Polymorphisms in the 5-hydroxytryptamine receptor 3B gene are associated with heroin dependence in the Chinese Han population

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Abstract

Previous studies suggested that the 5-hydroxytryptamine receptor 3B (HTR3B) is involved in heroin dependence by modulating dopamine (DA) release in the reward pathway and that the genetic polymorphisms in *HTR3B* play a plausible role in modulating the risk of developing heroin addiction. To identify markers that contribute to the genetic susceptibility to heroin dependence, we examined the potential associations between heroin dependence and 7 single nucleotide polymorphisms (SNPs) of the *HTR3B* gene using multiplex SNaPshot technology in a Chinese Han population. Participants included 418 heroin-dependent subjects and 422 healthy controls. The results suggested that the genotype distribution of *HTR3B* rs1176746 and rs1185027 were significantly different between heroin dependent

1 subjects and healthy controls ($p = 0.004$). The frequency of the GG of rs1176746 and
2 AA of rs1185027 genotype in heroin-dependent subjects were significantly higher
3 than that of healthy controls, while the GA of rs1176746 and AT of rs1185027
4 genotype distributions were much lower. Another SNP, rs10789970, showed a
5 nominally significant p -value in the genotype distribution between heroin dependent
6 subjects and controls ($p = 0.022$). These findings indicate the important role of
7 *HTR3B* polymorphisms in heroin dependence among the Chinese Han population and
8 provide valuable information for further genetic and neurobiological investigations of
9 heroin dependence.

11 **Keywords**

12 Heroin dependence; 5-hydroxytryptamine receptor 3B; Single nucleotide
13 polymorphisms; Chinese Han population

15 **Abbreviation:** HTR3B, 5-hydroxytryptamine receptor 3B; DA, dopamine; SNPs,
16 single nucleotide polymorphisms; 5-HT, 5-hydroxytryptamine /serotonin; MMT,
17 methadone maintenance treatment; DSM-IV, Diagnostic and Statistical Manual of
18 Mental Disorders, 4th revision; UTR, untranslated regions; CHB, Chinese Han of
19 Beijing; MAF, minor allele frequencies; HWE, Hardy-Weinberg equilibrium; χ^2 ,
20 Pearson chi squared; OR, odds ratio; CI, confidence interval; LD, linkage
21 disequilibrium.

23 **1. Introduction**

24 Heroin dependence is a chronic relapsing disorder characterized by compulsive
25 drug seeking behavior, drug abuse, tolerance, and physical dependence. Genetic

1 polymorphisms have plausible roles in modulating the risk of heroin dependence.
2 Twin and family studies have provided some evidence that the development of heroin
3 dependence was associated with both environmental and genetic factors [1, 2].
4 Previous studies have shown that the genetic risk of drug abuse is approximately 60%
5 [3]. Other studies have suggested that polymorphisms in the 5-hydroxytryptamine
6 (serotonin, 5-HT) receptor closely relate to schizophrenia and the abuse of drugs, such
7 as alcohol, cocaine and heroin [4-6]. Previously, we found that a polymorphism of the
8 5-HT receptor subtype 1B is associated with heroin dependence in the Chinese Han
9 population [7]. To further verify the association between heroin dependence and 5-HT,
10 we examined the relationship between 5-HT receptor 3B (*HTR3B*) gene
11 polymorphisms and heroin dependence.

12 5-HT₃ receptors are the only known ion-channel receptors of the serotonergic
13 receptor subtypes that could evoke fast excitation of serotonergic neurons in the
14 human nervous system [8]. The 5-HT₃ receptors are known to play an important role
15 in modulating dopamine (DA) release within the reward pathway, which is involved
16 in the addictive properties of drugs of abuse [9]. Previous studies showed that the
17 5-HT₃ receptor antagonists could attenuate cocaine or morphine induced increases in
18 extracellular levels of DA in the nucleus accumbens [10], suppress cocaine-induced
19 locomotor activity and reduce morphine-induced enhancement of behavioral activity,
20 including grooming, locomotion, rearing and sniffing in rats [11, 12]. 5-HT₃ receptor
21 overexpression in the forebrain of mice could decrease cocaine preference and
22 augment acute sensitivity with a corresponding increase in DA release in response to
23 cocaine [13]. Chronic heavy drinking depletes presynaptic serotonin reserves, causing
24 a hyposerotonergic state that may upregulate postsynaptic 5-HT₃ receptors [14].
25 Moreover, studies in both animals and humans have shown that 5-HT₃ antagonists

1 reduce alcohol intake [15-17].

