Journal of Addictive Diseases

ISSN: 1055-0887 (Print) 1545-0848 (Online) Journal homepage: http://www.tandfonline.com/loi/wjad20

Association of OPRK1 gene polymorphisms with opioid dependence in addicted men undergoing methadone treatment in an Iranian population

Ali Albonaim, Hedyeh Fazel, Alireza Sharafshah, Vahid Omarmeli, Sajjad Rezaei, Farzam Ajamian & Parvaneh Keshavarz

To cite this article: Ali Albonaim, Hedyeh Fazel, Alireza Sharafshah, Vahid Omarmeli, Sajjad Rezaei, Farzam Ajamian & Parvaneh Keshavarz (2017) Association of OPRK1 gene polymorphisms with opioid dependence in addicted men undergoing methadone treatment in an Iranian population, Journal of Addictive Diseases, 36:4, 227-235, DOI: 10.1080/10550887.2017.1361724

To link to this article: https://doi.org/10.1080/10550887.2017.1361724

Accepted author version posted online: 08 Aug 2017.
Published online: 30 Aug 2017.

JOURNAL OF ADDICTIVE DISEASES
2017, VOL. 36, NO. 4, 227–235
https://doi.org/10.1080/10550887.2017.1361724

Taylor & Francis
Taylor & Francis Group

ARTICLE

Association of OPRK1 gene polymorphisms with opioid dependence in addicted men undergoing methadone treatment in an Iranian population

Ali Albonaim, MSc[a], Hedyeh Fazel, MSc[b], Alireza Sharafshah, MSc[a], Vahid Omarmeli, MSc[a], Sajjad Rezaei, PhD[c], Farzam Ajamian, PhD[d], and Parvaneh Keshavarz, PhD [e]

[a]Cellular and Molecular Research Center, Faculty of Medicine, Guilan University of Medical Sciences/University of Guilan, Rasht, Iran; [b]Cellular and Molecular Research Center, Faculty of Medicine, Guilan University of Medical Sciences, Rasht, Iran; [c]Department of Psychology, University of Guilan, Rasht, Iran; [d]Department of Biology, Faculty of Sciences, University of Guilan, Rasht, Iran; [e]Cellular and Molecular Research Center, Faculty of Medicine, Guilan University of Medical Sciences, Guilan, Rasht, Iran

ABSTRACT

Previous studies have shown significant associations between OPRK1 and susceptibility to opioid dependence and the relationships between libido dysfunction and insomnia among opium addicts who underwent methadone maintenance treatment. The authors investigated the single locus and haplotype association of rs997917, rs6985606, and rs6473797 with susceptibility to opioid addiction. Samples were selected among 202 healthy individuals and 202 opium addicts undergoing methadone maintenance treatment. Genomic DNA was extracted from the whole blood samples of all subjects through a salting out procedure. All three variants were genotyped in the studied subjects using Amplification Refractory Mutation System-Polymerase Chain Reaction (ARMS-PCR). The whole analysis process was performed using SNPAlyze and SPSS ver.20 software packages. According to the single locus analyses, rs997917 and rs6985606 represented significant associations with opium addiction under recessive ($p = 0.0128$) and co-dominant ($p = 0.0001$) inheritance models, respectively. The haplotypes C-T-C (Permutation $p = 0.014$) and C-T-T (Permutation $p = 0.0002$) were significantly associated with opioid dependence. Among methadone maintenance treatment individuals, rs997917 was significantly associated with insomnia in both allelic and genotypic levels ($p = 0.0001$ and $p = 0.038$, respectively). Furthermore, rs6985606 had the only significant association with the co-incidence of insomnia and libido dysfunction in the methadone maintenance treatment group ($p = 0.038$). The OPRK1 gene variants showed significant association with susceptibility to opioid dependence among Iranians.

KEYWORDS

Variant; OPRK1; MMT; libido dysfunction; insomnia; association; opium addiction

Introduction

Opioid and psychotic drugs addiction is one of the major predicaments worldwide. According to the 2012 statistical reports, about 243 million individuals around the world have, at least once, experienced drug abuse between the ages 15- and 64-years-old. The rate of drug abuse is two to three times more in men compared to women. The drug-related mortality rate was calculated at about 183,000 people in 2012.[1] In this regard, it has been reported that the growth rate of substance abuse in Iran is than three times compared to the rate of the population growth over the past two decades.[2]

Morphine is one of the main opium-derived opioids[3] that causes alterations in gene expression through adaptive changes in some brain regions and leads to phenotypic changes in the next generation.[4] Opiates, such as morphine (that are extracted from the poppy plant), and opiates including endogenous peptides, such as endorphins, affect three types of neuron receptors: kappa, mu, and delta.[5] It was found that these receptors were expressed widely in the brain and endocrine glands like adrenal cortex and the whole body.[6] Methadone is the most commonly prescribed medication for opioid substitution treatment (OST).[7] This substance, as a synthetic opioid, was approved by the Federal Drug Administration (FDA) in 1972 to treat addiction to opiates.[8] It is reported that

