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# The Effect of Dopamine D2, D5 Receptor and Transporter (SLC6A3) Polymorphisms on the Cue-Elicited Heroin Craving in Chinese

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**Heroin dependence is resulted from the interaction between multiple genetic and environmental factors. Subjective craving is considered to be a central phenomenon, which contributes to the continuation of drug use in active abuser and the occurrence of relapse in detoxified abusers. Dopamine pathway has been implicated in the cue-elicited craving for a variety of addictive substances. The objective of this study was to test the hypothesis that heroin addicts carrying specific variants in dopamine-related genes would have higher levels of craving following exposure to a heroin-related cue. Craving induced by a series of exposure to heroin-related cue was assessed in a cohort of Chinese heroin abuser ($n = 420$) recruited from natural abstinence center at Shanghai. Significantly stronger cue-elicited heroin craving was found in individuals carrying D2 dopamine receptor gene (*DRD2*) TaqI RFLP A1 allele than the non-carriers ($P < 0.001$). Furthermore, we did not observed significant association of cue-elicited craving with the nine-repeat allelic variants in dopamine transporter gene (*DAT*) SLC6A3 and with the dinucleotide repeat polymorphism (DRP) 148bp allele in D5 dopamine receptor gene (*DRD5*). The results of our study suggest that human dopamine pathway be involved in cue-induced heroin craving, and indicate a potential genetic risk factor for persistent heroin behavior and relapse.** © 2006 Wiley-Liss, Inc.

**KEY WORDS: dopamine transporter; heroin; craving; polymorphism; dopamine receptor**

## INTRODUCTION

Opioid dependence represents a growing health and social problem, with heroin being its most common form [Imlah, 1989]. Although the etiology of heroin dependence remains controversial, drug dependence is believed to be resulted from the interaction between multiple genetic and environmental factors [Duaux et al., 2000]. The variation of liability to drug dependence in the population is attributable to genotypic differences of several candidate genes related to the function of the central nervous system (CNS) [Grove et al., 1990; Hutchison et al., 2002]. It is believed that all drugs of abuse stimulate dopaminergic system, and their reinforcing effects are related to dopamine release and the mesolimbic dopamine reward system [Koob and Nestler, 1997].

The function of dopamine is mediated by two classes of dopamine receptors—D1- and D2-like families. Both dopamine D1 receptor and D5 receptor belongs to the family of D1-like receptors which may mediate a reduction in drive to seek reinforcement, in contrast to the family of D2-like receptors (including D2, D3, and D4 receptors) which mediate the reward and reinforcement effects [Self et al., 1996]. The D5 receptor has been demonstrated to have 10 times higher affinity to dopamine than the D1 receptor [Sunahara et al., 1991], which suggests its putative role accounting for a portion of the variation in the liability to substance abuse. A dinucleotide repeat polymorphism (DRP) at the *DRD5* gene [Sherrington et al., 1993], which has been used as such in a number of studies of psychiatry, may be a representative of functional variation, with its 148-bp allele (A9) being selected as the reference allele, though it is unlikely to be of functional significance itself. [Vanyukov et al., 1998; Sullivan et al., 2001]. The candidate gene selected from D2-like receptor family is *DRD2* gene in which the presence of the TaqI RFLP A1 allele has more recently been reported to be positively associated with substance abuse [Lawford et al., 2000; Shahmoradgoli Najafabadi et al., 2005], Other studies, however, have failed to replicate these findings [Li et al., 2002; Gareeva et al., 2004]. Functional study showed that the A1 allele is associated with lower D2 receptor density and reduced CNS dopaminergic function [Noble, 2003]. Dopamine transporter (*DAT*), a 12-transmembrane domain neurotransmitter transporter, is responsible for the re-uptake of dopamine from the synapse in midbrain dopaminergic neurons [Giros et al., 1996], playing a key role in the homeostatic regulation of dopaminergic neurotransmission. The most investigated polymorphism is a 40-bp variable number tandem repeat (VNTR) in the 3′-untranslated region of *DAT1* gene (SLC6A3) [Vandenbergh et al., 1992], which may affect mRNA localization, transcript

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stability, or regulation of protein synthesis [Wu et al., 1998], is related to addictive behaviors [Lerman et al., 1999; Sabol et al., 1999], while this is not the case in other studies [Hong et al., 2003].

