

The Effect of OPRM1 rs648893 Gene Polymorphism on Opioid Addiction in an Iranian population in Zabol: A Case-Control Study

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Abstract

Introduction: Opioid addiction (OA) is a neurologically life-threatening challenge associated with socioeconomic and health concerns for individuals and society. The addictive drugs trigger neuromodulators and neurotransmitters through the opioid receptors and corresponding endogenous peptide ligands. In addition, drug addiction is reportedly related to the mu-opioid receptor (*OPRM1*) encoding gene and its variants. According to the role of the rs648893 polymorphism of the *OPRM1* gene in numerous disorders, it has been suggested as a candidate associated with drug addiction. The present case-control study was conducted to evaluate the role of *OPRM1* rs648893 polymorphism in the OA risk.

Methods: To this end, the rs648893 polymorphism was genotyped by tetra amplification refractory mutation system-polymerase chain reaction among 160 Iranian subjects consisting of 105 OA cases and 155 controls.

Results: According to our findings, there was no significant association between OA and the *OPRM1* rs648893 gene polymorphism. Moreover, a marginally insignificant difference was found between OA cases and controls in accordance with the allelic frequencies ($P=0.05$)

Conclusion: In general, our results reported no association between *OPRM1* rs648893 gene polymorphism and OA although further research among various ethnicities with larger sample sizes is needed to draw a definite conclusion on the association of rs648893 polymorphism and other *OPRM1* intronic variants with opioid and other addictions.

Keywords: Addiction, Opioid receptor, *OPRM1*, Polymorphism

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Introduction

Opioid addiction (OA) as an emerging epidemic has involved a significant number of people around the world in the current century. In the last decade, prescription and non-prescription opioids have been increasingly abused, especially among adults.¹ Nonetheless, the pathogenesis of addictive disorders and mechanisms of therapeutic approaches have not been well understood yet for drug addicts while serious socioeconomic problems are resulting from using illegal drugs in society.²

Opioids are highly addictive narcotics with applications to manage pain. However, opioid abuse can often result in developing overdose, dependence, and tolerance.³

They are naturally occurring compounds that physiologically influence the human body. The synthetic, semi-synthetic, and natural opioids apply their anti-nociception impacts on the central nervous system through opioid receptors.⁴

Accordingly, it is essential to scrutinize the genetic parameters involved in OA with an emphasize on the detection of new therapeutic targets, probably finding the genetic biomarkers of OA risk, and thus developing clinical purposes.⁵

The opioids are able to link with mu (μ), kappa (κ), and delta (δ) opioid receptors, thus changing neurotransmission processes.⁶ Both central and peripheral nervous systems carry such receptors⁷ affected by these opioids, thus resulting in

analgesia, tolerance, reward, and dependence.⁸

Almost all opiates have primarily high affinity to the μ -opioid receptor, which is originally an active binding site of endogenous and exogenous opioid drugs and peptides.⁹

Further, opioid receptor genes, due to genetic variations, can affect the structure, function, and expression of the receptors, thus leading to decreased or increased risk of opioid dependency.^{10,11}

The opioid dependence as medical and social concerns has affected people around the world, including Iran.¹² This highlights the necessity of further research in order to uncover genetic variables contributing to OA development, to show the possible genetic association, to raise the neurobiological knowledge of opioid dependence, and finally, to produce the analgesics with higher effectiveness and minimum side effects.

There are many documents on the importance of different genes related to the endogenous opioid system involved in the OA risk.¹³ Although the association between the OA and polymorphisms in opioid receptor genes has been reported among Iranian population,² there is no study regarding the correlation of *OPRM1* gene polymorphism and susceptibility to OA. Intronic sequences can be involved in alternative DNA splicing. The rs648893 (C/T) polymorphism is located at the third intron of the *OPRM1* gene, and some studies have previously reported the effect of this polymorphism on numerous disorders.^{14,15} Therefore, this study was the first one to examine the association of the *OPRM1* rs648893 gene polymorphism and OA in an Iranian population.

