http://pmjournal.ir Original Article
Autumn 2019, Volume 4, Issue 15 (4-6)

Investigation of the Relationship between PDYN Gene Polymorphisms and Tendency to Heroin Use

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DOI: 10.21859/pmj01032

Submitted: 2019/07/20
Accepted: 2019/08/15

Keywords:
Heroin Addicts
Gene Polymorphism
Prodynorphins
RFLP
VNTR

Abstract

Numerous studies have been conducted to investigate the relationship between genetic factors and drug use tendency, many of which have shown a significant correlation between genetic factors and drug use, especially heroin and cocaine. The most important site of drug action is the brain, which contains a variety of nerve receptors. Dynorphins are opioid peptides derived from the prodynorphins precursor (PDYN). These opioid peptides are capable of binding to the three types of opioid receptors. Studies have shown that heroin and cocaine use is associated with increased PDYN gene expression in specific areas of the brain. In this study, we investigated the association between rs910080 polymorphism in the 3'UTR region and the number of VNTR sequences in the promoter region with the tendency of heroin use in 155heroin addicts and 150 control with RFLP method. Results showed a significant relationship between heroin abuse and CC genotype in rs910080 polymorphism (P = 0.001), but no significant relationship between VNTR promoter repeats and heroin abuse. Rs910080 polymorphism can be used as a distinguishing factor.

INTRODUCTION

One of the social problems in all the countries of the world is drug addiction and its continuous use. Three types of factors contribute to the vulnerability and spread of dependence, including environmental factors, drug abuse, and genetic factors (1). Numerous studies have been conducted to investigate the relationship between genetic factors and drug use tendency, many of which have shown a significant correlation between genetic factors and drug use, especially heroin and cocaine (2). However, it is challenging to find genes related to the tendency to use drugs due to the complexity of inheriting these genes. The most famous site of drug action in the brain, which contains a variety of nerve receptors (3). One of these receptors is the opioid neurotransmitter receptors, which play a role in the mechanisms that reduce pain, happiness, and euphoria. Also, neurotransmitter produces androgenic opioids in the body, and exogenous opioids bind to opioid receptors in the brain and produce the same androgenic opioid effect (4). The mentioned alkaloids such as morphine and heroin. The opiate receptors are divided into three groups: delta, mu, and kappa. An opiate peptide can bind to more than one receptor and produce effects such as analgesia and euphoria (5). One downstream result of opioid binding to their receptors is a change in the expression of genes that cause behavioral changes in individuals. A characteristic feature of the drug is stimulating the release of dopamine in different areas of the brain (6). Dynorphins are opioid peptides derived from the prodynorphins precursor (PDYN). These opioid peptides are capable of binding to the three types of opioid receptors but are more likely to bind to the kappa-type opioid receptor. Studies have shown that heroin and cocaine use is associated with increased PDYN gene expression in specific areas of the brain (7). Therefore, numerous studies have been conducted on the association of polymorphisms of this gene with drug abuse, many of which indicate a positive association with drug abuse, and some studies have reported a negative association. This gene is located on the short arm of chromosome 20 and contains ten exons. In the promoter region of this gene, there is a polymorphic region that plays an essential role in regulating the expression of this gene (8). The 68bp sequence, known as a variable nucleotide tandem repeat (VNTR) that can contain one to four repeats in a cell, is associated with increased expression of this gene because it is a transcriptional activator, so grows the number of copies associated with increased expression of this gene (9).

On the other hand, there was a significant relationship between increased expression of this gene and an

increased tendency to heroin and cocaine use. Also, some polymorphisms of the 3'UTR region of this gene are significantly associated with reduced expression (10). In this study, we investigated the association between rs910080 polymorphism in the 3'UTR area and the number of VNTR sequences in the promoter region with a tendency to heroin use in the addicted population of Varamin city.

