# Exploring the Roles of 5-HTTLPR, STin2, 5-HT2A, and TPH2 Variants in Heroin Dependence

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# Abstract

**Background:** Substance abuse is a major worldwide public health and social problem. The serotonergic system, specifically the serotonin transporter (5-HTT) and postsynaptic activation of the serotonin 2A receptor (5-HT2A), may play a relevant role in substance dependence. This study investigated the association of 5-HTTLPR, serotonin transporter intron 2 (STin2), 5-HT2A, and tryptophan hydroxylase 2 (TPH2) variants with heroin dependence and determined genetic predispositions to heroin dependence and heroin co-occurring disorders.

**Methods:** This study comprised 36 case and 24 control subjects. All participants completed self-reported questionnaires which included items regarding demographic information, the Chinese Health Questionnaire (CHQ), Eysenck Personality Inventory, Parental Bonding Instrument, and the Taiwanese version of the Toronto Alexithymia Scale-20. Heroin-dependent subjects underwent further examinations including hepatitis B virus, hepatitis C virus, and venereal disease research laboratory tests. Genomic DNA was extracted from 3.5 mL of peripheral blood in all participants for genotyping of 5-HTTLPR, STin2, 5-HT2A, and TPH2.

**Results:** The heroin-dependent subjects were older than the control subjects ($t=19.091$, $p<0.001$). Statistically significant differences in education level ($t=83.66$, $p<0.001$), sex distribution ($\chi2=32.642$, $p<0.001$), marriage status ($\chi2=6.705$, $p=0.035$), and smoking status ($\chi2=60.00$, $p<0.001$) were observed between heroin-dependent and control subjects. The CHQ score in the heroin-dependent subjects was higher than that in the control group ($t=20.944$, $p<0.001$). Statistically significant differences in father care ($t=5.031$, $p=0.029$) and father protection ($t=4.170$, $p=0.046$) distributions were observed between heroin-dependent and control subjects.

**Conclusions:** Older males, divorced subjects, and smokers had higher risks of heroin dependence, while subjects who received more education had a lower risk of heroin dependence. Heroin-dependent subjects had higher CHQ scores and lower father care and higher father protection.


# Background

Substance abuse is a major worldwide public health and social problem. A study in Taiwan [1] reported that heroin dependence may cause significant economic costs and affect the quality of life. The authors also indicated that the economic cost of heroin dependency was comparable to that for schizophrenia. Heroin-dependent subjects also had a poorer quality of life than healthy normal controls.

Environmental and genetic risk factors influence substance dependence [2]. Previous studies have demonstrated clusters of heroin dependence in families [3]. The results of a genetic epidemiologic study [4] supported a high degree of heritable vulnerability for heroin dependence. Among relatives of the controls, the drug dependence rate was 3.5%, compared to 20.5% among relatives of heroin-dependent individuals. Other studies [5] support the importance of the genetic influence on substance dependence in general, and on heroin dependence in particular. Twin epidemiological studies showed that genetic

factors accounted for approximately 30–60% of the overall variance in the risk of drug addiction [6–8]. While the influences of environmental or genetic factors may vary at different stages [2], specific vulnerability to heroin has a major genetic component, greater than that of other types of substance abuse [6].

The neural-biochemical mechanisms of substance use disorders include dopamine (DA), 5-hydroxytryptamine, serotonin (5-HT), and norepinephrine (NE) neurotransmitter systems. Therefore, the receptor, transporter, and metabolic enzyme genes related to these neurotransmitter systems may be associated with substance use disorders. The serotonergic system, specifically the serotonin transporter (5-HTT) and postsynaptic activation of the serotonin 2A receptor (5-HT 2A), may play a relevant role in this substance dependence [2].

The serotonin transporter gene (SLC6A4) encodes the serotonin transporter which controls serotonin reuptake and inactivation from the synapse. A previous meta-analysis study [9] found a significant association in the combined studies of heroin dependence/abuse and 5-HTTLPR, which was more significant in European populations. They showed a weak association between STin2 variable number tandem repeat (VNTR) and heroin dependence/abuse in European studies of heroin dependence. However, Asian subjects with the STin2 VNTR 10 allele had a higher ratio of heroin dependence/abuse subjects than subjects with the STin2 VNTR 12 allele.

The promoter of the serotonin transporter gene is a repeat variant (5-HTTLPR) with either a short (S) or a long (L) form [10]. Recently, a variant (rs25531) was identified within the repeat which alters the transcriptional activity of the serotonin transporter gene. Combining this variant with the repeat yields a triallelic polymorphism which can be classified as a high transcriptional activity L_ or a low activity S_ allele [11].

Another study [12] observed an interaction between A-1438G (rs6311) of 5-HT2A and 5-HTT polymorphisms. The association between 5-HT2A A-1438G (rs6311) AA and AG/GG genotypes and heroin dependence was enhanced in the presence of 12-repeat 5-HTT VNTR (Stin2) and short-form 5-HTTLPR alleles. Their findings support the contribution of the 5-HT 2A gene to susceptibility to heroin dependence as well as a possible synergistic effect of 5-HT 2A and 5-HTT genes on susceptibility to heroin dependence.