2 The following five distinct subunits assemble the 5-HT₃ receptors: 5-HT₃A,
3 5-HT₃B, 5-HT₃C, 5-HT₃D and 5-HT₃E. The 5-HT₃A and 5-HT₃B subunits are the
4 most characterized 5-HT₃ receptor subunits, whereas the physiological relevance of
5 5-HT₃C, 5-HT₃D and 5-HT₃E subunits has only begun to be unraveled [18]. The
6 5-HT₃ receptors are mainly assembled in homopentamers formed by 5-HT₃A or
7 heteropentamers formed by 5-HT₃A/B subunits. Homomeric receptors 5-HT₃A are
8 more widely distributed in the central nervous system, but the expression of
9 heteromeric receptors 5-HT₃A/B was concentrated in the amygdala, the caudate and
10 the hippocampus, which are locations implicated in the reward pathway [5, 8].
11 Although the 5-HT₃B subunit fails to form either a functional receptor or a ligand
12 binding site, it is very important for trafficking and stabilizing the 5-HT₃A/B receptor
13 complex [19]. Moreover, in contrast to 5-HT₃A homopentamers, it appears that
14 heteromeric assemblies of 5-HT₃A and 5-HT₃B subunits display a greater single
15 channel conductivity and exhibit biophysical properties, which are much more similar
16 to those of native 5-HT₃ receptors [20]. Recent studies have shown that increased
17 synaptic 5-HT release coupled with increased 5-HT₃A/B receptor responsiveness
18 enhanced DA transmission in the reward pathway that is associated with a greater risk
19 of developing addiction [5]. Therefore, the 5-HT₃B subunit is a key target with
20 critical implications in heroin dependence.

21 Previous studies have shown that a polymorphism of the *HTR3B* gene was
22 associated with alcohol and heroin dependence. M.A. Enoch et al. genotyped 360
23 African American male patients with single and comorbid DSM-IV lifetime diagnoses
24 of alcohol, cocaine and heroin dependence and 187 African American male controls.
25 They found a significant association of the *HTR3B* rs1176744C with alcohol

dependence but not cocaine and heroin dependence, and the C allele was a risk factor [5]. O. Levran et al. found that the *HTR3B* single nucleotide polymorphisms (SNPs) rs11606194 in intron 2 showed a trend toward significance with heroin dependence in a Caucasian population [21]. More recently, O. Levran and his team further verified the association between *HTR3B* SNP rs11606194 and heroin dependence in a Caucasian population [4]. However, these studies only analyzed a few loci in the *HTR3B* gene, and, therefore, only provided limited SNPs evidence. Moreover, to the best of our knowledge, the association between the *HTR3B* SNPs and heroin dependence in the Chinese Han population is still poorly understood.

To verify the putative association between the *HTR3B* SNPs and heroin dependence, the present study investigated 7 SNPs (rs10789970, rs3758987, rs1176746, rs2276305, rs4936285, rs1185027, rs12795805) that cover the whole *HTR3B* gene and their relevant risk in heroin dependence in a Chinese Han population. Two SNPs were found significantly associated with heroin dependence, and one additional SNP showed a trend toward significance. These findings provide evidence of the interactive effects of variants in *HTR3B* affecting heroin dependence and encourage future efforts aimed at identifying functional polymorphisms in the *HTR3B* gene.

2. Materials and Method

2.1. Subject

A total of 418 male heroin dependence patients, who were receiving methadone maintenance treatment (MMT) in Xi'an Mental Health Center of China, were enrolled in this study. Their average age was 47.81 ± 0.04 years. These subjects were enrolled between October 2013 and May 2015. The diagnosis was based on the Diagnostic and

1 Statistical Manual of Mental Disorders, 4th revision (DSM-IV) diagnostic criteria for
2 heroin dependence, medical history, and urine test results. All patients were
3 interviewed by at least one experienced physician in a semi-structured interview.
4 Patients with dependence on substances other than heroin, histories of mental illness,
5 severe organic disorders or under 18 years of age were excluded from this study. The
6 healthy control group consisted of 422 males from the medical examination center of
7 First Affiliated Hospital, Xi'an Jiaotong University. The average age of the controls
8 was 48.38 ± 0.07 years. None of these subjects had any previous history of psychiatric
9 or neurological disorders.