MATERIALS AND METHODS

The study population included 155 heroin addicts with a mean age of 32.6 referring to Varamin Addiction Center and 150 healthy men with a mean age of 29.4 referring to Varamin Blood Transfusion Center, who had no history of drug abuse. All participants in this control-case study were given an after obtaining permission and clinical history including age, weight, Family history of addiction, obtain 2 ml peripheral blood in EDTA vials and were kept in the freezer until DNA extraction. DNA was extracted using the Salting out method. PCR and RFLP-PCR were used to determine the genotype of the polymorphisms. For this purpose, the target regions were amplified by the specific primers of Table 1. The PCR product of rs910080 polymorphism was treated with a Bsp1286I restriction enzyme. In the case of the T allele, only one 497 bp band was observed, and in the case of allele C, two bands 300 bp and 197 bp were observed on 2% agarose gel. To identify the number of VNTR copies of the promoter region, the PCR product loaded on 2% agarose gels, one, two, three, or four copies of this sequence will have 350, 418, 486 or 554 bp bands, respectively. Statistical analysis was performed by SPSS software version 19, and p values less than 0.05 were considered significant.

RESULTS

The study population included 155 male heroin users (mean age 32.6) and 150 healthy individuals as a control group (mean age 29.4 years). The genotype frequencies of rs910080 and VNTR promoter polymorphisms were evaluated by RFLP-PCR and PCR, respectively, and the results are as described in Table 2. Results showed a significant relationship between heroin abuse and CC genotype in rs910080 polymorphism (P = 0.001). But there was no significant relationship between VNTR promoter repeats and heroin abuse. On the other hand, there was no significant relationship between age (P = 0.43) weight (P = 0.94) and a family history of heroin use (P = 0.14).

Table 1. Primer Sequence

Polymorphism name	Sequence	Annealing
rs 9100800	F:CAATGCCCAGTGCGTATGT R:CTTTGGAGACGATGCTTTAGGT	65c
Promoter 68bp VNTR	F:ATCCAAGGTCTCTCCGATGGT R:CACCAGGCGGTTAGGTAGA	68c

Table 2. Genotype Frequency

Genotype	Cases	Controls	P value (≤ 0.05)
Rs910080			
TT	51 (32)	57 (38)	0.93
TC	73 (47)	86 (57)	0.74
CC	31 (21)	7 (5)	0.001
Promoter VNTR			
1/3	2 (1.3)	1 (0.6)	0.74
2/2	5 (3.2)	10 (6.6)	0.32
2/3	78 (50.3)	63 (42)	0.63
3/3	53 (34.1)	50 (33.3)	0.81
3/4	17 (10.9)	26 (17.3)	0.44

Data in table are presented as No. (%).

DISCUSSION

Genetics is one of the significant risk factors of drug abuse that has been considered in recent years. Through correlational studies, numerous genes associated with drug abuse have been identified, one of which is the prodynorphins (PDYN) gene (8). Studies have shown that increased expression of this gene is associated with drug abuse, especially heroin and cocaine. Hashemi et al. investigated the association between PDYN gene polymorphisms and heroin abuse in the Zahedan addict population. This group reported a significant relationship between rs910080 polymorphism but no significant relationship between VNTR promoter repeats and heroin abuse (11). The results of this group are similar to those obtained in our study, in which there was a significant relationship between heroin abuse and rs910080 polymorphism (P = 0.001). This polymorphism is located in the 3'UTR region of this gene. Yuferov et al., in a study of mice brain, showed that the nucleotide changes in the 3'UTR region of the PDYN gene were significantly correlated with decreased expression (12). However, Clarke et al. Did not report a significant association between rs910080 polymorphism and cocaine abuse. Since the 68bp sequence in the promoter of this gene binds to

transcriptional activators and enhances expression of this gene, and that increased expression of this gene is associated with drug abuse, an increase in the number of copies of this sequence is expected to be linked to heroin abuse (8). But in our study, there was no significant relationship between the increased number of copies of this gene and heroin abuse. Saify et al. investigated the association between the numbers of VNTR promoter variants of this gene with heroin abuse in the Shiraz addict population (9). The results of this study showed that there was a significant relationship between the number of repeats of this sequence and heroin abuse. In the present study, it was found that there was a significant relationship between rs910080 polymorphism and heroin abuse. This polymorphism can be used as a distinguishing factor.

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