One possible reason for the mixed results in the literature may be the lack of experimental control over the multiple factors that affect the addiction in the real-world settings [Lerman and Swan, 2002]. One of the approaches is to examine the genetic influence on a more narrowly defined and tightly characterized endophenotype. One such candidate is cue-elicited craving. The use of this endophenotype was employed in a previous report that CNS dopamine pathway plays a central role in the mechanism underlying cue-induced smoking craving [Erblich et al., 2005].

Numerous studies showed that craving plays a crucial role in persistent drug-taking and relapse and it has become available objective of pharmacological and behavioral therapy, although the definition and clinical relevance of craving are still a matter of controversy [Sayette et al., 2000]. Furthermore, both animal models and human studies demonstrated craving for heroin as well as other substance abuse could be induced in the laboratory by cue associated with drug taking behavior, stress, or priming dose of drug itself [Dackis and O'Brien, 2001], but the individual differences in nature and extent of the cue-elicited craving remain [Rees and Heather, 1995]. Therefore, cue, stress, or priming doses of drug-elicited craving seems to represent an underlying useable endophenotype for exploring genetic contribution to substance dependence. Craving has been conceptualized as an incentive sensitization model of addiction, which speculates that the burst firing of mesolimbic dopamine affects the motivational and appetitive attribute of heroin and other addictive substances by confining incentive salience to drug-related cue [Robinson and Berridge, 1993]. The sensitization of an incentive motivational process, which may mediate drug craving, is reported to depend on the mesolimbic dopamine system [Robinson and Berridge, 1993]. Thus, increasing attention has been focused on the relationship between craving and dopamine system genes.

The objective of the present study was, regarding cue-elicited heroin craving as an endophenotype, to test the hypothesis that heroin abuser carrying specific variants in dopamine-related genes would have higher levels of cue-induced craving. We hope to be able to understand fundamental knowledge of craving and to promote the treatment of heroin dependence.

## MATERIALS AND METHODS

### Overview

To test our hypothesis, 420 heroin addicts went through a laboratory cue exposure process, during which both heroin-related cues and neutral stimuli were presented to them. We measured heroin craving before and after each exposure. All the heroin addicts were genotyped for the *DRD2* TaqI A RFLP, *DRD5* DRP, and *DAT1* SLC6A3 VNTR polymorphisms. A factorial analysis of variance (ANOVA) was employed to estimate the effect of these variants on cue-induced heroin craving.

### Participants

Heroin addicts ($n = 420$) in Shanghai Voluntary Drug Dependence Treatment Center for abstinence were recruited. All subjects were of Han Chinese origin. The following inclusion criteria were used: age 18–60 years old, met DSM-IV (American Psychiatric Association, 1994) criteria for heroin dependence, abstinence from heroin for at least 1 month (to avoid obviously protracted withdrawal symptoms). Participants were excluded if they: met DSM-IV criteria for an additional Axis I disorder; had history of alcohol, cigarette, amphetamine, barbiturate, benzodiazepine, or marijuana dependence according to DSM-IV (some of the participants were smokers and alcohol consumer, but not dependent on those substances); were taking other prescribed medications that could affect the central nervous system, had a history of seizures, hematological diseases, liver, or kidney severe impairment; were pregnant for females. All subjects participated voluntarily and provided a written informed consent before enrollment. Protocols for this study were approved by the Ethics Committee of Fudan University (Shanghai).

### Measures

***Background questionnaire.*** Demographic questionnaire was employed to collect information including age, gender, education level, marital status, and vocation. Heroin taking history questionnaire was utilized to gather data on heroin amount per day, frequency of heroin taking per day, years of heroin taking, the route of heroin using and so on.

