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Polymorphisms of brain-derived neurotrophic factor associated with heroin dependence

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ABSTRACT

Brain-derived neurotrophic factor (BDNF) promotes synaptic remodeling and modulates the function of other neurotransmitters. It also plays a role in the reward response to many drugs, including heroin. To identify genetic variants associated with heroin dependence, we compared four single nucleotide polymorphisms (SNPs, rs13306221, rs6265, rs56164415, and rs16917204) of the BDNF gene in 487 subjects with heroin dependence and 492 healthy individuals. The analysis revealed the G allele of rs6265 was significantly more common in heroin-dependent subjects than in the healthy controls ($P = 0.001$ after Bonferroni correction). Among heroin-dependent individuals, the onset of dependence was significantly earlier in individuals with GG or GA genotypes compared to AA individuals ($P < 0.01$). Additionally, we found that the G allele of rs13306221 was significantly more frequent in heroin-dependent subjects than in controls ($P = 0.005$ after Bonferroni correction). These findings support a role of BDNF rs6265 and rs13306221 polymorphisms in heroin dependence and may guide future studies to identify other genetic risk factors for heroin dependence.

Heroin dependence is a chronically relapsing disease characterized by compulsive drug seeking, drug abuse, tolerance, and physical dependence. According to an adoption study, substance dependence, in general, and opioid dependence, in particular, have a genetic component [4]. Twin and family studies have provided some evidence that genetic factors may interact with environmental modulators together promoting heroin addiction and relapse after withdrawal [17,20]. Moreover, the studies have also indicated the genetic predispositions providing the basic conditions for dependence on most classes of drugs [32].

Brain-derived neurotrophic factor (BDNF) plays a role in the survival and function of several neurotransmitter systems, which can have an impact on substance dependence [24]. BDNF is a major regulator of the phosphatidylinositol 3′-kinase (PI3K), mitogen-activated protein kinase (MAPK), phospholipase Cγ (PLCγ), and nuclear factor kappa B (NFκB) signaling pathways, which influence a range of cellular functions, including neuronal survival, growth, differentiation, and structure. These signaling pathways regulate structural and behavioral plasticity in the context of drug addiction [27], suggesting that BDNF acts upon the reward system of the brain, which plays a key role in drug dependence. In naive rats, an increase in the concentration of BDNF in the VTA induced an opiate-dependent-like reward state [13]. Based on these studies, BDNF is a candidate locus for association with drug dependence.

Although there are known polymorphisms in BDNF, the exact role of these polymorphisms in heroin addiction is unknown. Neurons with at least one A allele of a common non-conservative polymorphism rs6265 of BDNF were characterized by lower depolarization-induced secretion [9,14] and reduced intracellular trafficking and packaging of the BDNF precursor (pro-BDNF). Consequently, these neurons also exhibited changes in the regulated secretion of the mature peptide and hippocampal function [29] and poorer performance on tests of episodic memory [9,14]. Strong evidence suggests that this polymorphism is also associated with addiction to other drugs [1,10,16,22], including opiates [7,8]. However, studies on smoking and alcohol abuse produced contradictory results [12,18,22,23]. In the putative promoter region, the polymorphism rs13306221 (712 bp upstream of the first exon) is associated with substance dependence [33]. In the first exon coding region, the polymorphism rs56164415 that may affect BDNF expression [31] is associated with psychiatric disorders [25,30]. In the 3′UTR, the polymorphism rs16917204 is associated with Alzheimer's disease-related depression [3].

Here, we evaluated the putative association between these four BDNF polymorphisms and heroin dependence using a large sample and a control group of the same ethnic origin.

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Table 1
Primer sequences used for genotyping of BDNF gene SNPs with the MALDI-TOF Sequenom platform.

Polymorphism Sites	Forward primers	Reverse primers	Extension primers
rs13306221	ACGTTGGATGGGGGCGTTCATCAATAAAA	ACGTTGGATGCTGACCTCTCTAGAGTTTGC	CGTTCATCAATAAAAAATGCT
rs6265	ACGTTGGATGCATCATTGGCTGACACTTTC	ACGTTGGATGTTTTCTTCATTGGGCCGAAC	CTCCGCCAACAGCTCTTCTATCA
rs56164415	ACGTTGGATGATTCCCAGCGCTTGCCTAC	ACGTTGGATGAATCGGAACCACGATGTGAC	TCCACACAAACCTCACG
rs16917204	ACGTTGGATGGTTTCTAATCACAGGGAATC	ACGTTGGATGACCCACCAGAAAGCTCAATC	TGAGCTCCTGAACGAGG

487 adult subjects (mean age: 39.6 ± 6.2 years, 364 males and 123 females) receiving rehabitation therapy in the Methadone Maintenance Treatment (MMT) Program provided by the Xi'an Mental Health Center were enrolled in the present study. The addiction status of each subject was assessed by a psychiatrist, and each subject met the DSM-IV diagnostic criteria [34] for opioid dependence. Major neurological and psychological presentations were evaluated at the beginning of rehabitation and reappraised once every year. The exclusion criteria for subject selection included: age less than 18 years, existence of a major central nervous system disease, psychosis, and using drugs other than heroin as the primary choice. All subjects had been on the MMT for at least six months at the time of the current study.