CONTACT Parvaneh Keshavarz, PhD keshavarz@gums.ac.ir Cellular and Molecular Research Center, Faculty of Medicine, Guilan University of Medical Sciences, 10th km Tehran road, Rasht, Iran, 3363.
Ali Albonaim, Hedyeh Fazel, Alireza Sharafshah, and Vahid Omarmeli have MSc degrees in Genetics; Sajjad Rezaei has a PhD in Psychology; Farzam Ajamian has a PhD in Molecular Genetics; and Parvaneh Keshavarz has a PhD in Medical Genetics.

methadone reduces dependency to the other opiates and declines the crime rates. It also transfers the risk of sexually transmitted diseases like Human Immunodeficiency Virus (HIV).[9] Methadone is absorbed by the intestinal-gastric system, with a plasma peak about 2 to 4 hours after the usage and a half-life between 10–18 hours.[10,11] Moreover, this substance has therapeutic function through declining opiate dependency because of its interactions with the opioid receptors.[12] Similar to morphine, methadone also has side effects including insomnia, constipation, sweating, cardiac arrhythmias, the risk of respiratory depression, and weight gain. However, insomnia problems and libido dysfunction are suggested as withdrawal indicators for many patients.[13,14]

Several studies have reported the association of opioid receptors genes with addiction dependence in different populations and the association of OPRD1, OPRK1, and OPRM1 variants in alcohol dependence.[15–18]

OPRK1 (or KOR) protein coding gene in human has four exons and is located on chromosome 8q11.23.[19] Studies have shown that polymorphism 36G>T of OPRK1 gene is associated with the risk of drug addiction. Moreover, some polymorphisms of this gene play a key role in alcoholism, anxiety, and pain.[18,20,21] According to the previous studies, rs6989250 polymorphism (C>G) plays an important role in the induction of stress and cocaine dependency.[22]

In a study on heroin-addicted Caucasian patients treated with methadone, some polymorphisms including rs6473797 in OPRK1 gene, rs2236861, rs2236857, and rs3766951 in OPRD1 gene, and also rs510769 and rs3778151 in OPRM1 gene were significantly associated with heroin addiction.[23]

Addiction is one of the most serious problems in Iran with vast effects in social, medical, and familial fields. Regarding the high economic costs of addiction due to drug abuse, searching better ways to solve this problem seems necessary. Moreover, insomnia problems and libido dysfunctions are important factors in methadone treatment withdrawal and more divorces in families. Altogether, the investigation of the susceptible polymorphisms before prescribing methadone can reduce the potential risk of its serious side effects remarkably. Therefore, the current study aimed to examine the association of OPRK1 gene variants including rs997917, rs6473797, and rs6985606 (all situated in intron 2) with the opioid dependence.

Materials and methods

Subjects

The current study was conducted in accordance with the tenets of the Declaration of Helsinki. The sample size of this study was assessed using the web-based software power and sample-size genetic program (PS; http://www.mc.vanderbilt.edu/prevmed/ps). All of the subjects were consented to participate in a process approved by the Ethics Committee for Human Genome/Gene Research at the Guilan University of Medical Science (No. 1930400417, on Jun 25, 2014). All volunteers who met the inclusion criteria for participating in this study were Iranian and resided in the Guilan Province. A total of 202 addicted subjects undergoing MMT (all males) and 202 healthy controls (all males) were selected from the Iranian individuals. Inclusion criteria for the addicts undergoing MMT included (1) over age 18; (2) MMT treatment period of at least 3 months with regular attendance of patients in the last 7 days before sampling;[24] (3) lack of concurrent use of other medications; and (4) lack of ongoing substance abuse apart from opium. Inclusion criteria for controls were as follows: (1) no history of drug abuse; (2) no history of drinking alcohol; (3) age over 18 (to be consistent with the studied group); (4) male (to be consistent with the experimental group); (5) no use of drugs which reduce or increase libido or sleep (like most psychotropic drugs); (6) lack of psychotic problems (since many psychotic problems induce libido dysfunction and insomnia problems; (7) no HIV and Hepatitis C Virus (HCV) history. Controls and patients were included in the study after they gave written informed consent and there was no missing data.

Genomic DNA was extracted from peripheral blood leukocytes through the salting out standard technique;[25] and analyzed by electrophoresis on 1% agarose gels stained with ethidium bromide. DNA concentration was determined using Nanodrop (ND-1000).

Genotyping of OPRK1 variants

Three variants (rs997917, rs6473797, and rs6985606) were selected for genotyping. Genotyping was performed using Amplification Refractory Mutation

System-Polymerase Chain Reaction (ARMS-PCR) protocol (95°C for 5 minutes, 95°C for 30 seconds, annealing time [58 for rs997917, 56 for rs6473797, and 57 for rs6985606] for 30 seconds, 72°C for 40 seconds, and 4°C for 5 minutes as holding time in 30 total cycles).