***Outcome measure.*** We applied a five-item 0–100 craving questionnaire to evaluate craving before and after each stimulus scene exposure. This questionnaire has been used to estimate craving level in a great deal of cue-elicited craving studies [Erblich et al., 2005], which employed diverse words such as "urge," "desire," and "craving" and had highly reliability in the present sample (Chronbach's alpha ranged from 0.91–0.93).

***Procedure.*** After offering written informed consent, heroin addicts took part in a laboratory cue-induced heroin craving program, which involved in exposure to both neutral (light bulb and pencil) and heroin-cue (heroin analogue, instruments such as tinfoil, lighter, and syringe used during heroin taking) stimuli. To avoid possible carryover effect, participants were always first exposed to neutral scene for 60 sec then heroin-related cue for 60 sec. Further, we asked subjects to watch nature scene video for 3 min between the neutral and heroin-cues. Participants fulfilled the craving scale before and after each of stimuli exposure at once. The subjects completed background questionnaires and donated 3 ml venous blood for genotyping right after finishing laboratory cue-induced craving procedure to ensure least disturbance.

### Genotype Assessment

Genomic DNA was extracted from leukocytes and the DNA sequence spanning the DRD2 TaqI A RFLP was amplified by polymerase chain reaction (PCR). Genotyping TaqIA polymorphism of the *DRD2* gene was carried out using a previously described method [Grandy et al., 1993]. Fluorescently labeled primers (sense primers 5′-FAM CGT GTA TGA TCC CTG CAG-3′ and anti-sense primer 5′-GCT CAT GAG AAG AAT GGA GTG-3′) were constructed to amplify the region containing the *DRD5* DRP polymorphism [Vanyukov et al., 1998] and the fluororescent-labeled sense primers (5′-FAM TGT GGT GTA GGG AAC GGC CTG AG-3′) and unlabeled anti-sense primer (5′-CTT CCT GGA GGT CAC GGC TCA AGG-3′) for the VNTR in the 3′ UTR of *DAT1*gene. PCR products were run on an ABI PRISM 3100 automated sequencer (Applied Biosystem, Foster City, CA) and analyzed using GENOTYPER (PE Applied Biosystems) software.

### Statistical Method

The statistical analyses were performed using SPSS for Window 10.0. The continuous variables were expressed as the mean ± SD and compared with the ANOVA. The categorical variables were expressed as percentage and the $\chi^2$ test was applied for the determination of significance of the associations. Pre- and post-exposure to heroin-related cue change

TABLE I. The Genotype Frequency for the SLC6A3 in *DAT1* gene, *DRD2* TaqIA and *DRD5* DRP Polymorphism for the Heroin Addicts

| | N | Frequency (%) |
|---|---|---|
| *DAT1* SLC6A*3* genotype | | |
| Non-9R/non-9R | 361 | 86% |
| Non-9R/9R | 56 | 13.3% |
| 9R/9R | 3 | 0.7% |
| *DRD5* DRP genotype | | |
| Non-A9/non-A9 | 152 | 36.2% |
| Non-A9/A9 | 196 | 46.7% |
| A9/A9 | 72 | 17.1% |
| *DRD2* TaqIA genotype | | |
| A1/A1 | 74 | 17.6% |
| A1/A2 | 204 | 48.6% |
| A2/A2 | 142 | 33.8% |

score was calculated as an index of craving reactivity. Craving score prior to stimulus scene exposure (baseline) was included to yield baseline-adjusted change scores, as reported previously [Erblich et al., 2005]. A factorial ANOVA was employed to estimate effect of these variants on cue-induced heroin craving. The criterion for significance was set at $P < 0.05$. The effect of the covariants such as baseline and neutral activity score was examined to exclude the possibility that the difference may be due to baseline or neutral.

## RESULTS

In total, 420 heroin addicts were recruited for our study. Seventy-six percent of the heroin abusers were male. Mean age of participants was $31.15 \pm 8.09$ years (range, 17–48). Among them, 42.7% were married and 53% obtained junior school (JS) diploma. The mean time of heroin using was $5.29 \pm 2.52$ years, and the mean dose of heroin was $0.85 \pm 0.28$ g/day.

