Mu Opioid Receptor Gene: New Point Mutations in Opioid Addicts

Amin Dinarvand[1], Ali Goodarzi[2], Nasim Vousooghi[3,4], Mehrdad Hashemi[5], Rasoul Dinarvand[6,7,8], Fahimeh Ostadzadeh[1], Ahad Khoshzaban[9], Mohammad-Reza Zarrindast[2,3,10,11,12*]

1. Science and Research Branch of Islamic Azad University, Tehran, Iran.
2. Iranian National Center for Addiction Studies, Tehran University of Medical Sciences, Tehran, Iran.
3. Department of Neuroscience, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran.
4. Genetics laboratory, Iranian National Center for Addiction Studies, Tehran University of Medical Sciences, Tehran, Iran.
5. Department of Genetics, Islamic Azad University, Tehran Medical Branch, Tehran, Iran.
6. Department of Pharmacoeconomics and Pharmaceutical Administration, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran.
7. Pharmaceutical Sciences Research Center, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran.
8. Nanotechnology Research Center, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran.
9. Eye Research Center, Farabi Eye Hospital, Tehran University of Medical Sciences, Tehran, Iran.
10. Department of Pharmacology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran.
11. Institute for Studies in Theoretical Physics and Mathematics, School of Cognitive Sciences, Tehran, Iran.
12. Institute for Cognitive Science Studies, Tehran, Iran.

Article info:
Received: 27 November 2012
First Revision: 02 March 2013
Accepted: 13 August 2013

Key Words:
Addiction,
OPRM1 Gene,
Polymorphism.

ABSTRACT

Introduction: Association between single-nucleotide polymorphisms (SNPs) in mu opioid receptor gene and drug addiction has been shown in various studies. Here, we have evaluated the existence of polymorphisms in exon 3 of this gene in Iranian population and investigated the possible association between these mutations and opioid addiction.

Methods: 79 opioid-dependent subjects (55 males, 24 females) and 134 non-addict or control individuals (74 males, 60 females) participated in the study. Genomic DNA was extracted from volunteers' peripheral blood and exon 3 of the mu opioid receptor gene was amplified by polymerase chain reaction (PCR) whose products were then sequenced.

Results: Three different heterozygote polymorphisms were observed in 3 male individuals: 759T>C and 877G>A mutations were found in 2 control volunteers and 1043G>C substitution was observed in an opioid-addicted subject. Association between genotype and opioid addiction for each mutation was not statistically significant.

Discussion: It seems that the sample size used in our study is not enough to confirm or reject any association between 759T>C, 877G>A and 1043G>C substitutions in exon 3 of the mu opioid receptor gene and opioid addiction susceptibility in Iranian population.

1. Introduction

There is a lot of evidence suggesting that genetic factors have a considerable role in driving a person toward opioid addiction, although the importance of environmental elements cannot be underestimated (Kendler, Jacobson, Prescott, & Neale, 2003; Tsuang et al., 1996). It has been shown that family members of opioid addicts are at greater risk of developing addiction in comparison to normal people (Luthar, Anton, Merikangas, & Rounsaville, 1992). Among all receptors involved in opioid addiction, mu opioid receptor (MOR) is the major one mediating opioid tolerance and dependence (Bart et al.,

* Corresponding Author:
Mohammad-Reza Zarrindast, PhD
Department of Neuroscience, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, 1417755469, Tehran, Iran.
Tel: (+9821)-8899-1118/ Fax: (+9821)-8899-1117
E-mail: zarinmr@ams.ac.ir

2004; Glatt et al., 2007). This receptor is encoded by a gene called OPRM1 which expands more than 80 kbp of nucleotide sequences on chromosome 6q24-25 and is composed of transcript regulatory region, introns and exons (Pan et al., 2005; Wang et al., 1994). In OPRM1 knockout mice, complete abolishment of morphine-induced physical dependence and place preference activity has been observed (Sora et al., 1997). It has been reported by many investigators that OPRM1 gene may have a potential role in individual susceptibility toward addiction (Compton, Geschwind, & Alarcon, 2003; Luo, Kranzler, Zhao, & Gelernter, 2003).