Linkage disequilibrium (LD) analysis

Pair-wise LD coefficients of |D'| and R^2 for three variants (rs997917, rs6473797, and rs6985606) from OPRK1 gene were assessed using SNPAlyze ver 8.1 Pro software (DYNACOM, Yokohama, Japan). This analysis was done according to Hardy-Weinberg equilibrium model.

Haplotype analysis

In order to estimate the multilocus association of OPRK1 gene variants with addiction dependence, haplotype analysis was performed using rs997917, rs6473797, and rs6985606 polymorphisms which are all study subjects. Eight haplotypes were predicted with frequencies higher than 0.5%. Haplotype analyses among case and control groups were performed based on the maximum-likelihood method with an expectation-maximization algorithm. Permutation p-values were calculated by comparing haplotype frequencies between control group and case group on the basis of 10,000 replications.

Statistical analysis

All statistical analyses and computations were performed using either SPSS (ver.20, http://www.spss.com/) or SNPAlyze software (ver.8.1, DYNACOM, Yokohama, Japan). Pearson's Chi-square test and binary logistic regression analysis were used to calculate the association between the OPRK1 gene variants at allelic and genotypic frequency levels and the diagnosis of opioid dependence (opium). The Chi-square goodness-of-fit was applied to the case group (addicts) to analyze their demographic characteristics. To evaluate the genotypic distribution of the cases (drug users under methadone maintenance treatment [MMT]) and controls, the Hardy-Weinberg equilibrium results were reported. All of the previous analyses were performed with the SNPAlyze (ver. 8.1, DYNACOM, Yokohama, Japan). The Shapiro-Wilk test revealed that insomnia and sexual reluctance data follows a normal distribution (Shapiro-Wilk, p-value > 0.05). Thus, after adjustment for demographic characteristics (such as age and daily methadone dose), the univariate analysis of covariance (ANCOVA) was used to determine the association between the OPRK1 gene variants at allelic and genotypic frequency levels and insomnia and sexual reluctance in drug user under MMT. Statistical analyses at a significance level of 0.05 were performed with the IBM SPSS ver. 20. The current study (a total sample size of 202 cases and 202 controls) had a statistical precision of more than 90% to detect an association with $p = 0.05$, for alleles with >10% frequency. The Addiction Severity Index (ASI) test,[26] the Treatment Outcomes Profile test (or TOP determines the amount of alcohol consumption and other opioids in the last 28 days),[27] the Clinical Opioid Withdrawal Scale (COWS; which examines the presence of indications for all of the 11 withdrawal complications)[28] were performed on all participants before prescribing methadone. The IIEF test (to identify libido dysfunction) and Insomnia Severity Index (PSQI, to determine the quality and inability to sleep) were then taken. The questions were read for illiterate people orally and the answers exactly wrote down in their profiles.

Results

Association analysis of OPRK1 gene variants in MMT patients and control individuals

In the present study, to perform the association of OPRK1 gene variants with susceptibility to addiction, the DNA of 202 addicted males undergoing MMT and 202 healthy male controls were analyzed. Three variants of the OPRK1 gene were analyzed in 404 participants. The distribution of allele and genotype frequencies of the variants in the case group and the control group is presented in Table 1.

The genotype frequencies of rs997917variant in MMT patients and control group were as follows: TT 13 and 15%, CT 45 and 30%, and CC 42 and 55%, respectively. The association analysis results indicated a significant difference in genotype frequencies of the two groups under a recessive hereditary model ($p = 0.01$). The genotype frequencies of rs6473797 in the two study groups, MMT patients, and control subjects were as follows: TT 50% and 45%, TC% 30 and 33%, and CC 20% and 22%, respectively. The frequency of this variant indicated no significant difference between the case and the control group based on all three hereditary models ($p < 0.05$).

Table 1. The association analysis of OPRK1 gene variants among MMT patients and control subjects.

		Genotype frequency			Allele frequency		*p*-value		Dominant model		Co-dominant model		Recessive model	
SNP	Subjects	MM	Mm	mm	Major	Minor	Genotype	Allele	*p*-value	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value	OR (95% CI)
rs997917 (T>C)→(C>T)	Case	0.13 (26)	0.45 (91)	0.42 (85)	0.35	0.65	0.0112	0.1156	0.5647	1.1807 (0.6706–2.0787)	0.140	0.81 (0.62–1.07)	**0.0128**	0.607 (0.41–0.090)
	Control	0.150 (30)	0.30 (62)	0.55 (110)	0.3	0.7								
rs6473797 (T>C)	Case	0.5 (102)	0.3 (59)	0.2 (41)	0.65	0.35	0.5377	0.3067	0.2732	0.8037 (0.5436–1.1883)	0.375	0.89 (0.70– 1.15)	0.714	0.914 (0.566– 1.476)
	Control	0.45 (91)	0.33 (67)	0.22 (44)	0.61	0.39								
rs6985606 (C>T)	Case	0.4 (82)	0.38 (76)	0.22 (44)	0.6	0.4	0.000883	.00008318	0.005279	1.7497 (1.1795– 2.5957)	**0.0001**	1.66 (1.26–2.19)	0.0006	2.682 (1.503– 4.783)
	Control	0.55 (110)	0.36 (73)	0.09 (19)	0.73	0.27								

