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Prodynorphin (PDYN) gene polymorphisms in Turkish patients with methamphetamine use disorder, changes in PDYN serum levels in withdrawal and the relationship between PDYN, temperament and depression

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ABSTRACT

Aim of the study is to compare prodynorphin (PDYN) rs1997794, rs1022563, rs6045819, rs2235749 polymorphisms in individuals with methamphetamine use disorder (MD) to that of healthy controls (HC), and to investigate the differences in serum PDYN levels in methamphetamine withdrawal. It is also aimed to explore the temperament characteristics and depression and their relationship with PDYN polymorphisms and PDYN serum levels in MD group. PDYN gene and serum levels were studied in 134 patients with MD and 97 HC. Patients with MD were administered Beck Depression Inventory (BDI) and Temperament Evaluation of Memphis, Pisa, Paris and San Diego Autoquestionnaire (TEMPS-A). For rs1022563 polymorphism, TT and CT genotype frequency and T allele frequency were significantly higher in the MD group than the frequencies in HC. It was found that rs2235749 polymorphism AA genotype was associated with increased risk of MD. PDYN rs1997794 CT genotypes had significantly higher scores of TEMPS-A irritable than CC genotypes and PDYN rs1022563 CC genotypes had significantly higher scores of TEMPS-A irritable than TT genotypes. PDYN levels among persons with MD were significantly higher than among the HC group when the withdrawal level increased and withdrawal symptoms improved. During the period in which the withdrawal level increased, there was a negative correlation between PDYN level and BDI and a positive relationship between PDYN level and TEMPS-A hyperthymic. It may be beneficial to screen temperament characteristics associated with increased risk of addiction in patients with MD and develop interventions based on temperament characteristics and the effects of PDYN.

KEYWORDS

Turkish; methamphetamine; dependence; prodynorphin; depression; temperament

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Introduction

Methamphetamine use has rapidly become widespread in Turkey as well as in the world and poses a serious threat. The development of substance-use disorders following substance use depend on social, psychological and environmental factors, as well as genetic factors. It was reported that genetic factors accounted for 44% of stimulant dependence (Tsuang et al., 1996). It has been suggested that methamphetamine use has a cultural background and use motivations varies interculturally (Evren & Bozkurt, 2018; Li et al., 2004). Prodynorphin (PDYN) is a precursor of dinorphin-related peptides associated with substance dependence. It yields endogeneous k opioid receptor agonists (Schwarzer, 2009). The activation of k opioid receptors within the dopamine terminals results in decreased dopamine release and aversive symptoms such as dysphoria and anhedonia (Pfeiffer et al., 1986).

Dinorphin peptides and C opioid receptors are involved in dopaminergic nigrostriatal and mesocortical-mesolimbic systems and are associated with stimulant, heroin and alcohol use disorder (Mathieu-Kia & Besson, 1998). There have been studies in which PDYN polymorphisms, were examined in people with heroin use disorder. It was reported that different polymorphisms in the PDYN gene were found to be correlated on people with heroin use disorder in varied ethnic groups such as China and Iran. PDYN rs2235749 polymorphism was found to be significant in a study on heroin use disorder in Iran (Hashemi et al., 2018) whereas PDYN rs1022563 polymorphism and rs1997794 polymorphism were found related in a study, conducted in China (Clarke et al., 2009). Further, rs1022563 polymorphism was reported to be related in European American patients (Clarke et al., 2012). Accordingly, the dinorphine system may contribute to emotional and motivational aspects of methamphetamine withdrawal (Nomura et al., 2006). A study in Iran that examined PDYN VNTR polymorphism in individuals with methamphetamine use disorder (MD) found no relationship (Saify & Saadat, 2015), but another study in Japan pointed out that PDYN VNTR 68 bp repeat 3 or 4 copies of allele polymorphism was more frequent in people with MD than healthy control (HC) group (Nomura et al., 2006).

