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Research report

Association between heroin dependence and prodynorphin gene polymorphisms

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ABSTRACT

Dynorphin peptides and k-opioid receptor are important in the rewarding effects of drugs of abuse such as heroin. This study examined potential association between heroin dependence and four single nucleotide polymorphisms (SNPs) of prodynorphin (PDYN) gene (rs35286281 in promoter region and rs1022563, rs2235749, rs910080 in 3′UTR). Participants included 304 heroin-dependent subjects and 300 healthy controls. Genotype, allele frequencies and difference between groups were analyzed by HaploView 4.0 and SPSS 11.5 software. The analysis indicated a significant higher frequency of the PDYN 68bp VNTR (rs35286281) H allele in heroin-dependent subjects than in controls ($p = 0.002$ after Bonferroni correction). Strong linkage disequilibrium was observed between rs1022563, rs2235749 and rs910080 polymorphism ($D' > 0.9$). Significantly more TCT haplotypes were found in heroin-dependent patients than in the controls ($p = 0.006$ after Bonferroni correction). We found significant pointwise correlation of these three variants (rs1022563, rs2235749 and rs910080) with heroin dependence. These findings support the important role of PDYN polymorphism in heroin dependence, and may guide future studies to identify genetic risk factors for heroin dependence.

1. Introduction

Heroin addiction is a chronic relapsing condition characterized by compulsive drug seeking, drug abuse, tolerance and physical dependence. An adoption study indicated a genetic component to substance dependence, especially opioid dependence [2]. Twin studies have suggested that genetic factors account for 30–60% of the overall variance in the risk of developing drug addiction [17,31,33]. Genetic component of opioid addition is even greater [31].

Prodynorphin (PDYN) is an opioid peptide precursor protein that gives rise to α- and β-neoendorphins, dynorphin A- and dynorphin B-related peptides [14]. These products of the PDYN genes have been shown to be able to inhibit neurotransmission by acting through kappa opioid receptors, and are implicated in reward, mood regulation, stress responses, and motor functions [7,13,16]. The dynorphins/kappa opioid receptors (Dyn/KOP) system plays a crucial role in addiction. Dysregulation of the Dyn/KOP system is induced by repeated drug abuse and involves the mesolimbic reward system. Thus, the dopaminergic pathway of the ventral tegmental area to the nucleus accumbens is seen as the main site of Dyn action in addiction. The importance of the Dyn/KOP systems is discussed not only with regard to habit learning and establishment, but also with regard to the reinstatement of addiction [28].

Several animal studies have shown that chronic administration of drugs of abuse, such as morphine [33], cocaine [1,11,18,19,26,27,37], amphetamine [32], nicotine and ethanol [9,10,20,23], altered the activity of PDYN in the brain. Drugs of abuse can increase prodynorphin expression in clinical models. Postmortem studies of human cocaine addicts demonstrated an increase in both mRNA and protein levels of dynorphin compared to controls [8,15]. Furthermore, polymorphisms in prodynorphin gene have been associated with opioid dependence in females [5]. In a human study, evidence suggested that dynorphin administered intravenously produced decreased withdrawal symptoms in heroin addicts [34]. It had been found that genetic variability of endogenous dynorphin opioid systems, which mediate dysphoria, may be related to opioid abuse, and thus has a strong heritability [12,30,31].

Human PDYN gene contains four exons and three introns. Zimprich et al. identified a functional polymorphism in PDYN gene promoter region with one to four repeats of a 68-bp element containing one binding site per repeat for transcription factor AP-1 (c-Fos/c-Jun) [39]. *In vitro* evidence has implied that this variable nucleotide tandem repeat (68-bp VNTR) polymorphism was able to induce transcriptional activation with four or three, but not less,

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Table 1
Primers and enzyme digestion information for the detection of PDYN gene SNPs.

Polymorphism sites	Primers	Restriction endonuclease	Digestion products (bp)
rs1022563C/T	F: 5′-TCAAGGCTGAGACGGGAGT-3′ R: 5′-AGGGAGCACAAGGGAACG-3′	DdeI	C: 256 = 172 + 177 + 6 T: 256 = 249 + 6
rs2235749A/G	F: 5′-TGGAAACCAAGACATCAGG-3′ R: 5′-TCATTGTTCAGAAAAGCACC-3′	NdeI	T: 571 = 206 + 365 C: 571 = 571
rs910080 A/G	F: 5′-CAATGCCCAGTGCGTATGT-3′ R: 5′-CTTTGGAGACGATGCTTTAGGT-3′	Bsp1286I	T: 497 = 497 C: 497 = 300 + 197

Note: All PCR experiments consisted of: a pre-denaturation step of 4 min at 95 °C, denaturation 30 s at 95 °C, 30 cycles and total extension 15 min at 72 °C.

copies of the repeats [38]. Such allelic variations could influence gene expression and contribute to individual psychophysiological variability [3,6]. However, studies on genetic association have failed to produce consistent results with regards to potential involvement of PDYN 68bp VNTR polymorphism in cocaine, methamphetamine and heroin dependence [3,6,23,25,32,39].

In addition to 68-bp VNTR polymorphism, the most well-studied one, other polymorphisms of PDYN genes in 3′UTR had also been associated with decreased PDYN mRNA expression in human brain tissues [37], suggesting that these PDYN gene variants might influence gene expression and thus PDYN function in human. Recent findings suggest that two SNPs (rs1022563 and rs1997794) in 3′UTR of this gene were associated with increased risk of developing opioid dependence in females [5]. In genotype and allelic tests, Yuferov et al. found experiment-wise significant association between three SNPs (rs910080, rs910079 and rs2235749) in 3′UTR and both cocaine dependence and cocaine/alcohol codependence in Caucasians, but not in African Americans [37]. However, genetic association studies have not been repeated in heroin dependence.

