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Investigating the Association between Two Polymorphisms Rs2235749 and Rs910080 in the PDYN Gene and Susceptibility to Addiction

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ABSTRACT

Background and Objective: Studies show that genetic factors, including polymorphisms, contribute to individual differences in the risk of developing addiction. One of the important genes whose polymorphisms are associated with the risk of developing addiction is the PDYN gene. The aim of this study is to investigate the association between two polymorphisms, rs2235749 and rs910080, in the PDYN gene and the susceptibility to addiction.

Methods: In this case-control study, blood samples from 80 individuals with a history of heroin or methamphetamine addiction and 80 healthy individuals with no history of use, living in Markazi province, were genotyped and compared. The genotypes of the individuals in the two groups were determined using PCR-RFLP and agarose gel electrophoresis techniques, and were examined and compared.

Findings: A significant difference was observed between the addicted and control groups for the genotypes of the rs910080 polymorphism ($p<0.000$). In this study, the frequencies of TT, CC, and CT genotypes were 43.75%, 15%, and 41.25% in the control group, and 25%, 46.25%, and 28.75% in the addicted group, respectively. No statistically significant difference was observed between the addicted and control groups for the genotypes of the rs2235749 polymorphism, and the frequencies of TT, CC, and TC genotypes were 23.75%, 40%, and 36.25% in the addicted group, and 22.5%, 33.75%, and 43.75% in the control group, respectively.

Conclusion: According to the results of this study, the CC genotype of the rs910080 polymorphism of the PDYN gene is significantly associated with addiction and increases the risk of developing addiction, while people with the CT and TT genotypes have a lower susceptibility to addiction.

Keywords: *Heroin, Polymorphism, Methamphetamine, PDYN.*

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Introduction

Addiction is a chronic, relapsing brain disease characterized by destructive behaviors. It represents a global public health crisis with devastating effects on individuals, families, and society (1, 2). The three main factors in the vulnerability and development of addiction include environmental factors, drug-induced physiological effects, and genetic factors, with genetic factors increasing the risk of addiction by approximately 40–60% (3-5). The inheritance of addiction has been investigated in many ways, including studies on families, adopted children, and twins, and it has been proven that genetics play a role in addiction (6-9). Given the role of genetics in many diseases, including addiction, identifying gene polymorphisms associated with addiction in each region is important for timely prevention and treatment (10-12).

According to studies by Yuanyuan et al. and David A Nielsen et al., polymorphisms of the PDYN gene increase the risk of developing addictive drugs, including heroin and cocaine (13, 14). The PDYN gene has four exons and is located at 20p13 and contains 15,530 bases. PDYN is the precursor of dynorphin-related peptides. PDYN plays a key role in some complex behaviors such as drug use. DYNs, which are post-translational products of the PDYN gene, bind to opioid receptors, but in the case of binding to Kappa-opioid receptors (KOR), this binding is carried out with very high affinity. DYNs inhibit dopamine release, thus playing an important role in the negative regulation of dopamine. DYNs and KORs are enriched in areas of the brain responsible for controlling emotions, motivation, and habits and behaviors related to drug use (15-18).

Two important polymorphisms of the PDYN gene are rs2235749 and rs910080, both of which play a role in regulating PDYN gene expression, and previous studies have shown that these polymorphisms of this gene are associated with addiction. The rs2235749 polymorphism and the rs910080 polymorphism are both located in the 3′-untranslated regions (3′-UTRs) of this gene and play a role in regulating PDYN gene expression (19-21).

Given the importance of the PDYN gene in addiction and the fact that racial and geographical differences play a role in the susceptibility to addiction, this study investigated the association between two important polymorphisms (rs2235749, rs910080) of this gene with heroin and methamphetamine addiction. This research can play a role in identifying genetic markers of addiction in each region, regional prevention, and treatment of this disease in the future.

The aim of this study is to investigate the association of rs910080 and rs2235749 polymorphisms of the PDYN gene with heroin and methamphetamine addiction and to help prevent it.