In a study conducted in the postmortem period, it was shown that dynorphin mRNA levels and dynorphin protein increased in chronic cocaine users (Frankel et al., 2008). Preclinical studies have evidenced that dynorphin peptides attenuate the extracellular dopamine increase in nucleus accumbens (Zhang et al., 2005). It has been suggested that the dynorphin K opioid system is a regulatory system that reverses substance-induced dopaminergic stimulation and may play a role in the development of dependence (Koob & Kreek, 2007). Chronic methamphetamine exposure stimulates dopamine receptors in nucleus accumbens, leading to an

increase in PDYN, which results in the emergence of withdrawal symptoms by decreasing dopamine through k opioid receptors (Nomura et al., 2006).

Temperament is defined as emotional reaction and behavioral patterns, which develop through the childhood. Temperament is generally recognized as having a hereditary basis and less changeable nature in a lifetime. It can also be conceptualized as the biological origin of personality. Reactions toward reward-related cues, which have an impact on the development and maintenance of dependence, are linked to temperament (Clarke et al., 2009; Kawamura et al., 2010). It is asserted that irritable, hyperthymic and cyclothymic types of temperament are associated with dependence to stimulants such as methamphetamine, which may augment the risk of MD (Yehya et al., 2019). Many people with MD experience depression as a comorbid condition in such a way that diagnostic criterion are met both inside and outside of withdrawal period. According to National Epidemiologic Data, the lifetime depression rate was 41.6% in individuals with amphetamine abuse or dependence (Conway et al., 2006). In a study on methamphetamine users, the depression rate was reported to be 56.9% (Chen et al., 2019). Researchers have demonstrated that temperament characteristics and depression levels are related to the course of treatment and relapse in dependence (Gao et al., 2018; Paulino et al., 2017). The theories of dependence imply that people may recurrently use substances for positive experiences associated with pleasurable effects during use or alleviation of unwanted effects associated with disuse. Dependence occurs through neurobiological changes of this process (Newton et al., 2009). In the present study, we aimed to compare PDYN rs1997794, rs1022563, rs6045819, rs2235749 polymorphisms in HC versus MD group, of which associations with heroin dependence (Clarke et al., 2009; Hashemi et al., 2018), alcohol dependence (Xuei et al., 2006) and the emergence of adverse effects during withdrawal have been indicated. To our knowledge, there have been no studies investigating these polymorphisms in people with MD. The other purpose of our present study was to investigate the difference between PDYN serum levels of people with MD in withdrawal and non-withdrawal period, and serum levels in HC. Moreover, we intended to assess temperament characteristics and depression, which have been associated with methamphetamine-abuse relapse, and their relationship with PDYN polymorphisms and PDYN serum levels.

Methods

Procedure and participants

Participants consisted of 134 male inpatients who were diagnosed with MD based on the criteria of DSM 5 (American Psychiatric Association, 2013) in

the Substance Use Disorders Treatment Center of University of Health Sciences Bursa Yuksek Ihtisas Training and Research Hospital and 97 healthy male individuals without any substance use history. Inclusion criteria included meeting criteria for Methamphetamine use disorder for at least 1 year and having used it at least 100 times. The HC group was recruited from the hospital staff and first or second degree relatives of hospital staff who had never had a history of psychiatric illness or substance use, as evaluated with psychiatric interview by psychiatrists. Exclusion criteria for all participants were having intellectual disability or a serious comorbidity (thyroid disorder, diabetes, etc.). All participants were male and Turkish. The blood samples were taken from all participants to examine PDYN genetic polymorphisms and serum levels after informing and getting written informed consent. Patients with MD were also administered Beck Depression Inventory (BDI) and Temperament Evaluation of Memphis, Pisa, Paris and San Diego Autoquestionnaire (TEMPS-A) and their relationship with PDYN polymorphisms and blood serum levels were analyzed. Similar to previous studies (Yoon et al., 2005), the study team administered the scales to the MD group and obtained their second blood sample at least 30 days following discontinuation of methamphetamine use to ensure withdrawal symptoms were absent. Ethical approval was acquired from the Clinical Research Ethics Committee of Bülent Ecevit University Research Hospital. The study was carried out with the financial support of the Scientific Research Projects Unit of the University of Health Sciences (Project Id: 2018/013).