Note: Number of subjects with each genotype and number of alleles (frequency in percent). OR: Odds ratio; CI: confidence interval. ORs for different modes of inheritance were calculated using the Web-Assotest program and SNPAlyze (ver.8.1). MM, Mm, and mm are Major homozygote, Heterozygote, and minor homozygote, respectively. The significance level of *p* is less than 0.05.

For rs6985606, the genotype frequencies among in the two study groups, MMT patients (CC 40%, CT 38%, and TT 22%) and healthy individuals (CC 55%, CT 36%, and TT 9%) showed a significant difference under a co-dominant hereditary model ($p = 0.0001$).

LD

Variants which were at a Hardy-Weinberg equilibrium and had Minor Allele Frequency higher than 5%, were analyzed using D' and R^2 tests. Among these three variants of the OPRK1 gene none of them were in a LD ($0.8 < D' \leq 1$, data are not shown). When LD was assessed by the more stringent measure of R^2, which also accounts for differences in allele frequencies, none of the LD structures of OPRK1 gene variants showed a higher degree of LD (data not shown).

Comparison of haplotype frequencies in MMT patients and the control group

Haplotype analysis was performed using SNPAlyze software. Among the haplotypes including three variants (rs997917, rs6473797, and rs6985606), there were eight haplotypes with frequencies higher than 5%. Among these haplotypes, two haplotypes indicated significant differences between two study groups including allele C-T-C ($p = 2.05E-3$ and permutation $p = 0.014$), allele C-T-T ($p = 5.4E-4$ and permutation $p = 2E-3$; Table 2).

Demographic characteristics of addicts undergoing MMT

There were 202 addicted males who underwent methadone treatment with mean age of 44.62 ± 14.56 compared with 202 healthy people with mean age of 38.71 ± 13.61. The Student's t-test showed that the mean age of case group was significantly higher than control group ($t = 4.21$, $df = 402$, $p < 0.0001$). Due to the significant difference between the study groups in terms of the mean age, results from adjusted odds ratio (AOR) were reported for assessing the odds (with the interval confidence of 5%). The mean age of onset and the mean age of addiction diagnosis of the addicts, undergoing MMT, were 27.93 ± 9.48 and 29.58 ± 9.34 years, respectively. The youngest and oldest addicts, in terms of both age of onset and age of addiction, were 18- and 66-years-old, respectively. Demographic characteristics of addicted males who underwent methadone treatment are represented in Table 3. The Chi-square goodness-of-fit results (Table 3) indicated that there were significant differences in the case group in terms of marital status, educational status, residence status, and job status. In other words, the frequency of widowers was lower than that of single and married ones ($x^2 = 26.27$, $df = 2$, $p < 0.0001$). A significant number of addicts were illiterate ($x^2 = 142.44$, $df = 5$, $p < 0.0001$), urbanites ($x^2 = 31.68$, $df = 1$, $p < 0.0001$), and day-worker ($x^2 = 119.42$, $df = 2$, $p < 0.0001$).

Table 2. Haplotype analysis of OPRK1 gene polymorphisms between healthy and MMT individuals.

	Haplotype	Overall	MMT individuals	Controls	p-value	Permutation p-value
1	C-T-C	0.2608	0.2143	0.3076	2.507E-3	0.014
2	C-C-C	0.1857	0.1617	0.2054	0.1086	0.22
3	C-T-T	0.1442	0.1704	0.1014	5.408E-4	2.E-3
4	T-T-C	0.1368	0.14	0.1368	0.8952	0.915
5	T-T-T	0.0919	0.1098	0.0705	0.0511	0.077
6	C-C-T	0.0814	0.0831	0.0835	0.9835	0.986
7	T-C-C	0.0764	0.0781	0.0754	0.8855	0.902
8	T-C-T	0.0229	0.0261	0.0193	0.5164	0.634

Note: Three SNPs (including rs997917, rs6473797, and rs6985606) were used to analyze haplotypes. Eight haplotypes with a frequency of more than 0.05% were found.

Table 3. Demographic characteristics of addicted males who underwent methadone treatment ($n = 202$).