10 All subjects, who were from three generations, were natives of Shaanxi or North
11 China but unrelated with each other. All Participants provided written informed
12 consent. The study was approved by the Medical Ethics Committee of Xi'an Jiaotong
13 University.

14

15 2.2. SNP selection

16 Based on the Chinese Han population in Beijing (CHB) of HapMap project, all
17 SNPs that were selected for the present study were located in the functional areas of
18 5-HT, including the promoter regions, untranslated regions (UTR), exon regions, and
19 intron-exon junctions (Fig. 1). The minor allele frequencies (MAF) of the SNPs were
20 greater than 0.05. These SNPs were further analyzed in this association study.

21

22 2.3. Genotyping

23 Three to five ml of whole blood were extracted from veins of every participant in
24 tubes coated with EDTA. All samples were frozen and stored at -80°C until use.
25 Employing the EZNA™ Blood DNAMidi Kit (OMEGA Bio-Tek, Norcross, GA,

USA) we extracted genome DNA from the blood leukocyte, according to the provided experiment protocol. Genomic DNA was purified using the TIANamp Blood DNA Kit (TIANGEN Biotech, Beijing, China). Genotyping of 7 SNPs in *HTR3B* was performed by multiplex SNaPshot technology using an ABI fluorescence-based assay discrimination method (Applied Biosystems, Foster City, CA,USA), which has been described in detail in the previous studies [22, 23]. This technology was based on the fluorescence assay discrimination method (Applied Biosystems, Foster City, CA,USA). The data were analyzed with an ABI3130XL sequencer and GeneMapperTM 4.0 Software (Applied Biosystems, Co. Ltd., USA).

2.4. Statistics

Deviations of genotype frequencies in controls from those expected under Hardy-Weinberg equilibrium (HWE) were assessed by the Pearson chi-squared (χ^2) test. The association between genetic polymorphisms and the risk of developing heroin dependence as examined using the χ^2 test. We used a binary logistic regression to calculate the odds ratio (OR) and 95% confidence interval (CI) for each independent association, and the age of onset was treated as a covariant in the binary logistic regression. *P*-values were calculated based on codominant and dominant for the rare allele and heterosis and recessive for the rare allele models of inheritance. Bonferroni correction was used for multiple comparisons, and the significant *p*-value threshold was set at 0.007 (0.05/7) in the association tests. Pair-wise linkage disequilibrium (LD) statistics (D' and r^2) and haplotype frequency were computed using Haploview 4.0 to construct haplotype blocks. Haplotype blocks were defined according to the controls. The significance of any haplotypic association was evaluated using a likelihood ratio test, followed by permutation testing that compared

estimated haplotype frequencies in cases and controls. Rare haplotypes were excluded (Frequency<0.05). All statistical tests were conducted using SPSS 18.0 software (SPSS Inc., Chicago, IL, USA).

3. Results

The genotype distributions of the 7 polymorphisms were consistent with the HWE in the controls. The distributions of genotypes and allelic frequencies with the results of statistical analysis are listed in Table 1.

Significant differences were found in the genotype distribution of *HTR3B* gene rs1176746 and rs1185027 between heroin-dependent subjects and healthy controls (both $p = 0.004$), and these differences remained statistically significant after Bonferroni correction. Specifically, the distribution of the GG of rs1176746 and AA of rs1185027 genotypes in heroin-dependent subjects was significantly higher than that in healthy controls (rs1176746: GG $p = 0.005$, OR =1.479, 95% and CI = 1.127-1.942; rs1185027: AA, $p = 0.006$, OR =1.494, 95% and CI = 1.124-1.987). Meanwhile, the GA of rs1176746 and AT of rs1185027 genotype in healthy controls was significantly higher than that in heroin-dependent subjects (rs1176746: GA $p = 0.001$, OR =0.627, 95% and CI =0.476-0.827; rs1185027: AT, $p = 0.001$, OR =0.637, 95% and CI = 0.485-0.836).

The genotype distribution of rs10789970 of heroin-dependence patients was nominally different from healthy controls ($p = 0.022$). The distribution of the TT of rs10789970 genotype in heroin-dependent subjects was significantly higher than that in healthy controls ($p = 0.018$, OR =1.395, 95% and CI = 1.058-1.840). The frequency of CT genotype in healthy controls was significantly higher than that in heroin-dependent subjects ($p = 0.007$, OR =0.685, 95% and CI = 0.521-0.9).