Methods

After approval by the Ethics Committee of Arak University with the code IR.ARAKMU.REC.1399.340, this case-control study was conducted on blood samples from 80 men with a history of heroin or methamphetamine use, who had been addicted to heroin or methamphetamine for more than a year and had been in addiction rehabilitation centers for less than 6 months, as the case group. These samples were collected from patients at the Ebrahimabad Addiction Treatment Center in Arak, clinics, and addiction treatment centers in Markazi Province. The control group included 80 healthy men and individuals referred

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to the Arak Blood Transfusion Center and a number of other individuals living in Markazi Province who had no history of addiction. The samples were matched for age and gender. The variables examined in this study were the CC, TT, and CT genotypes and the C and T alleles for both polymorphisms.

Genotyping: In this study, DNA was extracted from blood samples using a DNA extraction kit from Zistageneafarin-Iran. Genotyping of control and addicted individuals for both polymorphisms was performed using Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP). The mean age of the individuals in the addicted group was 44±1 years and in the control group was 43±1 years. First, specific primers were designed for both polymorphisms on the NCBI website and then synthesized by Sinaclon-Iran. The primer sequences and amplified fragment lengths of these two polymorphisms are listed in Table 1.

Table 1. Sequence of primers used in this study

Amplified fragment length	Primer sequence 5' to 3'	Polymorphism
497 bp	F:5’CAATGCCCAGTGCGTATGT3’ R:5’CTTTGGAGACGATGCTTTAGGT3’	rs910080
571 bp	F:5’TGGAAACCAAGACATCAGG3’ R:5’TCATTGTTCAGAAAAGCACC3’	rs2235749

The thermal cycle for performing the PCR reaction in the Eppendorf-Germany thermocycler was as follows for the two polymorphisms rs2235749 and rs910080: For rs2235749: initial denaturation at 95°C for 5 min, followed by 35 cycles of 95°C for 30 s, 53°C for 30 s, 72°C for 30 s, and final extension at 72°C for 5 min. For rs910080: annealing temperature was 56°C and other conditions were identical. The NdeI enzyme from Thermo Scientific-USA was used to identify the rs2235749 polymorphism, and the restriction enzyme (SduI) Bsp1286I from Thermo Scientific-USA was used to identify the rs910080 polymorphism.

The final reaction volume for enzymatic digestion was determined to be 10 µL (3 µL PCR product, 1 µL buffer, 0.2 µL enzyme, 5.8 µL water). For the rs910080 polymorphism, 3 µL of PCR product, 0.2 µL of Bsp1286I enzyme (SduI), one µL of enzyme buffer, and 6 µL of sterile distilled water were used. For the rs2235749 polymorphism, 3 µL of PCR product, 0.2 µL of NdeI enzyme, 1 µL of buffer, and 6 µL of sterile distilled water were used. The temperature and time conditions for both enzymes were 37°C for four hours. Finally, to determine the resulting genotypes, the digestion products were electrophoresed on agarose gel containing 3% and SafeStain dye from Sinaclon-Iran at a voltage of 110 V for 20 minutes using a Bio Rad Co-America device. Then, a GelDock device from Gen Flash Company from America was used to observe the cut pieces, and then the resulting bands were genotyped. To determine the size of the bands related to the different genotypes of both polymorphisms, 100 bp DNA Ladder from Sinaclon-Iran was used (Figures 2 and 1). In this study, the association between addiction and genotypes was calculated using odds ratio (OR) and 95% confidence interval (CI). The obtained results were analyzed using the chi-square test using SPSS version 26, and $p<0.05$ was considered significant.