Table I represents the distribution of genotype for the *DRD5* gene DRP and *DRD2* gene TaqIA RFLP, and *DAT1* gene SLC6A3 VNTR polymorphisms in the recruited heroin addicts. For the *DRD5* DRP, a total of 15 alleles were detected [132–160 bp]. Allele 9 (A9: 148 bp), the most common allele, was found to have a frequency of 40.5% in our sample. For the SLC6A3 VNTR polymorphism in the *DAT1* gene, we identify five alleles (7–11 repeat allele) [363–523 bp], with 483 bp (10 R) allele be the most frequent among all the subsample, followed by the 443 bp allele (9 R). For TaqIA RFLP of *DRD2* gene, three genotypes were obtained, A1/A1 (n = 74), A1/A2 (n = 204), and A2/A2 (n = 142). The "carriers" subjects were those who tested to be positive for the presence of the allelic variant (the 9 R allele of the *DAT1* gene, TaqI RFLP A1 allele of *DRD2* gene and A9 allele of *DRD5* gene). Since some genotypes and alleles were rare, the analysis was carried out by categorization according to the criteria reported previously [Vanyukov et al., 1998; Shahmoradgoli Najafabadi et al., 2005; Hong et al., 2003]. The distribution for the genotypes of the polymorphisms was in Hardy–Weinberg equilibrium. We found that 66.19% of the sample (n = 278) carried the DRD2-A1 allele (n = 74 homozygous), while 33.81% (n = 142) did not (A2/A2). For the SLC6A3 VNTR allele, 14.05% of the sample (n = 59) carried the nine-repeat allele (n = 3 homozygous), while the remaining 85.95% (n = 361) did not. For DRD5 DRP polymorphism, 63.80% (n = 262) was "carrier" (n = 64 homozygous), while the other 36.20%(n = 152) was "non-carrier."

To assess the possibility of genotype differences in background variables, we compared the demographic and heroin addiction characteristics by each genotype in the addicts group, as shown in Table II. *DRD5* DRP Allele 9 (148 bp) carriers consumed significantly more heroin per day than non-carriers ($P = 0.012$). There were no other differences between groups on background variables.

The present results show that heroin-related environmental factors could induce significant craving reaction, which is consistent with the hypothesis. As depicted in Figure 1, carrier of TaqI RFLP A1 allele in *DRD2* gene exhibited significant higher level of induced craving following exposure to a heroin-related cue, as compared to those of non-carrier ($F = 330.073$, $P < 0.001$), which is in consistent with the previous positive results among smokers [Erblich et al., 2005]. No difference of cue-induced heroin craving level was found in the homozygote A1/A1 genotype subgroup in comparison with heterozygote A1/A2 genotype subgroup among heroin-dependent subjects indicating that the TaqI RFLP A1 allele is dominant. No significant differences of the cue-elicited craving score were observed between different genotype groups of *DRD5* gene DRP and *DAT1* gene SLC6A3 VNTR polymorphism genetic variants (shown in Fig. 1). The association between carrier

TABLE II. Demographic and Heroin Abuse Characteristic by Each Genotype ($A1^+$ Denotes A1 Allele Carrier, and $A1^-$ Denotes A1 allele Non-carrier; $A9^+$ Denotes A9 Allele Carrier, and $A9^-$ Denotes A9 Allele Non-carrier; $9R^+$ Denotes 9R Carrier, and $9R^-$ Denotes 9R Non-carrier)