Pair-wise LD and haplotype block analysis of the 7 SNPs identified in the present study are shown in Fig.2 ($D' > 0.9$). LD data from the 422 healthy males showed that there were 3 haplotype blocks within the *HTR3B* gene region. These 7 SNPs are located in three separate haplotype blocks (Fig.2a and 2b). SNPs 1-2 (rs10789970, and rs3758987) are in block 1, SNPs 4-5 (rs2276305, and rs4936285) are in block 2, and SNPs6-7 (rs1185027, and rs12795805) are in block 3. The LD patterns of the 7 SNPs in the case participants (Fig. 2c and 2d) were very similar to those of the controls (Fig. 2a and 2b).The distribution of haplotype frequencies is listed in Table 2.

4. Discussion

HTR3B is one of the most important 5-HT₃ receptor subunits that may affect drug addiction [8, 24]. In the past decades, accumulating evidence has indicated that the *HTR3B* gene may serve as a genetic regulator of sensitivity to drugs of abuse[4], such as alcohol, cocaine, nicotine, morphine and heroin [21, 24, 25]. Seven SNPs, spanning both of the coding and non-coding regions of the *HTR3B* gene, were genotyped. Two SNPs (rs1176746, and rs1185027) were found to be significantly associated with heroin dependence, and one additional SNP rs10789970 showed a trend toward significance. Our results provide direct evidence that genetic polymorphisms in *HTR3B* were linked to heroin dependence in the Chinese Han population. We showed here in a cohort of Chinese Han population that *HTR3B* polymorphisms are relevant to heroin dependence. The CT of rs10789970, GA of rs1176746 and AT of rs1185027 act as protective factors. Meanwhile, TT of rs10789970, GG of rs1176746 and TT of rs1185027 may increase the risk of heroin dependence. To the best of our knowledge, this is the first report showing that these

1 SNPs are significantly associated with heroin dependence. Previous reports showed
2 that rs10789970 and rs1176746 SNPs were associated with major depression, poor
3 concentration of schizophrenia and so on [26, 27].

4 The *HTR3B* gene contains 9 exons, located on chromosome 11q23 [26].
5 Rs10789970 is located at the 5'UTR of the *HTR3B* and may modulate the *HTR3B*
6 expression. In this study, we found that rs10789970 was nominally associated with the
7 risk of heroin dependence; the TT genotype increased the risk of heroin dependence,
8 while the CT genotype acted as protected factors of heroin dependence. A previous
9 study has indicated that the C allele of rs10789970 was associated with poor attention
10 concentration in schizophrenia [27]. Using AliBaba2.1
11 (<http://www.gene-regulation.com/pub/programs/alibaba2/index.html>) to predict the
12 potential binding sites of the transcription factor in this promoter region, we found
13 that the T allele could facilitate binding of the transcription factor Oct-1, which may
14 lead to increased transcriptional activity of the *HTR3B* gene.

15 In our study, the GG genotype of rs1176746 was found to increase the risk of
16 heroin dependence. Additionally, GA genotype was associated with protection against
17 the propensity to develop heroin dependence. Rs1176746 is located in the junction of
18 intron4 and exon5 of *HTR3B* gene, which is the splice recognition site of mRNA. The
19 molecular mechanisms underlying allelic differences of this SNP are not yet
20 elucidated. It is possible that this SNP may alter the mRNA expression through
21 alternate splicing, and then affect the expression of *HTR3B*. The genotype of GA/GG
22 of rs1176746 was selected as a marker for the treatment and diagnosis of alcohol and
23 drug dependence [28]. Furthermore, rs1176746 provided evidence of genotypic
24 association in female bipolar patients [26]. Bipolar spectrum disorders and addiction
25 often co-occur [29] and constitute reciprocal risk factors that we believe may

1 associate with a common pathogenesis.

2 Few studies have investigated the association of rs1185027 with such disorders.
3 In the present study, the TT genotype of rs1185027 could increase the risk of heroin
4 dependence, while the AT genotype could decrease the risk of heroin dependence.
5 rs1185027 is located in the 3'UTR, which may affect the stabilization of mRNA. It
6 has been reported that the 3'UTR region of genes is rich in regulatory elements
7 essential for the regulation of mRNA stability and degradation, nuclear transport, and
8 translation [30, 31].