Despite the serotonin transporter, the availability of serotonin for postsynaptic activity also requires tryptophan hydroxylase (TPH). Both genes coding for these proteins have functional variants which may make lower levels of serotonin available in the synapse. TPH is the rate-limiting enzyme in the biosynthesis of serotonin [13] and is expressed in TPH2 in the brain [14]. Serotonin is involved in the regulation of various aspects of mood and impulsivity [15, 16]. Concentrations of cerebral spinal fluid 5-hydroxyindoleacetic acid (CSF 5-HIAA), a degradation product of serotonin, are low in depressed patients with a family history of alcoholism [17] and in those with behaviours characterised by a deficit in impulse control, such as aggression [18, 19] and impulsivity [20]. The synonymous variant 1125A >T (rs4290270) in exon 9 of the TPH2 gene is a marker for allelic expression imbalance [21]. The T allele is expressed at

approximately two-fold the levels of the A allele in heterozygous subjects. A allele carriers may produce less serotonin than those with a TT genotype.

Therefore, we explored the association of 5-HTTLPR, STin2, 5-HT2A, and TPH2 variants with heroin dependence by comparing the different distributions of these gene polymorphisms in the case and control groups. Using case-control methods to study associations between these gene polymorphisms and heroin use disorders is important to identify genetic predispositions to heroin dependence and genetic predispositions to heroin co-occurring disorders. We also wanted to understand the impact of 5-HTTLPR, STin2, 5-HT2A, and TPH2 on heroin use conditions, such as dose, duration of use, and age of first use.

## Methods

# Participants

The participants were recruited from a teaching hospital in Taiwan, in southern China. The inclusion criteria for the case group were heroin-dependent subjects who received methadone replacement therapy. The control group comprised volunteers from a hospital. We included 36 case and 24 control subjects. Patients were excluded if they had received a diagnosis of schizophrenia, schizoaffective disorder, mental retardation, or other cognitive disorders; were pregnant or breastfeeding; or had a serious and unstable medical condition.

# Materials

# Demographic information

All participants were required to complete self-reported questionnaires which included items regarding demographic information, heroin use information (daily dose, duration, and onset), Chinese Health Questionnaire (CHQ), and the Taiwanese version of the Toronto Alexithymia Scale-20 (TAS-20). The demographic information included details about participant age; sex; education and monthly income; current residential details; family background (including parental marital status, occupation, and education); and use of other substances such as drugs, alcohol, and tobacco.

# Chinese Health Questionnaire

The CHQ is a self-administered screening instrument used to identify minor psychiatric disorders in Chinese community settings. The General Health Questionnaire (GHQ) developed by Goldberg [22] has been widely used in surveys in Western countries. It was chosen as the questionnaire to be modified for use as the first-stage instrument. Given the cultural differences between China and the West and the interest in comparative studies of the GHQ between Western and Chinese communities, Cheng and William [23] designed an experimental CHQ. This item pool was then treated with discriminative function analysis to select a subset of 12 items. A simple 0-0-1-1 scoring method was applied to the CHQ. The optimum cut-off point (the best compromise between high sensitivity and a low false-positive rate) was

3/4 from the receiver operating characteristic (ROC) curves [24] Cheng, Wu, Chong, and Williams [25] demonstrated an internal consistency of 0.79 for the CHQ.

# Eyesenck Personality Questionnaire,EPQ

The Eysenck Personality Inventory (EPI) measures two personality traits: neuroticism (N trait) and extraversion (E trait). The 14 items which assess the N trait measure the emotional dysfunction of an individual, whereas the 11 items for the E trait measure sociability. This study used the simplified version of the EPI, which was modified by Lu (1994) and consists of 25 items. The inventory has been shown to demonstrate a high internal consistency of 0.90 [26].

# Parental Bonding Instrument,PBI

The Parental Bonding Instrument (PBI) used in this study is a modified Chinese-language version of the PBI developed by Parker and colleagues in 1979 [27]. This self-report inventory includes 25 items on a four-point Likert scale (0–3). Of the 25 items, 12 and 13 measure the levels of parental care and parental protection, respectively. Each participant was asked to complete two PBI surveys (one each for the father and mother). The scale contains tests for internal consistency (Cronbach's alpha = 0.65–0.73) and reliability (test-retest reliability = 0.66–0.88) [28]. In our study, the PBI difference score was calculated by subtracting the protection score from the care score. Parents with low PBI difference scores were considered unaffectionate and overprotective [29–31].

# Taiwan version of the Toronto Alexithymia Scale-20)

All subjects completed the traditional Chinese version of the TAS 20-item. This version was validated in a student population by Lin et al. [32] in Taiwan. The scores were calculated for three subfactors: (1) difficulty in identifying feelings and distinguishing between emotional and physical sensations (DIF), (2) difficulty in describing feelings (DDF), and (3) externally oriented thinking (EOT). The 20 items were rated from 1 (strongly disagree) to 5 (strongly agree). The sum of all 20 items, taking reversed items into account, was used to generate a total TAS score.