| | *DRD2*-A1 carrier status | | *SLC6A3*-9R carrier status | | *DRD5* DRP A9 carrier status | |
|---|---|---|---|---|---|---|
| | $A1^+$ | $A1^-$ | $9R^+$ | $9R^-$ | $A9^+$ | $A9^-$ |
| N | 278 | 142 | 59 | 361 | 268 | 152 |
| Age (years) | $30.87 \pm 7.89$ | $31.55 \pm 8.49$ | $31.18 \pm 8.15$ | $31.11 \pm 8.15$ | $30.75 \pm 8.25$ | $31.85 \pm 7.59$ |
| Sex (% female) | 22 | 24.5 | 23.1 | 27.1 | 21.7 | 27.0 |
| Marriage status (% married) | 48.9 | 52.4 | 51.9 | 45.8 | 51.3 | 50.7 |
| Education | | | | | | |
| Percentage did not complete JS** | 5.7 | 10.5 | 8.6 | 10.2 | 9.0 | 8.6 |
| Percentage completed JS** | 59.6 | 50.5 | 56.1 | 39.0 | 53.9 | 53.3 |
| Percentage completed HS** | 32.6 | 36.1 | 32.8 | 47.5 | 35.2 | 34.2 |
| Percentage completed college or > | 2.1 | 1.9 | 2.5 | 3.4 | 1.9 | 3.9 |
| Onset (years) | $26.92 \pm 7.67$ | $27.70 \pm 8.45$ | $27.31 \pm 8.09$ | $27.19 \pm 7.94$ | $27.25 \pm 8.24$ | $27.12 \pm 7.37$ |
| Dependence (years) | $5.50 \pm 2.50$ | $5.28 \pm 2.53$ | $5.14 \pm 2.28$ | $5.47 \pm 2.55$ | $5.42 \pm 2.43$ | $5.06 \pm 2.79$ |
| Amount (g/day) | $0.86 \pm 0.29$ | $0.85 \pm 0.25$ | $0.90 \pm 0.28$ | $0.85 \pm 0.28$ | $0.87 \pm 0.28$* | $0.80 \pm 0.29$* |
| Frequency (times/day) | $4.79 \pm 2.61$ | $4.64 \pm 2.16$ | $4.56 \pm 1.74$ | $4.76 \pm 2.57$ | $4.66 \pm 2.34$ | $4.87 \pm 2.53$ |

*Value differ at $P < 0.05$.
**HS denotes high school; JS denotes junior school.

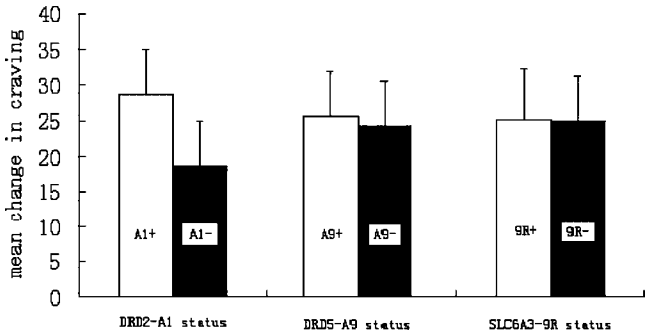


Fig. 1. Adjusted mean craving level following exposure to heroin-related cues grouped by *DRD2*-A1, *DRD5* DRP-A9, and SLC6A3-9R carrier statuses. Only groups of *DRD2* TaqI RFLP A1 allele status differ significantly ($A1^+$ denotes A1 allele carrier, and $A1^-$ denotes A1 allele non-carrier; $A9^+$ denotes A9 allele carrier, and $A9^-$ denotes A9 allele non-carrier; $9R^+$ denotes 9R carrier, and $9R^-$ denotes 9R non-carrier).

status and cue-induced craving was significant with gender, age, and education included as covariate, thus it is unlikely that the present results are confounded by these factors. There is no gene–gene interaction observed in our sample.

## DISCUSSION

The results of this study provide the first evidence that heroin addicts carrying specific polymorphism related to dopamine function in the CNS have higher cue-induced craving responses. Finding lends direct support to the hypothesis that dopamine system is involved in cue-induced heroin craving, and suggests a potential genetic risk factor for persistent heroin abuse and relapse, which is in coincidence with the previous report that TaqI RFLP A1 allele of *DRD2* gene is related to the cue-elicited smoking craving [Erblich et al., 2005]. Considering the important role of the craving in the heroin dependence, these results indicate that a possible behavioral mechanism underlying previously observed relations between these genetic polymorphisms and drug abuse behavior.