[DOI: 10.22088/jbums.27.1.65]

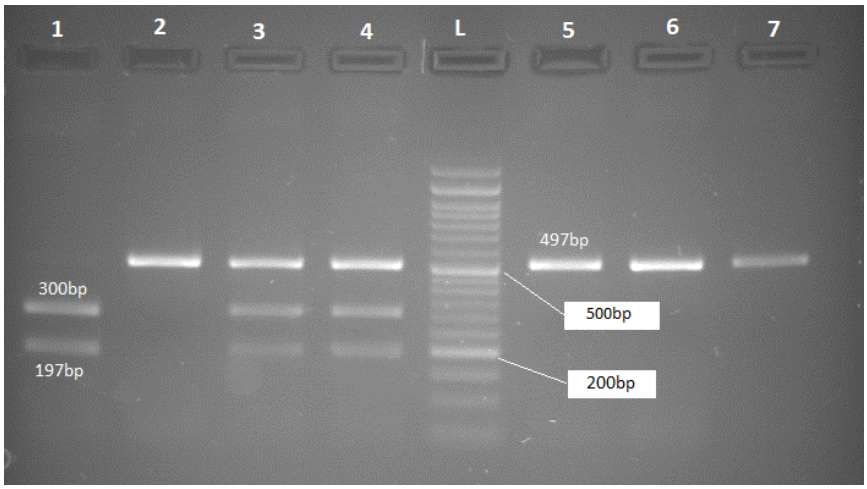

Figure 1. Fragments obtained from the digestion of the PCR product of the rs910080 polymorphism by the enzyme Bsp1286I. **The TT genotype contains one band of 497 bp, the CT genotype contains three bands of 497, 300, and 197 bp, and the CC genotype contains two bands of 300 and 197 bp. A 100 bp ladder was used to determine the size of the bands.**

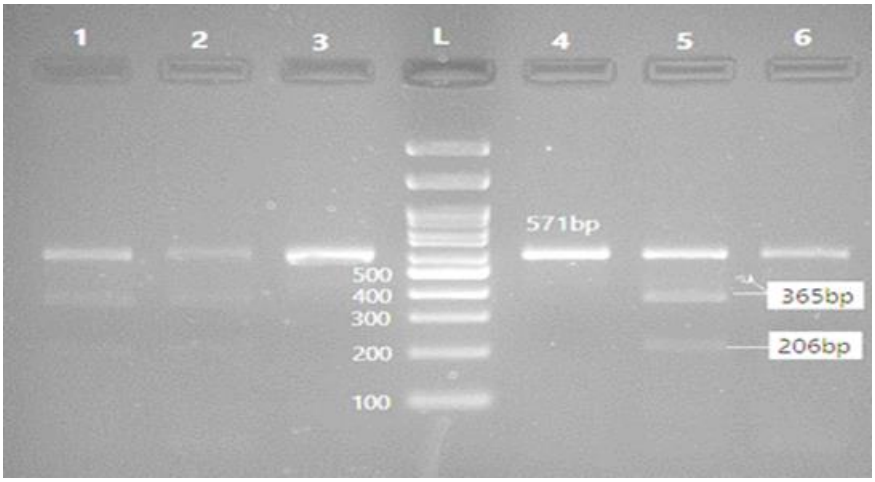

Figure 2. Bands corresponding to different genotypes of the rs2235749 polymorphism obtained by the action of the restriction enzyme NdeI on the PCR product. **In this gel, the CC genotype contains a band of 571 bp, the TC genotype contains three bands of 571, 365, and 206 bp, and the TT genotype contains two bands of 365 and 206 bp, and the DNA ladder used is 100 bp.**

Results

In the present study, the results showed a significant association between the CC genotype of the rs910080 polymorphism and the risk of addiction ($p<0.000$), while no significant association was observed between the genotypes of the rs2235749 polymorphism and the risk of addiction. In the case of the rs910080 polymorphism, the frequency of the T allele was different in the two control and addicted groups ($p<0.000$). In the case of the aforementioned polymorphism, the C allele increased the risk of addiction, and the results showed that the CC genotype was significantly associated with an increased risk of addiction compared to

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the CT and TT genotypes (p<0.000). Moreover, the dominant model for C allele (CC+CT) was significantly associated with an increased risk of addiction compared to TT genotype (OR=2.33, 95%CI: 1.19-4.57, p=0.013). The frequency of TT, CC, and CT genotypes was 43.75%, 15%, and 41.25% in the control group, and 25%, 46.25%, and 28.75% in the addicted group, respectively.