Measures

Beck Depression Inventory (BDI), a 21-item scale, was developed to evaluate emotional, cognitive, somatic and motivational symptoms of depression. BDI is a Likert-type self-report scale scored between 0 and 3. The total score is calculated by the sum of item scores (Beck et al., 1961). Hisli (1988) demonstrated the validity and reliability of a Turkish version of the BDI.

Temperament Evaluation of Memphis, Pisa, Paris and San Diego Autoquestionnaire (TEMPS-A) is a self-report scale consisting of 110 items with true and false statements (Akiskal et al., 2005). Vahip et al. (2005) demonstrated the validity and reliability of a Turkish version of the TEMPS-A.

Genotyping

A 2-ml blood sample was collected from all voluntary participants, using EDTA tubes and DNA isolation was extracted from peripheral blood using

Table 1. Primer sequences and binding temperatures for PCR.

SNP ID	Primer sequences (5′-3′)	Binding temperatures (°C)
rs1997794 C > T	F: GTGAACTAGCCTCCTAACTG R: TACCCATCTCCCTATCTCTG	56
rs1022563 C > T	F: TAAATGCCATCCACCACTACC R: GTCCCAGTTTACACTGTCAC	56
rs6045819 A > G/T	F: TGAGCTGAGCATGGGGAAGG R: GACGGGGATAGCATGGGTCA	59
rs2235749 A > G	F: GAAACCAAGACATCAGGAGG R: ATCAAGGAGGGAGAAAGGC	56

SNP = Single nucleotide polymorphism.

GeneAll® Exgene^{TM} Clinic SV Mini Kit. Suitable primers have been designed to amplify regions containing PDYN gene polymorphisms (rs1997794, rs1022563, rs6045819, rs2235749). For each sample, PCR (polymerase chain reaction) was performed at appropriate primary binding temperatures in a total volume of 25 ilel with 20–100 ng DNA, 100 100 M dNTP, 20 pmol primers, 1X DreamTaq Buffer and DreamTaq DNA Polymerase (Thermo Fisher Scientific, USA) (Table 1). PCR program was performed for 35 cycles with a 1 min denaturation at 95 °C following a 3 min initial denaturation at 95 °C, 1.5 min binding at the appropriate temperatures to primers, an 1 min elongation at 72 °C followed by a 7 min final elongation at 72 ^{0}C. PCR was allowed to incubate with the enzymes given in the Table 2 under optimal conditions. After restriction, polymorphisms were detected by 120 V 50 min electrophoresis on 3% and 3.5% agarose gel of the products. The agarose gel was displayed with Vilber Lourmat E-BOX VX5 under UV light (Figures 1–5).

Serum PDYN measurement

Serum PDYN levels were evaluated by Sandwich Enzyme Linked Immunosorbent Assay (ELISA). YL-Biont's commercial Human PDYN elisa kit (Ca, USA, Cat. No. YLA3974HU) was used for measurements. The PDYN concentrations of the samples were calculated from the absorbance measurement at 450 nm wavelength and the plotted standard curve chart after the incubations made before the study.

Statistical analyses

The statistical analyses were carried out using SPSS 18.0 package (SPSS Inc., Chicago, IL). The Shapiro-Wilk goodness-of-fit test was used to examine the normality in numerical variables. The descriptive statistics were described as median (min-max) for numerical variables, and numbers and percentages for categorical data. Mann-Whitney U test was used for comparison of age and education level, also for comparing the serum PDYN levels. Comparison of TEMPS-A irritable scores in TT, CT and CC genotypes of PDYN rs1997794 and PDYN rs1022563 were compared via Kruskal Wallis test and Mann-Whitney test with Bonferroni correction.

Table 2. Enzymes specific to polymorphisms and restriction conditions.

SNP ID	Restrictive enzyme	Restriction conditions
rs1997794 C > T	BseSI (Thermo Scientific)	2 h incubation at 55 °C
rs1022563 C > T	DdeI (Thermo Scientific)	Overnight incubation at 37 ° C
rs6045819 A > G/T	PagI (Thermo Scientific) BauI (Thermo Scientific)	Overnight incubation at 37 ° C
rs2235749 A > G	NdeI (Thermo Scientific)	Overnight incubation at 37 ° C

SNP = Single nucleotide polymorphism.