492 healthy blood donors (mean age: 40.2 ± 5.8 years, 360 males and 132 females) were recruited at the First Affiliated Hospital of Medical College of Xi'an Jiaotong University. Collection of medical history included psychotic symptoms, alcohol and nicotine addiction, as well as the use of illicit drugs. Subjects who were pregnant, had substance abuse, participated in other studies or suffered from chronic brain diseases were excluded from the present study.

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

Three to five milliliters of peripheral blood 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. Genotyping of the rs16917204, rs56164415, rs6265 and rs13306221 polymorphisms were performed using the MassARRAY system (Sequenom Inc., San Diego, CA, USA). Briefly, following PCR amplification, primer extension products were analysed by chip-based matrix-assisted laser desorption ionisation time-of-flight (MALDI-TOF) mass spectrometry. Primers were designed using Sequenom software (as shown in Table 1), and the extension reaction produced allele-specific products with masses differing by, 30 Da, or approximately one single nucleotide. Primer extension and PCR were performed according to the manufacturer's instructions, using iPLEX enzyme (Sequenom) and HotStarTaq DNA polymerase (Qiagen). After desalting of the reaction products (SpectroCLEAN; Sequenom), ∼10 nL were loaded into a SpectroCHIP (Sequenom) and analysed in the MALDI-TOF MassARRAY system in the fully automated mode. Genotypes were automatically identified by the SpectroTYPER software (Sequenom).

We determined if allelic and genotypic frequencies for each polymorphism were at Hardy-Weinberg equilibrium with a χ^2-test. Association between heroin dependence and each polymorphism was analysed using Fisher's exact test or Pearson χ^2-test. Statistical analyses were done in SPSS13.0 (publisher). Differences were significant when $P < 0.0125$ after Bonferroni correction.

The genotypic and allelic frequencies of BDNF polymorphisms rs13306221, rs6265, rs56164415, and rs16917204 (Table 2) were all in Hardy–Weinberg equilibrium.

The genotypic and allelic frequencies of rs6265 were significantly different between heroin-dependent subjects and healthy controls (Table 2; Genotypic: $P = 0.004$; Allelic: $P = 0.001$ after Bonferroni correction). The frequency of the G allele in

Table 2
The distribution of genotype and allele frequencies of the BDNF gene polymorphisms.

Position	Genotype/allele	Heroin-dependen (487, %)	Control (492, %)
rs13306221 genotype	GG	448 (91.99)	424 (86.18)
	GA	39 (8.01)	68 (13.82)
	AA	0 (0.00)	0 (0.00)
	χ^2, *P*	8.495, 0.004[a]	
rs13306221 allele	G	935 (96.00)	916 (93.09)
	A	39 (4.00)	68 (6.91)
	χ^2, *P*, *OR*, *CI*	8.004, 0.005[a], 1.780, 1.188–2.666	
rs6265 genotype	GG	184 (37.78)	156 (31.71)
	GA	225 (46.20)	217 (44.11[b])
	AA	78 (16.02)	119 (24.19[b])
	χ^2, *P*	10.958, 0.004[a]	
rs6265 allele	G	593 (60.88)	529 (53.76)
	A	381 (39.12)	455 (46.24)
	χ^2, *P*, *OR*, *CI*	10.150, 0.001[a], 1.339, 1.119–1.602	
rs56164415 genotype	CC	452 (92.81)	445 (90.45)
	CT	35 (7.19)	47 (9.55)
	TT	0 (0.00)	0 (0.00)
	χ^2, *P*	1.785, 0.205	
rs56164415 allele	C	939 (96.41)	937 (95.22)
	T	35 (3.59)	47 (4.78)
	χ^2, *P*, *OR*, *CI*	1.707, 0.191, 1.346, 0.861–2.104	
rs16917204 genotype	GG	219 (44.97)	256 (52.03)
	GC	214 (43.94)	186 (37.81)
	CC	54 (11.09)	50 (10.16)
	χ^2, *P*	4.971, 0.083	
rs16917204 allele	G	652 (66.94)	698 (70.93)
	C	322 (33.06)	286 (29.07)
	χ^2, *P*, *OR*, *CI*	3.648, 0.056, 1.205, 0.995–1.460	

[a] Heroin dependence compared with control, differences reaching statistical significance are $p < 0.0125$.
[b] Rounded off to two decimal.