Variables	Sub-type	Frequency (%)	χ^2	p-value
Marital status	Single	85 (42.1)	26.27	0.0001
	Married	84 (41.6)		
	Widower	33 (16.3)		
Education status	Illiterate	85 (42.1)	142.44	0.0001
	Elementary	11 (5.4)		
	Guidance	8 (4.0)		
	High school	9 (4.5)		
	Diploma	53 (26.2)		
	Academic	36 (17.8)		
Residence condition	Rural	61 (30.2)	31.68	0.0001
	Urban	141 (69.8)		
Job status	Jobless	55 (27.2)	119.42	0.0001
	Day-worker	136 (67.3)		
	Night- and day-worker	11 (5.4)		

Phenotype-genotype sub-analysis association of OPRK1 gene variants with development of insomnia and libido dysfunction among MMT individuals

Among the 202 male drug users under MMT, only 82 fully and properly completed the insomnia and sexual reluctance questionnaires. The average daily intake of

Table 4. Main effects of allelic and genotypic levels of OPRK1 gene variants on libido dysfunction scores.

	n	Mean of erectile function (pair-wise comparisons)	*df*	MS	F	*p*-value
Allelic level						
rs997917			1	2661.15	13.51	0.0001
C	72	24.98 ± 1398				
T	10	33.20 ± 18.21				
rs6473797			1	1.710	0.008	0.927
C	39	26.48 ± 15.54				
T	43	25.53 ± 14.04				
rs6985606			1	2473.21	0.001	0.976
C	52	25.88 ± 15.64				
T	30	26.16 ± 13.10				
Genotypic level						
rs997917			2	639.47	3.40	0.038
TT	10	33.20 ± 18.21				
TC	37	22.16 ± 11.73				
CC	35	27.97 ± 15.64 (TT=TC, TT=CC, **TC<CC**)				
rs6473797			2	288.54	1.46	0.237
TT	43	25.53 ± 14.04				
TC	26	29.11 ± 16.38				
CC	13	21.23 ± 12.68 (TT=TC, TT=CC, TC=CC)				
rs6985606			2	57.17	0.28	0.755
CC	30	26.17 ± 13.10				
TC	32	27.09 ± 17.05				
TT	20	23.95 ± 13.29 (CC=TC, CC=TT, TC=TT)				

Note: Significant $p < 0.05$.

Table 5. Main effects of allelic and genotypic levels of OPRK1 gene variants on insomnia scores.

	n	Mean of insomnia severity (pair-wise comparisons)	*df*	MS	*f*	*p*-value
Allelic level						
rs997917			1	367.06	1.910	0.171
C	72	14.61 ± 6.58				
T	10	16.00 ± 5.35				
rs6473797			1	1.710	0.008	0.927
C	39	14.49 ± 7.02				
T	43	15.04 ± 5.92				
rs6985606			1	11.18	0.28	0.600
C	55	15.16 ± 5.92				
T	26	14.54 ± 7.01				
Genotypic level						
rs997917			2	37.102	0.880	0.419
TT	10	16.00 ± 5.35				
TC	37	15.51 ± 6.53				
CC	35	13.65 ± 6.60 (TT=TC, TT=CC, TC=CC)				
rs6473797			2	8.612	0.201	0.819
TT	43	15.04 ± 5.92				
TC	26	14.77 ± 6.99				
CC	13	13.92 ± 7.34 (TT=TC, TT=CC, TC=CC)				
rs6985606			2	146.042	3.713	**0.029**
CC	30	15.43 ± 5.59				
TC	32	16.25 ± 6.88				
TT	20	11.45 ± 5.96 (**TT<TC**, TT=CC, TC=CC)				

Note: Significant $p < 0.05$.

methadone in these individuals was 93.05 ± 35.92. Before analysis of variance (ANOVA) of the main effects of OPRK1 gene variants on the dependent variables of insomnia and sexual reluctance, the Pearson's correlation coefficient revealed that demographic variables of age and daily dosage of methadone show a relatively low but statistically significant correlation (in the range of $r = 0.13$ to 0.23) with dependent variables. The univariate ANCOVA was used to eliminate their confounding effects. Table 4 shows the main effects of the genotypic and allelic levels of OPRK1 gene variants on the sexual reluctance in two ANCOVA reports.

According to the ANCOVA results in Table 4, among the three alleles, only the main effect of rs997917 on sexual reluctance scores is significant even after controlling demographic variables ($F_{(1.75)} = 13.51$, $p = 0.0001$). According to mean values, this result implies that the T allele of rs997917 plays a protective role against the poor erectile function of the male drug users. The results obtained for this analysis from Levene's test also showed that the error variance obtained for libido dysfunction (as the dependent variable) for the groups were equal ($F = 0.002$; df1 = 1; df2 = 80; $p = 0.962$). The ANCOVA results at the genotypic level indicated that only the main effect of rs997917 genotype ($F_{(2.71)} = 3.40$, $p = 0.038$) on the sexual reluctance scores is significant. According to mean values, this result implies that the TT genotype of rs997917 plays a protective role against the poor erectile function of male drug users. In the meantime, the results of the Bonferroni correction test for the pair-wise comparisons showed that the difference between the sexual desire mean scores in the TC and CC genotype groups with a destructive role in the erectile function is significantly lower than TT homozygous in the rs997917. Table 5 shows the main effects of the genotypic and allelic levels of OPRK1 gene variants on insomnia scores.