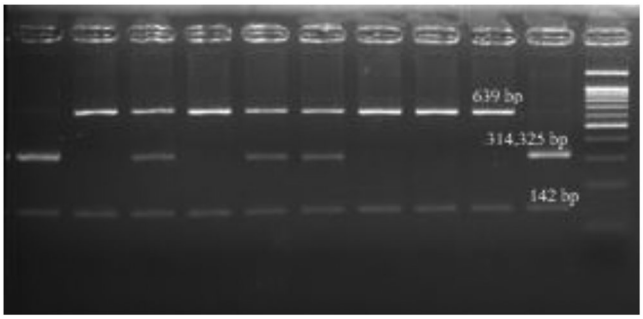

Figure 1. Gel image of the PDYN rs1997794 polymorphism. (1st well CC, 2nd well TT, 3rd well CT, 11th well 100 bp DNA marker).

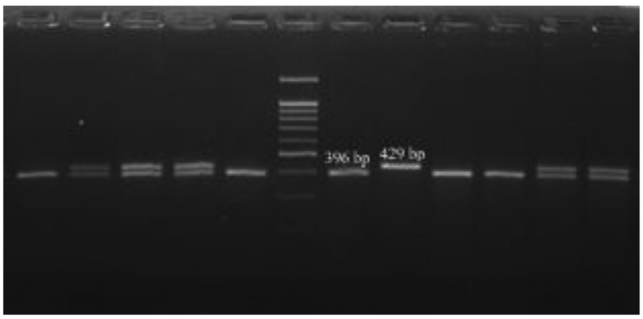

Figure 2. Gel image of PDYN rs1022563 polymorphism. (1st well CC, 2nd well CT, 6th well 100 bp DNA marker, 8th well TT).

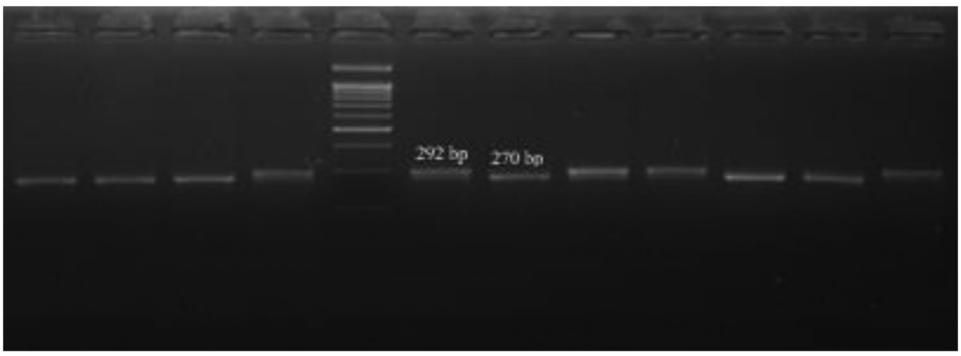

Figure 3. PagI cut gel image of PDYN rs6045819 polymorphism. (5th well 100 bp DNA marker, 6th well A-, 7th well AA, 12th well -).