In view of the crucial role of the PDYN in addiction disorders, as well as the controversy in genetic association studies, we conducted a study with relatively large sample size and same ethnic origin to verify the putative association between PDYN polymorphisms and heroin dependence.

2. Patients and methods

2.1. Subjects

Three hundred and four adult individuals with heroin addiction (mean age of 37.1 ± 6.3, 181 males and 123 females) receiving treatment in the Methadone Maintenance Treatment (MMT) Program at Xi'an Mental Health Center participated in this study. Opioid addiction was diagnosed based on the DSM-IV criteria from the medical history, along with urine test, and interview. A case vignette was made to assist with diagnosis, using a semistructured interview with questions on (a) the age at initiation and duration of heroin use, (b) quantity of drug used over this period, (c) route of administration (nasal inhalation or injection), (d) whether other substances were used or abused, and (e) comorbidity for any other psychiatric disorder. Major central nervous system (CNS) diseases and psychoses were evaluated by a senior psychiatrist at the beginning of the methadone management program. Participants were excluded if they: met DSM-IV criteria for an additional Axis I disorder; had a history of alcohol, cigarette, amphetamine, barbiturate, benzodiazepine, or marijuana dependence according to DSM-IV; were taking other prescribed medications that could affect the central nervous system; had a history of seizures, hematological diseases, or liver or kidney severe impairment; or were pregnant. Each subject participated in this study voluntarily and provided written informed consent before enrollment. All subjects had been on MMT for at least six months when enrolled in this study.

Three hundred healthy blood donors (mean age of 36.5 ± 5.6, 168 males and 132 females) were recruited at the First Hospital Affiliated to the Medical College of Xi'an Jiaotong University. Collection of the medical history included past psychotic symptoms, alcohol and nicotine addiction, as well as the use of illicit drugs. Subjects who had substance abuse, participated in other studies or suffered from chronic brain diseases were excluded.

All participants are Han Chinese from Shaanxi Province and not genetically related. Written informed consent was obtained from all participants. The study was approved by the Ethical Committee of Xi'an, China.

2.2. Methods

3–5 ml peripheral blood from each subject were collected with tubes coated with EDTA. Genome DNA was extracted with TIANamp Blood DNA Kit (TIANGEN, Beijing, China), and then stored at −20 °C until use. PCR primes were designed by Primer 5.0 software. PCR amplification system has a total volume of 24 μl, containing 100–400 ng of genome DNA, 1× PCR buffer solution, 15 mmol/L $MgCl_2$, 5 umol/L primer, 1.0U Tag enzyme (TIANGEN). The PCR products were subjected to 2% agarose gel electrophoresis and then visualized with a gel imaging system to determine genotypes. Primer sequences of 68bp VNTR in the promoter region of PDYN gene using the following primers: forward primer 5′-AGC AAT CAG AGG TTG AAG TTG GAC GC-3′ and reverse primer 5′-GCA CCA GGC GGT TAG GTA GAG TTG TC-3′. The PCR conditions consisted of an initial denaturation step of 94 °C for 5 min, followed by 35 cycles of 94 °C for 30 s, 62 °C for 30 s and 72 °C for 30 s, followed by a final extension of 72 °C for 5 min. Alleles, containing 1, 2, 3 or 4 repeats, produced PCR amplicons of 385, 454, 522 and 590 bp, respectively. Bands corresponding to each number of repeats (1, 2, 3, or 4) were isolated from gels, subcloned using TA-TOPO cloning kit (Invitrogen Inc., Carlsbad, CA, USA) and sequenced to confirm their identities. Primer sequences, amplification condition and the length (bp) of restriction digestion products for detection of rs1022563, rs2235749, rs910080 in 3′UTR SNPs are listed in Table 1.

2.3. Statistical analyses

The HaploView 4.0 software was used for haplotype test, χ^2 tests was performed to test allelic and genotypic differences between experimental and control groups using SPSS 11.5 software. $p < 0.0125$ denotes significant difference after Bonferroni correction.

3. Results

The distribution of genotypes, allelic frequencies and haplotype in control and patient groups, together with the results of statistical analysis were listed in Tables 2 and 3.

Significant differences were found in the distribution of genotype and allele frequencies of PDYN gene 68-bp VNTR between heroin-dependent subjects and healthy controls ($p = 0.004$ and 0.002, respectively). The frequency of the H allele in heroin-dependent subjects was significantly higher than in the controls ($\chi^2 = 9.435$, $p = 0.002$, $OR = 1.537$, 95% $CI = 1.167–2.025$).

Furthermore, interestingly, the results of linkage disequilibrium tests demonstrated that PDYN gene rs1022563, rs2235749 and rs910080 were in strong linkage disequilibrium ($D' > 0.9$). Significantly more TCT haplotype was found in heroin-dependent patients than in the controls ($\chi^2 = 7.451$, $p = 0.006$, $OR = 1.711$, 95% $CI = 1.161–2.522$). We found significant pointwise correlation of these three variants (rs1022563, rs2235749 and rs910080) with heroin dependence. These differences retained statistical significance after Bonferroni correction.

4. Discussion

Dynorphin and k-opioid receptor (KOPr) are located in several areas of the dopaminergic nigrostriatal and mesolimbic–mesocortical systems, and play a modulatory role in heroin, cocaine and other rewarding stimuli [20]. The binding between PDYN and k-opioid receptors makes dopaminergic system important for the reinforcing and rewarding effects of drugs of abuse such as heroin [28].

After adjusting for multiple testing (threshold significance p value was set at 0.0125 in Tables 2 and 3), the most intriguing finding of the present study is the association of PDYN polymorphism with heroin dependence. This element contains an AP-1 transcrip-