In the case of rs2235749, according to the obtained results, no significant association was observed between the alleles of this single nucleotide polymorphism and the risk of addiction. According to the statistical results, there was no significant association between the CT genotype and the increased risk of addiction. The combination of TC + TT genotypes also did not show a significant association (p=0.413, 95% CI: 0.401-0.455, OR=0.764) and the frequency of TT, CC, TC genotypes in the addicted group was 23.75%, 40%, 36.25%, respectively, and in the control group it was 22.5%, 33.75%, 43.75% (Table 2).

Table 2. Genotypic and allelic distribution of PDYN gene polymorphisms in the addicted and control groups

Variable	Control Number(%)	Addict Number(%)	p-value	OR	95% CI
rs910080 genotype					
TT	35(43.75)	20(25%)			
CC	12(15)	37(46.25%)	0.000	2.782	-4.377 1.769
TC	33(41.25)	23(28.75%)			
rs910080 Allele					
C	35.6%	60.6%	0.000	20.028=X^2	1=df
T	64.4%	39.4%			
rs2235749 Allele					
C	56.9%	58.1%	0.652	0.204=X^2	1=df
T	43.1%	41.9%			
rs2235749 genotype					
TT	18(22.5)	19(23.75)			
CC	27(33.75)	32(40)	0.603	0.903	-1.406 0.580
CT	35(43.75)	29(36.25)			

Discussion

In this study, the results show that there is a significant association between the CC genotype of the rs910080 polymorphism and the susceptibility to addiction, while no significant association was observed between the CC, CT, and TT genotypes of the rs2235749 polymorphism and the risk of addiction. In the meta-analysis conducted by Wang et al., 3129 addicted individuals and 3289 control individuals were examined. The findings of this study showed that the rs910080 polymorphism is significantly correlated with the risk of addiction among the Asian population, and there was no association between the rs2235749 polymorphism and the susceptibility to addiction (22). This study is also consistent with the present study, and its cause may be related to Asian ethnicity in the two populations.

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In a study by Nagaya et al. on 459 men with opioid dependence and 543 healthy men as controls to determine the association between rs910080 polymorphisms and opioid dependence in the Malaysian population, the results showed that there was a significant association between opioid dependence and rs910080 (23). The reason for this concordance of the results could be related to the close ethnicity of the two populations studied and the similarity of the subject of the study (addiction) and the studied polymorphisms, which confirms the results of the present study.

The results of a study by Esfahani et al. on 155 heroin addicts and 150 controls in Tehran population also show that there is a significant association between the rs910080 polymorphism in the region and the tendency to use heroin (24). The results of this study are consistent with our results and this could be due to the close racial background of the Tehran and Arak populations.

The findings of a study by Hashemi et al. showed that rs910080 significantly increased the risk of heroin dependence, while the rs2235749 polymorphism of the PDYN gene was not associated with heroin dependence (25). This study is consistent with our results, which could be due to the selected race living in the same country with similar environmental conditions.

In a study by Şenormancı et al. on 134 methamphetamine addicts and 97 control subjects in Turkey, the results showed that the rs2235749 polymorphism increased the risk of methamphetamine dependence (26). The results of this study are not consistent with the present study, and the reason for this could be related to the racial and geographical differences of the populations studied.

The results of the study by Kaya-Akyüzlü et al. on 100 heroin addicts and 108 control individuals in Turkey are also consistent with the results of our study and show that there is a significant relationship between the genotypes of the rs910080 polymorphism and heroin addiction (27). The reason for this agreement is related to the similarity of the polymorphisms studied and the close race of the two populations studied.

Considering the importance of the PDYN gene and its role in addiction and the association of the rs910080 polymorphism with an increased risk of addiction, this polymorphism can be used as a marker in predicting the risk of addiction.

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