Several studies have shown the presence of single nucleotide polymorphisms (SNPs) on different parts of the OPRM1 gene (Hoehe et al., 2000). Some of these SNPs lead to the substitution of amino acids in receptor molecule which may affect receptor function, while others may influence gene transcription and receptor expression (Lotsch & Geisslinger, 2005). A variety of these SNPs have been shown to be associated with drug addiction in humans. For example, it has been reported that A118G substitution in exon 1 of the OPRM1 gene is significantly associated with heroin addiction in Hong Kong Chinese population (Szeto, Tang, Lee, & Stadlin, 2001). Involvement of this mutation in alcohol dependence has also been evaluated (Town et al., 1999). Other investigators have found other polymorphisms like C17T in exon 1 to be significantly associated with drug dependence (Bond et al., 1998).

Although the SNPs of the OPRM1 gene have been reported in many populations, there are very limited studies regarding the state and frequencies of mutations on the OPRM1 among Iranian people. Besides, there is no published paper focusing on the relationship between mutations of this gene and opioid addiction in Iranian opioid addicts. In the present study, we have investigated the polymorphisms of the exon 3 of Iranian OPRM1gene and evaluated the possible association of these mutations with opioid dependence in this population.

2. Methods

2.1. Subjects

A total of 213 Iranian volunteers participated in the present study. They were divided into two groups: 79 unrelated opioid-dependent subjects (55 males, 24 females; mean age 31.3 years) recruited from Iranian National Center for Addiction Studies (INCAS) and 134 non-addict or control individuals (74 males, 60 females; mean age 32.7 years) who were healthy blood donors at the Boghrat laboratory with no history of substance abuse. Subjects were first visited by a professional psychiatrist to evaluate their drug habit situation. Patients were included in the opioid-dependent group of the study if the result of their urine test was positive for opioids and also they fulfilled DSM- IV (Diagnostic and Statistical Manual of Mental Disorders, 4th Edition) criteria for substance abuse disorder. Urine analysis was also used to exclude multiple drug abuse in the mentioned group. A history of psychiatric diseases, smoking and gambling were other exclusion criteria of the study. This study was approved by Tehran University of Medical Sciences Ethics Committee. All subjects gave a written informed consent before participating in the study.

2.2. DNA Extraction and Genotyping

5 ml peripheral blood was drawn from each subject and anticoagulated with EDTA. DNA was extracted from blood samples using the G-DEXTM Genomic DNA Extraction Kit for Blood (iNtRON Biotechnology, Korea). 50 ng of genomic DNA was used to perform Polymerase chain reaction (PCR) with LA Taq (Takara Bio Inc., Shiga, Japan). Exon 3 of the MOR1 was amplified using a forward primer: 5'- TGGCAGTATTAACACCTTATG-3' and the reverse primer: 5'- TACCTGATGATTAGTTCTATCC-3'. PCR was carried out in a thermalcycler. The amplification program was: denaturation for 5 min at 95°C, 26 cycles for 12 s at 95°C, annealing for 15 s at 50°C, extension for 15 s at 72°C and final elongation for 10 min at 72°C. The PCR products were then sequenced using an automated DNA sequencer ABIPrism 3130XL (Applied Biosystems, Foster City, Ca, USA). In order to detect polymorphisms in PCR products, the observed sequences were compared with the native OPRM1 DNA sequence (Gene Bank, AY587764). To confirm the observed data, all samples with a mutation were re-sequenced.

2.3. Statistical Analysis

Chi-square analysis was done to compare genotypic and allelic distribution between control subjects and addicts using SPSS for Windows 14.0. Statistical significance level was set at $P<0.05$.

3. Results

In all 213 studied samples, mutations in exon 3 of the OPRM1 gene were observed only in 3 male individuals. All detected mutations were heterozygote and each one of the mentioned 3 subjects showed a different polymorphism: 759T>C and 877G>A substitutions were

observed in 2 healthy subjects and 1043G>C mutation was detected in an opioid-addict individual. Thus, the frequency of each mutation in the whole samples is less than 1%. Statistical analysis revealed no significant relationship between genotype and opioid-addiction for each observed mutation in the studied population (Chi-square test, P>0.05).