Materials and Methods

Study Population

The study was conducted on 105 OA subjects, including 9 females and 96 males, who referred to the Center for Drug Addiction Treatment in Zabol, Sistan and Baluchestan province, Iran. Their drug dependency was recognized on the basis of the Diagnostic and Statistical Manual of Mental Disorders and the International Statistical Classification of Diseases.^{16,17} The exclusion criteria were major psychiatric disorders affecting neurotransmitter systems.^{16,17} On the other hand, 155 self-reported cases, including 26 females and 129 males, with no history of drug abuse and major psychiatric disorders were included

as controls. All samples represented a willingness to participate in the study and signed written consent form.

DNA Extraction and Genotyping

The blood samples were taken from the research units and maintained at -20°C until testing. The salting-out method was employed to extract genomic DNA.

Then, the samples were genotyped by the tetra amplification refractory mutation system-polymerase chain reaction (ARMS-PCR). The primers were designed (Table 1) by the online Soton website (<http://primer1.soton.ac.uk/primer1.html>). Next, the genes were amplified by the PCR process using a mixture with the 20- μL final volume, containing 1 μL of F/R primer, 10 mL of 2 \times Taq PCR Master Mix (Genetbio, Denmark), 1 μL of template DNA, and 5 μL of water. The thermal program of Thermocycler was 30-second denaturation at 94°C , 30-second annealing at 67°C , and 50-second extension at 72°C within 30 cycles. The DNA products were run on a 3% agarose gel electrophoresis, stained by the green viewer, and the resulting gel pictures were obtained by the Life Technologies E-gel imager instrument (Figure 1). The randomly repeated genotyping for about 20% of the samples showed no mistake in genotyping.

Statistical Analysis

The collected data were statistically analyzed by SPSS software, version 20. The Hardy-Weinberg equilibrium was explored for study groups (OA patients and controls) using the Chi-square test. The association of rs648893 polymorphism with OA was examined by logistic regression analysis through calculating a 95% confidence interval (CI) and the odds ratio (OR) at the statistically significant level of $P < 0.05$.

Results

The frequency of *OPRM1* rs648893 genotypes among the study populations is shown in Table 2. No significant deviation from Hardy-Weinberg equilibrium was found in the SNP data for cases and controls.

According to Table 2, the frequencies of TT (ancestral), TC, and CC genotypes of *OPRM1* rs648893 were 80%, 18.1%, and 1.9% in cases versus 88.4%, 11%, and 0.6% in controls, respectively. Based on logistic regression findings, the OA showed no significant association with

Table 1. Primers Sequences

Primer Name	Primer Sequence 5' \rightarrow 3'	PCR Product (bp)
Forward outer primer	312 TCTGATCATTTCTGTAGCTGGTCACACA 339	343
Reverse outer primer	654 GACTAAGCAAGCAAGTAGGTTGCTTTGC 627	
Forward inner primer (C allele)	476 TGTGTACTGCAGTCTGGTCCCATAGC 501	180
Reverse inner primer (T allele)	526 CTACTCCCAAATCCCAAGGCACTA 501	215

Note. PCR: Polymerase chain reaction.

Table 2. The Frequency of Alleles and Genotypes of *OPRM1* rs648893 Polymorphism in Case and Control Groups

<i>OPRM1</i> , rs648893	Case (n=105)	Control (n=155)	P Value	OR (95% CI)
TT (%)	84 (80)	137 (88.4)		Reference
TC (%)	19 (18.1)	17 (11)	0.1	1.8 (0.9-3.7)
CC (%)	2 (1.9)	1 (0.6)	0.3	3.3 (0.3-36.5)
Dominant (TC+CC vs. TT)			0.07	1.9 (0.9-3.7)
Recessive (CC vs. TT+TC)			0.4	3 (0.3-33.4)
T (%)	187 (89)	291 (93.9)		Reference
C (%)	23 (11)	19 (6.1)	0.05	1.9 (1-3.5)

Note. *OPRM1*: Mu-opioid receptor; OR: odds ratio; CI: confidence interval.

the *OPRM1* rs648893 gene polymorphism. However, the higher frequency of TC+CC genotype versus TT was found in cases in the dominant model compared to the OA group although it was not significant ($P=0.07$). Moreover, there were marginally insignificant differences in rs648893 allele distributions between controls and cases ($P=0.05$).