Compared with the previous relative mixed results on the relation between TaqI RFLP A1 allele of *DRD2* gene and dependence behavior, the strong results observed here may due to the utility of a narrowly defined phenotype under controlled conditions. The heroin addiction is a complex disorder, which is determined by the multiple genetic and environmental factors. Different pathway may play different role in different circumstance, which may be the leading cause of the mixed results in the previous study. That is to say, despite the evidence for genetic factor to drug abuse, any one specific gene cannot interpret all forms of substance dependence [Schuckit, 1998; Li, 2000]. A certain pathway (such as *DRD2* gene) may be important for some abusers, whereas for other addicts (without the environmental cue), this pathway may be less important. Behavioral scientists have recently recommended examining specific genetic effects on a particular, narrowly defined phenotype or an endophenotype under controlled conditions, which is experientially relevant to the clinical presentation of the disorder. This provides further insights into the mechanisms underlying addiction behavior, and may contribute to developing new pharmacogenetic approaches on the treatment or prevention of drug abuse.

Craving is defined as the desire or urge to experience the effect of a previously experienced psychoactive substance (UNDCP/WHO, 1992). Craving is one of the most characteristic experiences in addiction, and can be regarded as an important mediator of continued substance use and relapse after abstinence [Sayette et al., 2000]. Environment-related cues are mostly responsible for craving [Lu et al., 2002]. It has been stipulated when exposure to related cue, mesolimbic dopamine system can be activated, which makes incentive salience be confined to the neural area related to drug-related stimuli. It has also been postulated that dopamine release in the nucleus accumbens attributes incentive salience to drug-associated stimuli [Robinson and Berridge, 1993].

It has been reported that the rewarding and reinforcing effects of opiate are, at least partially, mediated by non-dopaminergic mechanisms, whereas the incentive to seek opiates due to the release of dopamine [Di Chiara and North, 1992]; The *DRD2* has been reported to be related to craving in smoker, alcoholics, and cocaine abuser [Erblich et al., 2005; Heinz et al., 2004; Milivojevic et al., 2004]. Our present data seem to support the view that the *DRD2* play an important role in the cue reactivity to environmental heroin-related stimuli. Positron emission tomography (PET) studies have demonstrated that a significant reduction of D2 receptor density was observed in the A1/A2 genotype group as compared to the A2/A2 group. The association between the A1 allele and low D2 receptor availability indicates that the A1 allele of the TaqIA polymorphism might be in linkage disequilibrium with a mutation in the promoter/regulatory gene element that affects dopamine D2 receptor expression [Pohjalainen et al., 1998]. The previous study has demonstrated that alcohol craving is associated with a low availability of D2 receptors [Heinz et al., 2004] and low availability of D2 receptors in the brain reward system mediates excessive attribution of incentive salience to environmental stimuli [Robinson and Berridge, 1993; Heinz et al., 2004]. Thus may be the explanation for the findings that significant stronger cue-elicited heroin craving was found for individuals carrying TaqI RFLP A1 allele of *DRD2* gene. This is in agreement with the observation of Schultz et al. [1997] that brain reward system can be activated by conditioned reward-indicating stimuli and the strength of the activation was inversely correlated with the availability of dopamine receptors. A lack of D2 receptors in the nucleus accumbens may interfere with the normal error-detection procedure of dopaminergic neurotransmission indicating the availability of reward.

The results of the present study indicate that dopamine system is involved in mechanism underlying the cue-induced craving for heroin and thus may be one of the pathways responsible for opiate addiction. Further animal and human studies are necessary to elucidate the mechanism of the effect of TaqI RFLP A1 allele of *DRD2* gene on the cue-induced craving. Since the cue-elicited craving plays an important role in the persistence of drug abuse and relapse, D2 receptor agonists may be useful in reducing cue-induced craving so as to facilitate abstinence or relapse prevention. The present study may provide insight into the mechanism underlying the heroin dependence as well as other addiction and may help in establishing novel pharmacogenetic approaches on addiction therapy and relapse prevention. Further study was warranted to investigate the role of polymorphisms in the other candidate gene of dopamine system as well as other pathway involving in the mechanism underlying the cue-induced craving for heroin.

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