequence. Various mode of epigenetics including DNA methylation, histone modification, chromatin remodeling and non-coding RNA-mediated processes are supposed to influence gene expression [29–31]. Structural and functional changes in the brain accompany repeated exposure to drugs of abuse propose that alterations in gene regulation contribute substantially to the addictive phenotype [29,31].

Recently, Ebrahimi et al [32] showed promoter hypermethylation of *OPRM1* in opium use disorder.

The inconsistency between the findings may be related to genetic and environmental differences between the populations studied. One of the limitations of this study is relatively small sample sizes. The power of the study was not enough to detect a mild or moderate association between *OPMR1* and heroin addiction.

In conclusion, our findings did not support an association between *OPRM1* rs1799971 variant and risk of heroin dependence in a sample of Iranian population, but this is not definitive. Larger sample sizes with different ethnicities are required to validate our findings.

Acknowledgements

This work was supported by a dissertation grant (MD thesis of ZR#7258) from Zahedan University of Medical Sciences.

Conflict of Interest

The authors declare that there is no conflict of interest to disclose.

References

1. Jones JD, Luba RR, Vogelman JL, et al. (2016) Searching for evidence of genetic mediation of opioid withdrawal by opioid receptor gene polymorphisms. *Am J Addict* 25: 41-48.
2. Ridenour TA, Maldonado-Molina M, Compton WM, et al. (2005) Factors associated with the transition from abuse to dependence among substance abusers: implications for a measure of addictive liability. *Drug Alcohol Depend* 80: 1-14.
3. Kendler KS, Jacobson KC, Prescott CA, et al. (2003) Specificity of genetic and environmental risk factors for use and abuse/dependence of cannabis, cocaine, hallucinogens, sedatives, stimulants, and opiates in male twins. *Am J Psychiatry* 160: 687-695.
4. Tsuang MT, Lyons MJ, Meyer JM, et al. (1998) Co-occurrence of abuse of different drugs in men: the role of drug-specific and shared vulnerabilities. *Arch Gen Psychiatry* 55: 967-972.
5. van den Bree MB, Johnson EO, Neale MC, et al. (1998) Genetic and environmental influences on drug use and abuse/dependence in male and female twins. *Drug Alcohol Depend* 52: 231-241.
6. Bond C, LaForge KS, Tian M, et al. (1998) Single-nucleotide polymorphism in the human mu opioid receptor gene alters beta-endorphin binding and activity: possible implications for opiate addiction. *Proc Natl Acad Sci U S A* 95: 9608-9613.
7. Selley DE, Bidlack JM (1992) Effects of beta-endorphin on mu and delta opioid receptor-coupled G-protein activity: low-Km GTPase studies. *J Pharmacol Exp Ther* 263: 99-104.
8. Zadina JE, Hackler L, Ge LJ, et al. (1997) A potent and selective endogenous agonist for the mu-opiate receptor. *Nature* 386: 499-502.
9. Basbaum AI, Fields HL (1984) Endogenous pain control systems: brainstem spinal pathways and endorphin circuitry. *Annu Rev Neurosci* 7: 309-338.
10. Pasternak GW (1993) Pharmacological mechanisms of opioid analgesics. *Clin Neuropharmacol* 16: 1-18.