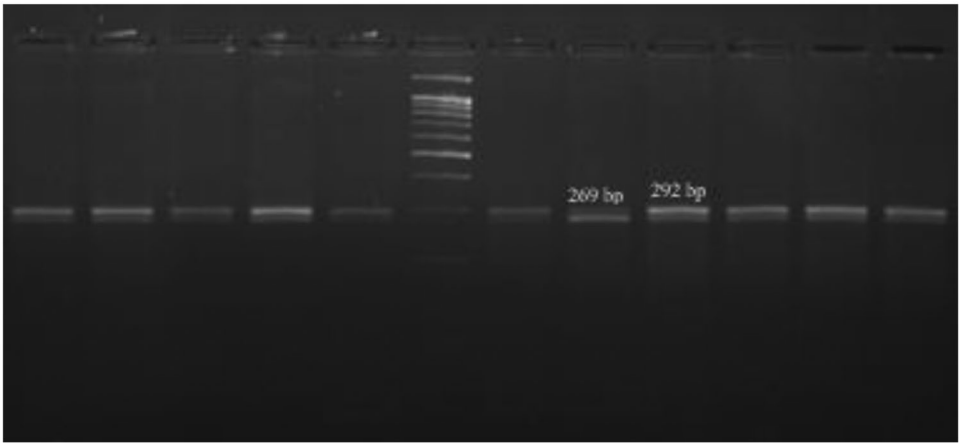

Figure 4. Baul cut gel image of PDYN rs6045819 polymorphism. (6th well 100 bp DNA marker, 8th well GG, 9th well G-).

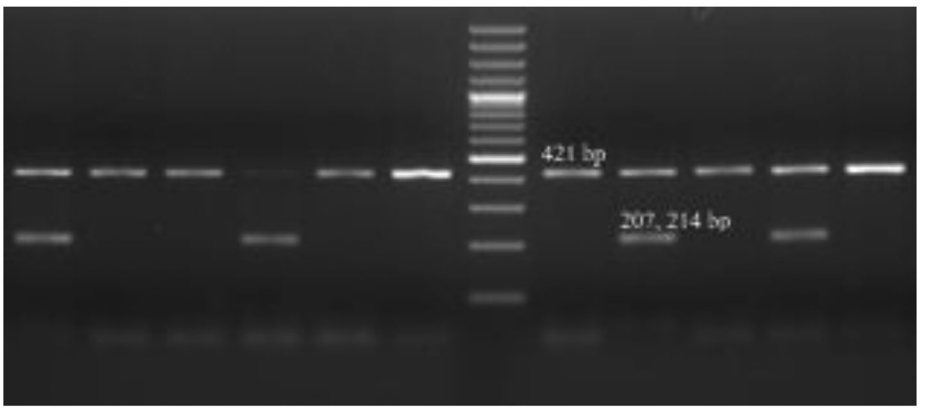

Figure 5. Gel image of the PDYN rs2235749 polymorphism. (1st well AG, 2nd well GG, 4th well AA, 7th well 100 bp DNA marker).

The association of PDYN level with scale scores and demographic data was examined with Spearman correlation analysis. $\chi 2$ (chi-square) test was used to contrast marital status and the genotype frequency of polymorphisms (rs1997794, rs1022563, rs6045819, rs2235749) between individuals with MD and HC. The relationship between polymorphisms and MD was modeled through binary logistic regression analysis. To compare the risk of dependence among genotypes, OR value and 95% confidence intervals were calculated and p value <0.05 was considered statistically significant.

Results

Age of the MD group was 34 (21–59) and duration of education was 5 (5–20) years. In HC group, age was 26 (18–50) years and duration of

Table 3. Clinical features of people with methamphetamine dependence (n = 134).

	Med/n	min-max/%
The onset age of methamphetamine	24	10–48
Amount of methamphetamine use (daily)		
0.5–1 gr	96	71.6
<1gr	38	28.4
Years of methamphetamine use	3	1–8
Number of hospitalizations in the Substance Dependence Treatment Center in the past	0	0–4
Using methamphetamine by oral route or inhalation	134	100

education was 8 (5–15) years. Also, 91 (67.9%) patients in the MD group were single, 30 (22.4%) were married, and 13 (9.7) were divorced. In HC group, 23 (23.7%) were single, 74 (76.3%) were married. There were significant differences in terms of age and marital status between the groups ($p < 0.001$), and no differences in terms of duration of education ($p > 0.05$).

The clinical features of people with MD were given in Table 3.