According to the ANCOVA results in Table 5, the main effect of none of the three alleles on the insomnia scores is significant even after controlling demographic variables. According to the mean values, this result shows the same insomnia of the carriers of above alleles in male drug users. The results were also analyzed at three allele levels by Levene's test. The variance of errors for the dependent variable of insomnia is the same in both groups ($p > 0.05$). The ANCOVA results at the genotypic level indicated that only the main effect of rs6985606 genotype ($F_{(2.71)} = 3.713$,

$p = 0.029$) on the insomnia scores is significant. According to the mean values, this result implies that the TC genotype of the rs6985606 plays a protective role against the poor quality of sleep in male drug users. In the meantime, the results of the Bonferroni correction test for the pair-wise comparisons showed that the difference between the insomnia scores in the TT genotype group with a destructive role in the sleep quality is significantly lower than TC homozygous, but does not show a significant difference with the CC genotype in the rs6985606.

Discussion

The current study represented the significant association of three OPRK1 gene variants (rs997917, rs6473797, and rs6985606 all situated in intron 2) with susceptibility to addiction dependence among addicted individuals undergoing MMT in Northern Iranians. The Chi-square goodness-of-fit results revealed that there were significant differences in the demographic characteristics of addicted males who underwent methadone treatment in terms of marital status, educational status, residence status, and job status. In order to investigate some clinical side effects of MMT, such as insomnia and libido dysfunction, a phenotype-genotype sub-analysis association was also performed among addicted individuals undergoing MMT. Moreover, two significant haplotypes were found.

Because drug addiction is a complex condition, genetic results may be contradictory with some previous studies; however, some studies have confirmed the association of genetic changes with this condition.[29] Studies have shown the key role of a number of OPRK1 variants in alcoholism, anxiety, and pain.[18, 20, 21] In addition, Xu et al. indicated that rs6989250 polymorphism (C > G) plays an important role in the induction of stress and cocaine dependence.[22]

In a study on heroin-addicted Caucasian patients who underwent methadone maintenance, some polymorphisms including rs6473797 in OPRK1 gene, rs2236861, rs2236857, and rs3766951 in OPRD1 gene, and rs510769 and rs3778151 in OPRM1 gene were significantly associated with heroin addiction.[23] Gerra et al. studied the 36G>T (a polymorphism of OPRK1 gene) in a heroin-addicted population of Western Europe and reported an association between heroin and 36G>T at OPRK1 gene ($p = 0.39$).[30] Xuei et al. conducted a study in a European-American population with alcohol dependence and found the relationship of several OPRK1 gene variants, such as rs2235749, and its ligand PDYN with alcohol ($p = 0.004$).[20]

The dependence of seven OPRK1 gene polymorphisms in the European-American population were studied. Among the seven studied polymorphisms, the three variants including rs1051660, rs6985606, and rs997917 were associated with addiction and alcohol dependence.[31] In the current study, the authors examined the two variants, including rs997917 and rs6985606, in opium-addicted people who underwent methadone maintenance. rs997917 and rs6985606 had a statistically significant relationship with addiction among study subjects ($p = 0.0128$ and $p = 0.0006$, respectively); therefore, the current findings confirmed the results of Zhang and colleagues.[18]

In addition, regarding the association of rs997917 polymorphism with smoking and drug addiction, Ashenhurst et al. reported the correlation of this polymorphism with alcoholism.[32] Among many variants in various genes, Levran et al. reported that only seven polymorphisms including rs6473797 in OPRK1 gene have been associated with drug addiction.[23] The rs6473797 of OPRK1 gene was also studied in MMT individuals and healthy people. The results regarding the association of rs6473797 with opioid dependence indicated no significant difference between the frequency of the two studied groups in the male population of northern Iran ($p = 0.71$). Therefore, the current findings are not consistent with the study of Levran et al.[23]

Wang et al. conducted a study on a Caucasian population of 336 addicted people who underwent MMT and analysis of 15 common Single Nucleotide Polymorphisms (SNPs) of the OPRM1 gene, they reported a significant association of some SNPs, such as rs1074287 and rs6912029, with changes in libido ($p < 0.042$).[24] They also found a significant association between insomnia and the methadone dosage ($p = 0.011$).[24] In 2012, they found a significant association between some OPRK1 gene variants, such as rs495491 and rs589046, with insomnia ($p < 0.009$).[24] The current data showed that among MMT individuals, rs6985606 was significantly associated with insomnia ($p = 0.029$) and rs997917 regarding allele and genotype distributions was significantly associated with libido dysfunction ($p = 0.0001$ and $p = 0.038$, respectively).

Analysis of the studied SNPs in this research showed that rs6473797 and rs6985606 which were associated with a high risk of addiction to methadone overlapped with CpG dinucleotides. Alterations in methylation of OPRK1 gene meSNPs due to environmental factors acting through epigenetic mechanisms may affect OPRK1 transcription and lead to methadone susceptibility.