4. Discussion

The role of OPRM1 gene in opioid effects including addiction has been well documented (Pasternak, 1993). During recent years, investigators have found various polymorphisms in OPRM1gene (Lotsch & Geisslinger, 2005). In the present study, we looked for the possible mutations in exon 3 of the OPRM1gene and their relationship with opioid addiction in Iranian population. Exon 3 of this gene spans from 642 to 1126 and encodes 2⁄3 of the second extracellular loop, transmembrane domains V–VII, and 2⁄3 of the C-terminal tail. The importance of this exon in MOR mutation studies comes from reports claiming that SNPs in this exon like 779G>A or 794G>A can result in impaired receptor signaling (Wang, Quillan, Winans, Lucas, & Sadee, 2001). Another example is 802T>C SNP which leads to the loss of receptor desensitization by Ca2+/calmodulin-dependent protein-kinase-II (Koch et al., 2000) and significant reduction of opioid efficacy and potency (Befort et al., 2001). 820G>A and 942G>A are other SNPs in exon 3 mentioned in previous reports (Bond et al., 1998; Wang, Quillan, Winans, Lucas, & Sadee, 2001). None of these SNPs were found in our studied samples which is probably due to different ethnic populations or limited number of individuals in our study.

To our knowledge, this may be the first study reporting two novel mutations (759T>C and 1043G>C) in exon 3 of the human OPRM1 gene. Although the allele frequencies of both mutations were less than 1% in our population, another study with higher number of samples is required to confirm the occurrence rate of described mutations in Iranian people. Whether these two substitutions affect receptor function or signaling also needs to be studied in the future.

In addition to these two novel mutations, we observed another polymorphism (877G>A) in exon 3 that has been reported previously in Han Chinese samples (Shi et al., 2002) with a frequency similar to our results (<1%). There is no information about the effect of this mutation on ligand binding or receptor function.

It has been shown in previous studies that mutations in OPRM1 gene may affect human susceptibility to opioid addiction (Uhl, Sora , & Wang, 1999). For example, the frequency of mutant allele of the 17C>T SNP in exon 1was found to be higher in opioid addicts in comparison to non-addicts (Bond et al., 1998). It has been reported that opioid dependence is associated with mutant alleles of the 118A>G (exon 1) and 691C>G (intron 2) SNPs in Chinese heroin addicts (Szeto, Tang, Lee, & Stadlin, 2001). Another study has indicated that heroin consumption rate is directly related to the presence of both mutated 118G and 31A (intron 2) alleles in addicted individuals (Shi et al., 2002).The mutated allele of 118A>G SNP has also been observed more often in Indian heroin addicts than in control subjects (Tan, Tan, Karupathivan, & Yap, 2003). Although several papers have been published which address the association between OPRM1 gene mutations and drug addiction, relationship between mutations in exon 3 of this gene and opioid addiction has been rarely investigated. This may be due to the low frequency of mutated alleles in evaluated populations (Shi et al., 2002). In the present study, we did not find a significant relationship between genotype and opioid addiction for all 3 observed low frequency mutations which may be due to the small sample size of our study. Thus we cannot conclude whether our observed genetic variations in exon 3 of the OPRM1 gene play any role in driving a subject toward opioid addiction. To answer this question, the study should be repeated with a higher number of samples (at least 400) in the future. Also a genetic screening for SNPs in other exons and introns of the OPRM1 gene and the evaluation of OPRM1 expression level in subjects with different genotypes is suggested to further clarify the importance of gene mutations in opioid addiction.

References

Bart, G., Heilig, M., LaForge, K.S., Pollak, L., Leal, S.M., Ott, J., et al. (2004). Substantial attributable risk related to a functional mu-opioid receptor gene polymorphism in association with heroin addiction in central Sweden. Molecular Psychiatry, 9(6), 547-9.

Befort, K., Filliol, D., Decaillot, F.M., Gaveriaux-Ruff, C., Hoehe, M.R., Kieffer, B.L. (2001). A single nucleotide polymorphic mutation in the human mu-opioid receptor severely impairs receptor signaling. Journal of Biological Chemistry, 276(5), 3130-7.

Bond, C., LaForge, K.S., Tian, M., Melia, D., Zhang, S., Borg, L., et al. (1998). Single-nucleotide polymorphism in the human mu opioid receptor gene alters beta-endorphin binding and activity: possible implications for opiate addiction. Proceedings of the National Academy of Sciences of the United States of America, 95(16), 9608-13.

Compton, P., Geschwind, D.H., Alarcon, M. (2003). Association between human mu-opioid receptor gene polymorphism, pain tolerance, and opioid addiction. American Journal of Medical Genetics Part B: Neuropsychiatric Genetics,121B(1), 76-82.

Glatt, S.J., Bousman, C., Wang, R.S., Murthy, K.K., Rana, B.K., Lasky-Su, J.A., et al. (2007). Evaluation of OPRM1 variants in heroin dependence by family-based association testing and meta-analysis. Drug and Alcohol Dependence, 90(2-3), 159-65.