Evaluation of PDYN polymorphisms

For rs1022563 polymorphism, a statistically significant increase ($p = 0.042$ and $p = 0.015$, respectively) in TT and CT genotype frequency and T allele frequency was observed in the patient group compared to the control group. PDYN genotype and allele frequencies of MD and HC group were presented in Table 4. As the relationship between PDYN genotypes and the MD was examined by "logistic regression analysis", it was found for rs1022563 polymorphism that the dependence risk was increased by CT genotype (1.99 times), TT genotype (2.73 times) and T allele (1.8 times). Accordingly, for the rs2235749 polymorphism, AA genotypes lead to a 3.55 times greater dependence risk (OR = 3.553; 95% CI = 1.124–11.232).

Comparison of TEMPS-A irritable scores of PDYN rs1997794 TT, CT and CC genotypes showed that the TEMPS-A irritable score of the CT genotype was significantly higher than the CC genotype ($p = 0.006$). When TEMPS-A irritable scores of PDYN rs1022563 TT, CT and CC genotypes were compared, TEMPS-A irritable score of the CC genotype was significantly higher than that of the TT genotype ($p = 0.010$) (Tables 5 and 6).

Evaluation of serum PDYN levels

The mean serum PDYN levels of the group with MD were attained as 212.7 (57.2–590.7) pg/ml in the period of increased withdrawal within detoxification process and 194.8 (54.0–551.3) pg/ml in the remission period following withdrawal, compared to 139.9 (71.7–869.5) pg/ml for the HC. No significant correlation was found across the serum PDYN levels obtained in the periods of increased level of withdrawal and symptom

Table 4. PDYN genotype and allele frequencies of methamphetamine (n = 134) and control (n = 97) groups.

SNP (PDYN)	Genotype/Allele	Methamphetamine	Control	OR (95 CI%)	p
	TT	47 (35.1%)	40 (41.2%)	Reference	
rs1997794	TC	62 (46.3%)	45 (46.4%)	1.173 (0.663–2.074)	0.370
	CC	25 (18.7%)	12 (12.45%)	1.773 (0.791–3.974)	
	CC	80 (59.7%)	73 (75.3%)	Reference	
rs1022563	CT	48 (35.8%)	22 (22.7%)	1.991 (1.097–3.613)	0.042
	TT	6 (4.5%)	2 (2.1%)	2.737 (0.536–13.992)	
	AA	100 (74.6%)	76 (78.4%)	Reference	
rs6045819	AG	32 (23.9%)	21 (21.6%)	1.158 (0.619–2.166)	0.301
	GG	2 (1.5%)	0 (0%)		
	GG	61 (45.5%)	51 (52.6%)	Reference	
rs2235749	GA	56 (41.8%)	42 (43.3%)	1.115 (0.646–1.925)	0.060
	AA	17 (12.7%)	4 (4.1%)	3.553 (1.124–11.232)	
rs1997794	T	156 (58.2%)	125 (64.4%)	Reference	0.209
	C	112 (41.8%)	69 (35.6%)	1.301 (0.888–1.904)	
rs1022563	C	208 (77.6%)	168 (86.6%)	Reference	0.015
	T	60 (22.4%)	26 (13.4%)	1.864 (1.127–3.083)	
rs6045819	A	232 (86.6%)	173 (89.2%)	Reference	0.474
	G	36 (13.4%)	21 (10.8%)	1.278 (0.721–2.267)	
rs2235749	G	178 (66.4%)	144 (74.2%)	Reference	0.081
	A	90 (33.6%)	50 (25.8%)	1.456 (0.967–2.193)	

$p < 0.05$, PDYN = Prodynorphin, SNP = Single nucleotide polymorphism, OR (%95 CI)=Odds ratio 95% confidence interval.

Table 5. Comparison of TEMPS-A irritable scores of PDYN rs1997794 and rs1022563 polymorphism genotypes (n = 134).

	Med (min–max) (n)	Med (min–max) (n)	Med (min–max) (n)	
PDYN	TT	CT	CC	p
rs1997794	9 (0–16) (47)	10 (3–18) (62)	5 (0–14) (25)	0.021
rs1022563	5 (0–8) (6)	10 (1–16) (48)	9 (0–18) (80)	0.049

Kruskal-Wallis test, $p < 0.05$, PDYN = Prodynorphin.
PDYN = Prodynorphin, TEMPS = Temperament Evaluation of Memphis, Pisa, Paris and San Diego Autoquestionnaire.