To the best of the authors' knowledge, addiction is a multifactorial disease and its development depends on various factors, including environmental, genetic, and epigenetic elements, which cause the tendency of individuals to drug dependence variable. The understanding of the mechanisms of these substances effects on the body is important in order to apply them in the prevention and treatment of the related diseases, and to provide an optimal and functional solution. Therefore, the response to these associations should be found at the molecular level. Taken together, the current results indicated the associations of studied OPRK1 gene variants with opioid dependence in addicted Iranian men. However, it seems that more studies are required regarding the relationship of genetic and epigenetic factors with addiction. These findings definitely help in making a decision regarding factors affecting addiction problems; which are the main topics in the social and individual levels worldwide and particularly in Iran.

The present study had some limitations. Body mass index (BMI) analysis was not possible because there was not BMI data available for each sample. Another limitation was a loss of libido dysfunction and insomnia scoring in healthy people. However, insomnia and libido functions could be influenced by more than one gene and more than one environmental factor, such as smoking, alcohol, etc. Due to social conditions, healthy people refused to cooperate with the project. Based on this, there was no information of libido dysfunction and insomnia in healthy subjects to compare between the two study groups.

In conclusion, rs997917 and rs6985606 showed associations with susceptibility to opioid dependence. Moreover, rs997917 represented a key role in the development of libido dysfunction (rs997917) and insomnia (rs6985606) in MMT individuals. The results revealed a significant haplotype association of the three variants of OPRK1 (rs997917, rs6473797, and rs6985606) that might represent a strong potential of these polymorphisms to be clinical candidates for this gene. However, this requires more studies in larger sample size with more addiction markers other populations.

Acknowledgments

The authors appreciate the cooperation and support of Dr. Soltani, the head of Cellular and Molecular Research Center, Dr. Mirzajani, and Mrs. Habibi, in Biochemistry and Biophysics lab, Faculty of Medicine, Guilan University of Medical Sciences.

Funding and support

The authors of the current study have no conflict of interest to declare.

ORCID

Parvaneh Keshavarz, PhD http://orcid.org/0000-0002-1093-6051

References

1. Burns L. World Drug Report 2013 By United Nations Office on Drugs and Crime New York: United Nations, 2013ISBN: 978−92−1−056168−6, 151 pp. Grey literature. Drug Alcohol Rev 2014; 33(2):216.
2. Abolghasemi A, Rajabi S. The role of self-regulation and affective control in predicting interpersonal reactivity of drug addicts. Int J High Risk Behav Addiction 2013; 2 (1):28.
3. Cunha-Oliveira T, Cristina Rego A, R Oliveira C. Oxidative stress and drugs of abuse: an update. Mini-Reviews Organic Chem 2013; 10(4):321–34.
4. McClung CA, Nestler EJ, Zachariou V. Regulation of gene expression by chronic morphine and morphine withdrawal in the locus ceruleus and ventral tegmental area. J Neurosci 2005; 25(25):6005–15.
5. Vainio A, Rosenberg P, Kalso E, Ollila J, Matikainen E. Driving ability in cancer patients receiving long-term morphine analgesia. The Lancet 1995; 346(8976):667–70.
6. Kapas S, Purbrick A, Hinson JP. Action of opioid peptides on the rat adrenal cortex: stimulation of steroid secretion through a specific μ opioid receptor. J Endocrin 1995; 144 (3):503–10.
7. Alho H, Sinclair D, Vuori E, Holopainen A. Abuse liability of buprenorphine–naloxone tablets in untreated IV drug users. Drug Alcohol Depend 2007; 88(1):75–8.
8. Ries RK, Miller SC, Fiellin DA. Principles of addiction medicine. Philadelphia, PA: Lippincott Williams & Wilkins, 2009.
9. Daley M, Argeriou M, McCarty D, Callahan JJ, Shepard DS, Williams CN. The costs of crime and the benefits of substance abuse treatment for pregnant women. J Substance Abuse Treat 2000; 19(4):445–58.