Discussion

Drug addiction as one of the life-threatening disorders in the nervous system is affected by environmental, behavioral, and genetic factors.¹⁸ The drug dependence has been reportedly shown to be significantly affected by genetic factors according to family, twin, and adoption investigations.¹⁹ Recent studies have revealed the interactions between the genetics of complex disorders and certain variants significantly affecting the OA risk, which are probably more ready in many replication studies.²⁰

Regarding the effectiveness of drug addiction treatment, the drug addiction risk and the interpersonal variations depend on various genetic factors.²¹ There are reports on the association between drug addiction and the polymorphisms of some genes such as those related to

opioid receptors and ligands.²²

The opioid μ -receptor encoded by the *OPRM1* gene on chromosome 6q24-q25 not only can regulate the analgesic response to pain but also can control the rewarding impacts of different addictive drugs such as opioids and nicotine, as well as alcohol consumption.²³

Studies on the numerous ethnic groups through the genome sequencing of the *OPRM1* gene reported 3324 polymorphisms occupied a 200-kb area on chromosome 6q24-q25 (<http://www.1000genomes.org>). The frequency of many of such polymorphisms is very low with restricted relevance in the populations. However, the global populations have exhibited minor allele frequencies >1% for 1395 genetic variants. The sensitivity to opioids can be significantly affected by inter-individual differences in the *OPRM1* gene.

Additionally, the *OPRM1* SNPs have been indicated to be important in mediating differences in the OA, which might be related to opioid dependence and ethnic variations in opioid dependence.²⁴

Although there is a strong association between *OPRM1* exonic SNPs and the risk of opioid dependency,^{18,25-27} there are a few studies regarding the association between *OPRM1* intronic SNPs and the risk of opioid dependency.²⁸ Moreover, previous results showed an association between *OPRM1* intronic SNPs and heroin dependency.²⁹ According to Zhang et al, the intronic variants of the *OPRM1* gene including rs648893 polymorphism have roles in susceptibility to alcohol and drug dependency in European populations.¹⁵

Based on the literature review, this is the first study to evaluate the effect of *OPRM1* rs648893 polymorphism on the OA in an Iranian population. Although no association was found between the OA and the *OPRM1* rs648893 gene polymorphism. The distribution of the rs648893 allele suggested slightly significant differences between OA cases and healthy controls ($P=0.05$). Arias et al evaluated the association between rs648893 and suicide-associated outcomes in 426 European-Americans and reported no genotypic/allelic association with phenotypes of suicide-associated outcomes.³⁰ In consistent to our results, Xuei

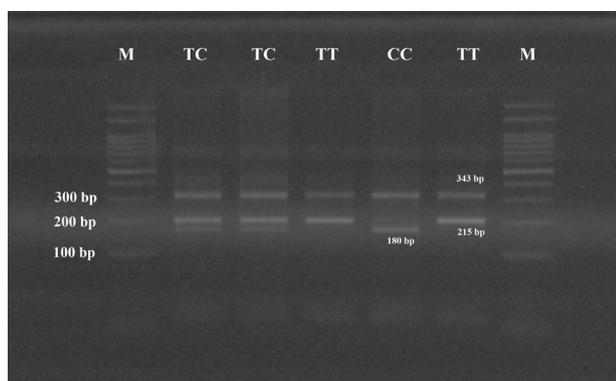


Figure 1. Tetra Amplification Refractory Mutation System-Polymerase Chain Reaction Results for *OPRM1*-rs648893 Polymorphism. M: 100 bp DNA marker.

et al found no significant association between rs648893 polymorphism and drug or alcohol dependency among European Americans.³¹

Conclusion

The vulnerability to reliance on substance can be affected by *OPRM1* genetic variation, which can influence the treatment response to opioid antagonists. According to the results of this study, there was no significant association between the OA and the *OPRM1* rs648893 gene polymorphism. However, further research on various ethnicities and with larger sample sizes is needed to draw a definite conclusion on the association between rs648893 polymorphism and other *OPRM1* intronic variants with opioids and other addictions.

Ethical Approval

The Ethics Committee of Zabol University approved our research project with the code of IR.UOZ.Rec.1393.01 according to the Declaration of Helsinki.

Conflict of Interest Disclosure

We have no conflict of interests.

Informed Consent

The participants provided written informed consent.

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Authors' Contribution

AR: the conception and design of the study, data analysis, and manuscript preparation

FD: data collection

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