9 In our results, rs3758987 was not significantly associated with heroin
10 dependence. However, O. Levran et al. found that rs3758987 in the 5'-region near the
11 *HTR3B* had a nominally significant association with heroin dependence in a
12 Caucasian population [21]. However, in a more recent study, rs3758987 did not show
13 any association with alcohol and heroin dependence in African American [5].
14 Moreover, rs3758987 was marginally associated with alcohol dependence in
15 Caucasians[32]. This apparent inconsistency may have resulted from different
16 population stratification and sample sizes, which are important factors to be
17 considered in human genetic surveys [33]. To some extent, our finding further
18 supports a disputative role of *HTR3B* promoter polymorphism in drug dependence.

19 We showed that rs2276305 was not associated with heroin dependence. In Kazuo
20 Yamada's study, rs2276305 produced nominally significant allelic associations with
21 the combined mood disorder and more significantly with major depression in a
22 Japanese female [26]. However, The A allele of rs2276305 in *HTR3B* was nominally
23 significantly associated with alcohol dependence as a protective factor in Caucasians
24 [32]. We propose three possible explanations for these contradictory results. First, the
25 hormone levels, neuronal system adaptations and the pharmacokinetics of abused

substances vary. Second, association studies can be significantly affected by different sample sizes and genetic heterogeneity. Third, the gender ratio and age compositions among these studies are different and may affect the results. Moreover, rs4936285 and rs12795805 are both located at the 3'UTR of *HTR3B*. There was no significant correlation between these two SNPs and heroin dependence in our study. Few studies have investigated the association of these two SNPs with such disorders.

However, our study has several limitations. In the present study, only male participants were recruited because males account for a substantial proportion of heroin dependence in the Chinese population. In addition, the functions of the positive SNPs were not verified. More systematic approaches need to be employed for further functional analysis.

5. Conclusion

In conclusion, the present study provides direct evidence that genetic polymorphisms in *HTR3B* were linked to heroin dependence in the Chinese Han population. Our results are in line with the serotonergic hypothesis, which was developed to understand the acute and chronic effects of heroin on the brain. The data also encourage future efforts aimed at identifying functional polymorphisms within, and close to, the *HTR3B* gene using a systemic approach in a larger sample set.

Author contributions statement

Conceived and designed the experiments: Fangyuan Yin, Jianghua Lai, and Shuguang Wei. Analyzed the data: Fangyuan Yin, Hao Guo. Conducted the experiments: Yuanyuan Ji, Jing Zhang. Wrote the paper: Fangyuan Yin and Yuanyuan Ji. Managed

the literature search: Jing Zhang and Xin Huang. Contributed to and approved the final manuscript: Fangyuan Yin, Yuanyuan Ji, Jing Zhang, Hao Guo, Xin Huang, Jianghua Lai, Shuguang Wei.

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7

8 Figure legends

9 Fig. 1. The human *HTR3B* gene structure and the distribution of the 7 SNPs used in
10 this study.

11 Fig. 2. The LD plot of the 7 SNPs in the *HTR3B* gene of the healthy controls and
12 patients. Values in the squares are the pair-wise calculation of D' (Fig2.a and 2.c) or r^2
13 (Fig2.b and 2.d).

Figure1

Figure2

Table1

Table 1 Genotypic and allelic frequencies of *HTR3B* polymorphisms in the controls and patients with heroin dependence

Variable	Location	MAF	Cases (n=418)		Controls (n=422)		<i>H-W</i>	<i>P-value</i> ^a	OR, 95% CI
			No.	%	No.	%			
rs10789970	5'-UTR	0.386					0.308	0.022	
TT			186	44.5	154	36.5		0.018	1.395,1.058-1.840
CT			169	40.4	210	49.8		0.007	0.685,0.521-0.900
CC			63	15.1	58	13.7		0.584	1.114,0.757-1.638
T allele			541	64.7	518	61.4		0.156	1.154,0.947-1.407
C allele			295	35.3	326	38.6			
rs3758987	5'-UTR	0.179					0.617	0.247	
TT			279	66.7	283	67.1		0.923	0.986,0.740-1.314
CT			118	28.2	127	30.1		0.552	0.914,0.678-1.230
CC			21	5.0	12	2.8		0.108	1.807,0.877-3.723
T allele			676	80.9	693	82.1		0.510	0.921,0.720-1.178
C allele			160	19.1	151	17.9			
rs1176746	Intron4	0.333					0.205	0.004	
GG			221	52.9	182	43.1		0.005	1.479,1.127-1.942
GA			150	35.9	199	47.2		0.001	0.627,0.476-0.827
AA			47	11.2	41	9.7		0.470	1.177,0.756-1.833
G allele			592	70.8	563	66.7		0.069	1.211,0.985-1.489
A allele			244	29.2	281	33.3			
rs2276305	exon5	0.262					0.603	0.382	
GG			214	51.2	232	55.0		0.272	0.859,0.655-1.127
GA			164	39.2	159	37.7		0.643	1.068,0.809-1.410