11. Kreek MJ (1996) Opioid receptors: some perspectives from early studies of their role in normal physiology, stress responsivity, and in specific addictive diseases. *Neurochem Res* 21: 1469-1488.
12. Haerian BS, Haerian MS (2013) OPRM1 rs1799971 polymorphism and opioid dependence: evidence from a meta-analysis. *Pharmacogenomics* 14: 813-824.
13. Zhang Y, Picetti R, Butelman ER, et al. (2015) Mouse model of the OPRM1 (A118G) polymorphism: differential heroin self-administration behavior compared with wild-type mice. *Neuropsychopharmacology* 40: 1091-1100.
14. Franke P, Wang T, Nothen MM, et al. (2001) Nonreplication of association between mu-opioid-receptor gene (OPRM1) A118G polymorphism and substance dependence. *Am J Med Genet* 105: 114-119.
15. Xu K, Liu XH, Nagarajan S, et al. (2002) Relationship of the delta-opioid receptor gene to heroin abuse in a large Chinese case/control sample. *Am J Med Genet* 110: 45-50.
16. Clarke TK, Crist RC, Kampman KM, et al. (2013) Low frequency genetic variants in the mu-opioid receptor (OPRM1) affect risk for addiction to heroin and cocaine. *Neurosci Lett* 542: 71-75.
17. Shi J, Hui L, Xu Y, et al. (2002) Sequence variations in the mu-opioid receptor gene (OPRM1) associated with human addiction to heroin. *Hum Mutat* 19: 459-460.
18. Hashemi M, Hanafi Bojd H, Eskandari Nasab E, et al. (2013) Association of Adiponectin rs1501299 and rs266729 Gene Polymorphisms With Nonalcoholic Fatty Liver Disease. *Hepat Mon* 13: e9527.
19. Beer B, Erb R, Pavlic M, et al. (2013) Association of polymorphisms in pharmacogenetic candidate genes (OPRD1, GAL, ABCB1, OPRM1) with opioid dependence in European population: a case-control study. *PLoS One* 8: e75359.
20. Hancock DB, Levy JL, Gaddis NC, et al. (2015) Cis-Expression Quantitative Trait Loci Mapping Reveals Replicable Associations with Heroin Addiction in OPRM1. *Biol Psychiatry* 78: 474-484.
21. Wei SG, Zhu YS, Lai JH, et al. (2011) Association between heroin dependence and prodynorphin gene polymorphisms. *Brain Res Bull* 85: 238-242.
22. Yuferov V, Ji F, Nielsen DA, et al. (2009) A functional haplotype implicated in vulnerability to develop cocaine dependence is associated with reduced PDYN expression in human brain. *Neuropsychopharmacology* 34: 1185-1197.
23. Glatt SJ, Bousman C, Wang RS, et al. (2007) Evaluation of OPRM1 variants in heroin dependence by family-based association testing and meta-analysis. *Drug Alcohol Depend* 90: 159-165.
24. Nikolov MA, Beltcheva O, Galabova A, et al. (2011) No evidence of association between 118 A > G OPRM1 polymorphism and heroin dependence in a large Bulgarian case-control sample. *Drug Alcohol Depend* 117: 62-65.

25. Crowley JJ, Oslin DW, Patkar AA, et al. (2003) A genetic association study of the mu opioid receptor and severe opioid dependence. *Psychiatr Genet* 13: 169-173.
26. Kumar D, Chakraborty J, Das S (2012) Epistatic effects between variants of kappa-opioid receptor gene and A118G of mu-opioid receptor gene increase susceptibility to addiction in Indian population. *Prog Neuropsychopharmacol Biol Psychiatry* 36: 225-230.
27. Bart G, Kreek MJ, Ott J, et al. (2005) Increased attributable risk related to a functional mu-opioid receptor gene polymorphism in association with alcohol dependence in central Sweden. *Neuropsychopharmacology* 30: 417-422.
28. Woodcock EA, Lundahl LH, Burmeister M, et al. (2015) Functional mu opioid receptor polymorphism (OPRM1 A(118) G) associated with heroin use outcomes in Caucasian males: A pilot study. *Am J Addict* 24: 329-335.
29. Nestler EJ (2014) Epigenetic mechanisms of drug addiction. *Neuropharmacology* 76: 259-268.
30. Robison AJ, Nestler EJ (2011) Transcriptional and epigenetic mechanisms of addiction. *Nat Rev Neurosci* 12: 623-637.
31. Nielsen DA, Utrankar A, Reyes JA, et al. (2012) Epigenetics of drug abuse: predisposition or response. *Pharmacogenomics* 13: 1149-1160.
32. Ebrahimi G, Asadikaram G, Akbari H, et al. (2017) Elevated levels of DNA methylation at the OPRM1 promoter region in men with opioid use disorder. *Am J Drug Alcohol Abuse* 25: 1-7.

AIMS Press