10. Loimer N, Schmid R. The use of plasma levels to optimize methadone maintenance treatment. Drug Alcohol Depend 1992; 30(3):241–6.
11. Robinson AE, Williams FM. The distribution of methadone in man. J Pharm Pharmacol 1971; 23(5):353–8.
12. Martin TJ, Kahn WR, Xiao R, Childers SR. Differential regional effects of methadone maintenance compared to heroin dependence on μ−opioid receptor desensitization in rat brain. Synapse 2007; 61(3):176–84.
13. Huo S, Scialli AR, McGarvey S, Hill E, Tügertimur B, Hogenmiller A, Hirsch AI, Fugh-Berman A. Treatment of men for "low testosterone:" a systematic review. PloS One 2016; 11(9):e0162480.
14. Wong ML, Lau KN, Espie CA, Luik AI, Kyle SD, Lau EY. Psychometric properties of the Sleep Condition Indicator and Insomnia Severity Index in the evaluation of insomnia disorder. Sleep Med 2017; 33:76–81.
15. Sharafshah A, Fazel H, Albonaim A, Omarmeli V, Rezaei S, Mirzajani E, Ajamian F, Keshavarz P. Association of OPRD1 gene variants with opioid dependence in addicted male individuals undergoing methadone treatment in the North of Iran. J Psychoactive Drugs 2017; 49(4):242–251.
16. Crist RC, Ambrose-Lanci LM, Vaswani M, Clarke TK, Zeng A, Yuan C, Ferraro TN, Hakonarson H, Kampman KM, Dackis CA, Pettinati HM. Case–control association analysis of polymorphisms in the delta-opioid receptor, OPRD1, with cocaine and opioid addicted populations. Drug Alcohol Depend 2013; 127(1):122–8.
17. Roussotte FF, Jahanshad N, Hibar DP, Sowell ER, Kohannim O, Barysheva M, Hansell NK, McMahon KL, Zubicaray GI, Montgomery GW, Martin NG. A commonly carried genetic variant in the delta opioid receptor gene, OPRD1, is associated with smaller regional brain volumes: replication in elderly and young populations. Human Brain Mapping 2014; 35(4):1226–36.
18. Zhang H, Kranzler HR, Yang BZ, Luo X, Gelernter J. The OPRD1 and OPRK1 loci in alcohol or drug dependence: OPRD1 variation modulates substance dependence risk. Molecular Psych 2008; 13(5):531–43.
19. Cox BM, Christie MJ, Devi L, Toll L, Traynor JR. Challenges for opioid receptor nomenclature: IUPHAR Review 9. British J Pharmacol 2015; 172(2):317–23.
20. Xuei X, Dick D, Flury-Wetherill L, Tian HJ, Agrawal A, Bierut L, Goate A, Bucholz K, Schuckit M, Nurnberger J, Tischfield J. Association of the κ-opioid system with alcohol dependence. Molecular Psych 2006; 11(11):1016–24.
21. Holliday KL, McBeth J, Macfarlane G, Huhtaniemi IT, Bartfai G, Casanueva FF, Forti G, Kula K, Punab M, Vanderschueren D, Wu FC. Investigating the role of pain−modulating pathway genes in musculoskeletal pain. European J Pain 2013; 17(1):28–34.
22. Xu K, Seo D, Hodgkinson C, Hu Y, Goldman D, Sinha R. A variant on the kappa opioid receptor gene (OPRK1) is associated with stress response and related drug craving, limbic brain activation and cocaine relapse risk. Translational Psych 2013; 3(8):e292.
23. Levran O, Londono D, O'hara K, Nielsen DA, Peles E, Rotrosen J, Casadonte P, Linzy S, Randesi M, Ott J, Adelson M. Genetic susceptibility to heroin addiction: a candidate gene association study. Genes, Brain Behav 2008; 7(7):720–9.
24. Wang SC, Tsou HH, Chen CH, Chen YT, Ho K, Hsiao CF, Chou SY, Lin YF, Fang KC, Huang CL, Su LW. Genetic polymorphisms in the opioid receptor mu1 gene are associated with changes in libido and insomnia in methadone maintenance patients. European Neuropsychopharmacol 2012; 22(10):695–703.
25. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988; 16(3):1215.
26. McLellan AT, Kushner H, Metzger D, Peters R, Smith I, Grissom G, Pettinati H, Argeriou M. The fifth edition of the Addiction Severity Index. Journal Substance Abuse Treat 1992; 9(3):199–213.
27. Marsden J, Farrell M, Bradbury C, Dale−Perera A, Eastwood B, Roxburgh M, Taylor S. Development of the treatment outcomes profile. Addiction 2008; 103(9):1450–60.
28. Wesson DR, Ling W. The clinical opiate withdrawal scale (COWS). J Psychoactive Drugs 2003; 35(2):253–9.
29. Macedo, C. A. A. F. D. Pharmacogenomics of drug addiction. Master's Thesis, 2014.
30. Gerra G, Leonardi C, Cortese E, D'Amore A, Lucchini A, Strepparola G, Serio G, Farina G, Magnelli F, Zaimovic A, Mancini A. Human kappa opioid receptor gene (OPRK1) polymorphism is associated with opiate addiction. Am J Med Genetics Part B: Neuropsych Genetics 2007; 144(6):771–5.
31. Li Z, Zhang H. Analyzing interaction of μ-, δ- and κ-opioid receptor gene variants on alcohol or drug dependence using a pattern discovery-based method. J Addiction Res Therapy 2013; (Suppl 7):007.
32. Ashenhurst JR, Bujarski S, Ray LA. Delta and kappa opioid receptor polymorphisms influence the effects of naltrexone on subjective responses to alcohol. Pharmacol Biochem Behav 2012; 103(2):253–9.