Hoehe, M.R., Kopke, K., Wendel, B., Rohde, K., Flachmeier, C., Kidd, K.K., et al. (2000). Sequence variability and candidate gene analysis in complex disease: association of mu opioid receptor gene variation with substance dependence. Human Molecular Genetics, 9(19), 2895-908.

Kendler, K.S., Jacobson, K.C., Prescott, C.A., Neale, M.C. (2003). Specificity of genetic and environmental risk factors for use and abuse/dependence of cannabis, cocaine, hallucinogens, sedatives, stimulants, and opiates in male twins. American Journal of Psychiatry, 160(4), 687-95.

Koch, T., Kroslak, T., Averbeck, M., Mayer, P., Schroder, H., Raulf, E., et al. (2000). Allelic variation S268P of the human mu-opioid receptor affects both desensitization and G protein coupling. Molecular Pharmacology, 58(2), 328-34.

Lotsch, J., Geisslinger, G. (2005). Are mu-opioid receptor polymorphisms important for clinical opioid therapy? Trends in Molecular Medicine, 11(2), 82-9.

Luo, X., Kranzler, H.R., Zhao, H., Gelernter, J. (2003). Haplotypes at the OPRM1 locus are associated with susceptibility to substance dependence in European-Americans. American Journal of Medical Genetics Part B: Neuropsychiatric Genetics, 120B(1), 97-108.

Luthar, S.S., Anton, S.F., Merikangas, K.R., Rounsaville, B.J. (1992). Vulnerability to substance abuse and psychopathology among siblings of opioid abusers. Journal of Nervous and Mental Disease, 180(3), 153-61.

Pan, L., Xu, J., Yu, R., Xu, M.M., Pan, Y.X., Pasternak, G.W., et al. (2005). Identification and characterization of six new alternatively spliced variants of the human mu opioid receptor gene, Oprm. Neuroscience, 133(1), 209-20.

Pasternak, G.W. (1993). Pharmacological mechanisms of opioid analgesics. Clinical Neuropharmacology, 16(1), 1-18.

Shi, J., Hui, L., Xu, Y., Wang, F., Huang, W., Hu, G. (2002). Sequence variations in the mu-opioid receptor gene (OPRM1) associated with human addiction to heroin. Human Mutation, 19(4), 459-60.

Sora, I., Takahashi, N., Funada, M., Ujike, H., Revay, R.S., Donovan, D.M., et al. (1997). Opiate receptor knockout mice define mu receptor roles in endogenous nociceptive responses and morphine-induced analgesia. Proceedings of the National Academy of Sciences of the United States of America, 94(4), 1544-9.

Szeto, C.Y., Tang, N.L., Lee, D.T., Stadlin, A. (2001). Association between mu opioid receptor gene polymorphisms and Chinese heroin addicts. Neuroreport, 12(6), 1103-6.

Tan, E.C., Tan, C.H., Karupathivan, U., Yap, E.P. (2003). Mu opioid receptor gene polymorphisms and heroin dependence in Asian populations. Neuroreport, 14(4), 569-72.

Town, T., Abdullah, L., Crawford, F., Schinka, J., Ordorica, P.I., Francis, E., et al. Association of a functional mu-opioid receptor allele (+118A) with alcohol dependency. American Journal of Medical Genetics, 88(5), 458-61.

Tsuang, M.T., Lyons, M.J., Eisen, S.A., Goldberg, J., True, W., Lin, N., et al. (1996). Genetic influences on DSM-III-R drug abuse and dependence: a study of 3,372 twin pairs. American Journal of Medical Genetics, 67(5), 473-7.

Uhl, G.R., Sora, I., Wang, Z. (1999). The mu opiate receptor as a candidate gene for pain: polymorphisms, variations in expression, nociception, and opiate responses. Proceedings of the National Academy of Sciences of the United States of America, 96(14), 7752-5.

Wang, D., Quillan, J.M., Winans, K., Lucas, J.L., Sadee, W. (2001). Single nucleotide polymorphisms in the human mu opioid receptor gene alter basal G protein coupling and calmodulin binding. Journal of Biological Chemistry, 276(37), 34624-30.

Wang, J.B., Johnson, P.S., Persico, A.M., Hawkins, A.L., Griffin, C.A., Uhl, G.R., et al. (1994). Human mu opiate receptor. cDNA and genomic clones, pharmacologic characterization and chromosomal assignment. FEBS Letters, 338(2), 217-22.