Table 6. Pairwise comparison of TEMPS-A irritable scores.

TEMPS-A irritable	TT × CT, p	TT × CC, p	CT × CC, p
rs1997794	0.271	0.082	0.006
rs1022563	0.032	0.010	0.779

Bonferroni correction $\alpha^* = \alpha/3 = 0.05/3 = 0.016$.
TEMPS = Temperament Evaluation of Memphis, Pisa, Paris and San Diego Autoquestionnaire.

recovery in MD group ($p > 0.05$). However, PDYN levels were significantly higher than the HC in the periods of increased level of withdrawal and improved withdrawal symptoms ($p = 0.003$ and $p = 0.015$, respectively). There was a negative correlation between PDYN level, gotten in the period of increased level of withdrawal, and BDI ($r = -0.37$, $p = 0.012$); a positive correlation between PDYN level and TEMPS-A hyperthymic ($r = 0.31$, $p = 0.035$).

PDYN, which was taken in the period of increased withdrawal level, had a negative relationship with age ($r = -0.35$, $p = 0.019$). PDYN, in the period of improved withdrawal symptoms, had a negative relationship with age

(r= −0.44, p = 0.003). There was a negative relationship between the age of onset of methamphetamine and PDYN taken in the period of increased level of withdrawal (r= −0.36, p = 0.014). There was a negative relationship between the age of onset of methamphetamine and PDYN taken when withdrawal symptoms were improved (r= −0.42, p = 0.004).

Discussion

All of the participants with MD were using methamphetamine by oral route or inhalation. Our dependence center, located in the southern Marmara region of Turkey, is the only substance dependence treatment center, which provides inpatient treatment in the region. Although we have not conducted any research on the motivations for methamphetamine use preferences, our clinical observations point out that people with MD, who have applied to our clinic, justify that intravenous use is harmful and they avoid it.

In the studies conducted with people with alcohol use disorder, PDYN rs6045819 polymorphism (Xuei et al., 2006) and PDYN rs1997794 polymorphism (Babbitt et al., 2010; Faisal et al., 2014; Karpyak et al., 2013; Xuei et al., 2006) were associated with alcohol use disorder; but those were not significant in our study.

Despite the fact that both cocaine and methamphetamine are considered as psychostimulants, PDYN gene expressions caused by both substances are not similar (Turchan et al., 2002). In a study consisting of White and African Americans diagnosed with cocaine dependence or cocaine and alcohol dependence as comorbid conditions, and the HC, rs1997794 and rs6045819 polymorphisms were not significant, as in our study. Whereas PDYN rs2235749 polymorphism, also investigated in our study, was reported to be associated with the diagnosis of cocaine dependence and alcohol dependence accompanying cocaine dependence in Whites, but not in African Americans (Yuferov et al., 2009). In the present study, it has been evidenced in a Turkish sample that AA genotype of PDYN rs2235749 polymorphism augments the risk for dependence to methamphetamine, another stimulant.

The literature review has referred to the fact that PDYN polymorphisms vary according to factors such as type of substance, gender and race (Taqi et al., 2011). No relation was detected in the study that examined PDYN VNTR polymorphism in patients with MD in Iran (Saify & Saadat, 2015). As for the study on Japanese patients with MD, polymorphism of alleles of PDYN VNTR 68 bp repeat 3 or 4 copies was more frequent than HC group (Nomura et al., 2006). To our knowledge, the polymorphisms, assessed in our study, have not previously been evaluated in a sample of patients with