AA			40	9.6	31	7.3		0.248	1.335,0.818-2.178
G allele			592	70.8	623	73.8		0.169	0.861,0.695-1.066
A allele			244	29.2	221	26.2			
rs4936285	3'-UTR	0.179					0.137	0.849	
TT			281	67.2	289	68.5		0.696	0.944,0.707-1.261
CT			116	27.8	115	27.3		0.871	1.025,0.757-1.388
CC			21	5.0	18	4.3		0.602	1.187,0.623-2.262
T allele			678	81.1	693	82.1		0.594	0.935,0.730-1.197
C allele			158	18.9	151	17.9			
rs1185027	3'-UTR	0.425					0.063	0.004	
AA			167	40.0	130	30.8		0.006	1.494,1.124-1.987
AT			176	42.1	225	53.3		0.001	0.637,0.485-0.836
TT			75	17.9	67	15.9		0.425	1.159,0.807-1.663
A allele			510	61.0	485	57.5		0.140	1.158,0.953-1.407
T allele			326	39.0	359	42.5			
rs12795805	3'-UTR	0.165					0.845	0.266	
TT			285	68.2	295	69.9		0.589	0.923,0.688-1.236
CT			112	26.8	115	27.3		0.881	0.977,0.721-1.325
CC			21	5.0	12	2.8		0.108	1.807,0.877-3.723
T allele			682	81.6	705	83.5		0.292	0.873,0.678-1.124
C allele			154	18.4	139	16.5			

^a *P*-value was calculated by 2×3 and 2×2 chi-squared tests based on codominant, dominant for the rare allele, heterosis and recessive for the rare allele models of inheritance.

Alpha value is adjusted by Bonferroni correction and statistically significant results ($P < 0.007$)

Table 2 HTR3B haplotype in block 1-3 frequencies and the results of their associations with risk of heroin dependence

Blocks	Haplotype	Cases(n, %)	Controls(n, %)	Statistics			
				χ^2	<i>P</i>	OR	95%CI
Block 1	TT	191(45.694)	184(43.602)	0.372	0.542	1.088	0.829-1.429
	CT	147(35.167)	163(38.626)	1.078	0.299	0.862	0.342-0.644
	TC	80(19.139)	75(17.772)	0.260	0.610	1.095	0.773-1.553
Block 2	AT	122(29.187)	111(26.303)	0.871	0.351	1.151	0.853-1.563
	GT	217(51.914)	236(55.924)	1.359	0.244	0.851	0.649-1.116
	GC	79(18.900)	75(17.772)	0.178	0.673	1.078	0.760-1.529
Block 3	AT	178(42.584)	173(40.995)	0.218	0.641	1.067	0.811-1.404
	TT	163(38.995)	179(42.417)	1.019	0.313	0.868	0.659-1.143
	AC	77(18.421)	70(16.588)	0.489	0.484	1.135	0.795-1.622

* *P* value is adjusted by Bonferroni correction and statistically significant results ($P < 0.017$).

Table 3 Demographic and addiction characteristics of rs1176746 and rs1185027

Variable	rs1176746			rs1185027		
	GG	GA	AA	AA	AT	TT
Age (year)	47.0±6.14	46.5±5.72	48.8±5.91	47.5±5.85	46.6±6.19	47.2±5.79
Occupation (%)						
Employed	55.1	31.6	13.3	40.8	41.8	17.3
Unemployed	50.4	38.2	11.4	37.8	42.5	19.7
Marital status (%)						
Unmarried	51.2	38.8	10.0	36.2	48.8	15.0
Married	54.0	32.9	13.1	40.1	40.5	19.4
Divorced or widowed	38.9	52.8	8.3	36.1	38.9	25.0
Pattern of addiction (%) ^a						
Oral suck	45.5	44.5	10.0	34.5	47.3	18.2
Injection	57.3	31.8	10.9	44.1	37.9	18.0
Mixture	64.7	19.6	15.7	45.1	37.3	17.6
Onset age (year)	23.4±7.96	24.3±7.96	23.7±6.89	22.6±7.69	24.5±8.18	24.4±7.13

^a Significantly associated with pattern of addiction of rs1176746, P=0.026.

The number that follows the ± sign is a standard deviation (s.d.).