MD. In the present study, it has been found that CT and TT genotypes for PDYN rs1022563 polymorphism; T allele and AA genotype for PDYN rs2235749 polymorphism increased the risk of dependence. According to a community-based study in the United States, it was found out that PDYN 68 bp tandem repeat element polymorphisms were correlated with Zuckerman Sensation Seeking Scale (ZSS-V) scores. Even though there was no relationship between PDYN and alcohol dependence in the individuals diagnosed with alcohol dependence, PDYN was identified to be associated with risky, disinhibited behaviors. The authors suggested that PDYN gene variations are a dimensional feature covering all types of dependence and may be linked to substance use and risky behaviors (Flory et al., 2011). Irritable temperament has been asserted to be the most related temperament type with dependence (Moore et al., 2005). Irritable temperament is characterized by over reactivity to aversive stimuli in cases of emotional dysregulation, impulsivity, and negative affect (Rihmer et al., 2010). PDYN rs1997794 CT genotypes had significantly higher scores of TEMPS-A irritable than CC genotypes, and PDYN rs1022563 CC genotypes, which was associated with increased dependence risk, had significantly higher scores of TEMPS-A irritable than TT genotypes. In conclusion, considering the characteristics of irritable temperament, it can be explained that PDYN is associated with the increasing risky behaviors, regardless of the MD risk.

In the current study, as we investigated whether there was a relationship between the change in withdrawal symptoms and PDYN levels in the patients who were hospitalized in the substance dependence center and recently gave up using methamphetamine, the PDYN level in the blood sample taken at the time of increased withdrawal symptoms was higher than the one taken in the period without symptoms, but this difference was not significant. The blood PDYN levels in those periods were significantly higher than the HC. This consequence may be indicative of a relationship between PDYN level and withdrawal findings.

Hyperthymic temperament is one of the temperament characteristics being associated with MD (Yehya et al., 2019). Hyperthymic temperament is strongly influenced by the dopaminergic system in contrast to other characteristics (Rihmer et al., 2010). People with hyperthymic temperament have features such as difficulty in suppression, vulnerability to stimuli, impulsivity, and hyperthymic temperament is negatively associated with depressive symptoms. (Rovai et al., 2013). As all features reviewed together, it can be said that he high levels of PDYN in methamphetamine withdrawal may be related to the hyperthymic temperament and low levels of depression. To the best of our knowledge, no studies investigating PDYN level in the withdrawal period and of this relationship with temperament and depression level exist in the literature. New studies on this subject may

be beneficial in terms of developing more effective strategies in the methamphetamine withdrawal.

There was a negative correlation between age and age of onset of methamphetamine, and PDYN levels at the time of increased withdrawal level and improved withdrawal symptoms. PDYN levels were not related to the number of years of methamphetamine use and the number of hospitalizations in the substance dependence treatment center in the past. As for HC group, no link between age and PDYN level was confirmed. The sample of our study was not homogeneous and the age of onset of methamphetamine was as early as 10-year and as wide as 48-year. The PDYN's association only with age and age of onset of methamphetamine may stem from the disruptive effects of methamphetamine on the brain, especially on the dopamine system, in those who begin using methamphetamine at an early age (Beynon, 2009).

Our study has some limitations. There was significant difference between the groups in age and marital status of participants. The study was performed on individuals who completed the detoxification process in the inpatient substance dependence unit in compliance with its method. Since the inpatients taking treatment in MD were mostly men, not female participant was included. The studies involving both genders may be useful, as gender-specific differences in PDYN polymorphisms and temperament characteristics have already been known. In the study, depression and temperament characteristics of the HC group were not evaluated. It will be beneficial to conduct studies on healthy individuals to compare their PDYN polymorphisms with depression and temperament characteristics. The first blood sample taken from the patients were taken according to subjective experience and clinical observation. The lack of a standardized measurement for assessment of withdrawal symptoms can be regarded as a limitation. Another limitation is that the medical and substance use history of the participants were taken via self-report measures. Our study was conducted on Turkish people with MD. The studies involving other ethnic groups may be useful for assessing ethnic differences.

To conclude, as disturbing withdrawal symptoms are one of the important factors that cause re-use of methamphetamine, screening of temperament characteristics thought to be associated with dependence risk and setting up preventive and curative interventions in accordance with temperament characteristics may be advantageous in patients with MD. The development of interventions that may reduce the effects of PDYN, such as the use of K opioid receptor antagonists in individuals with MD, may alleviate withdrawal symptoms and then relapse rates (Erikson et al., 2018; Shippenberg et al., 2007).

Disclosure statement

No conflict of interest.

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