# Genotyping

# Blood sampling and DNA Extraction

Genomic DNA was prepared from 3.5 mL of peripheral blood collected from all participants. DNA extraction was performed according to the manufacturer's protocol using the QIAmp DNA extraction kit (Qiagen). All extracted genomic DNA was stored at -86℃.

# 5-HTTLPR

The 5-HTTLPR variants were genotyped in a two-step process. The classic L and S allele variants (rs4795541) [10] were determined by polymerase chain reaction (PCR) amplification of DNA to yield 181 and 138 bp fragments for the L and S alleles, respectively [33]. The internal A→G single nucleotide

polymorphism at 25531 in the L allele, which creates an HpaII restriction site, was determined by digestion of the amplified DNA with HpaII (New England Biolabs, Ipswich, MA, USA). The G allele, designated ('LG') [34], is digested into 96 and 85 bp fragments, while the A ('LA') allele remains undigested at 181 bp. The fragment sizes were determined by electrophoresis on a 4–20% polyacrylamide Tris/Borate/EDTA (TBE) gel. The distribution of these 5-HTTLPR genotypes among the two treatment groups is shown in Table 3. Since the LG and S alleles have lower transcriptional expression levels than that of the LA allele [11], the functionally similar LG and S alleles were designated as S_ and LA alleles as L_.

# 5-HT2A

Determination of the 5-HT2A A-1438G (rs2070040) polymorphism was performed as previously described [35]. The sense oligonucleotide primer for 5-HT2A A-1438G was 5'-CAT AAG CTG CAA GGTAGC AAC AGC-3', while the antisense primer was 5'-GAAACC AAC TTA TTT CCT ACC AC-3'. The PCR products were digested for 10–12 h 37 °C with MspI. The 474 bp fragment indicated the presence of the A allele (no MspI restriction site) while the 228 and 246 bp fragments indicated the presence of the G allele (presence of MspI restriction site). MspI-treated PCR fragments were run on 3% agarose gels and stained with ethidium bromide.

# TPH2

PCR-based restriction fragment length polymorphism assays were performed to genotype TPH2 1125A > T (rs4290270), as described previously [36]. The forward and reverse primer sequences were: F: 5'-AATTATGCACAGCCCACCATTT-3' and R5'-TTTAGGCCTGCCATTTGTTACC-3'. The amplification mixture contained 1 μL of 100 ng/μL DNA, 5 μL of 10 × Ex Taq buffer, 4 μL of 0.4 mM Ex dNTP mixture, 2 μL primer, 37.75 μL distilled water, and 0.25 μL Taq polymerase (Fermentas, USA). The PCR amplification protocol included denaturation at 95 °C for 5 min, followed by 36 cycles at 95 C for 30 s, 57 °C for 30 s, and 72 °C 30 s, followed by elongation at 72 °C for 5 min. The PCR product of single nucleotide polymorphism (SNP) (rs4290270) was digested with NdeI (New England Biolabs) 37 °C overnight and run on 2% agarose gels at 120 V for 45 min, yielding different restriction patterns for the AA (328 bp + 153 bp) and TT (481 bp) genotypes.

# Statistical analysis

The observed frequencies were compared to the expected according to Hardy–Weinberg equilibrium by $\chi 2$ tests. The allele and genotype frequency comparisons between patients and controls were performed using $\chi 2$ tests and odds ratio calculations. The Genecounting Program [37] was used to compare estimated haplotype frequencies in patients and controls and to test for differences with an LRT as well as to calculate the pairwise linkage disequilibrium (LD) between all pairs of markers. The two groups (case and control groups) were compared for baseline differences in CHQ, TAS score, history of heroin use, and demographics using $\chi 2$ or t-tests as appropriate. We also performed logistic regression to analyse the possible impact of 5-HTTLPR, STin2, 5-HT2A, and TPH2 on heroin use behaviours.

# Results

Demographic characteristics

The demographic data including sex, age, marriage status, education level, smoking status, and alcohol use between the case and control groups are shown in Table 1). T-test analysis showed that the case group was older than the control group ($t = 19.091$, $p < 0.001$). Statistically significant differences in education were observed between heroin-dependent and control subjects ($t = 83.66$, $p < 0.001$). $\chi 2$ tests showed significant differences in sex distribution ($\chi 2 = 32.642$, $p < 0.001$), marital status ($\chi 2 = 6.705$, $p = 0.035$)m and smoking status between the case and control groups ($\chi 2 = 60.00$, $p < 0.001$) but not for alcohol use ($\chi 2 = 0.589$, $p = 0.332$).

Table 1
Demographic characteristics of the heroin-dependent and control groups.

| | **Case group (n = 36)** | **Control group (n = 24)** | **t-value** | **χ2** | **P-value** |
|---|---|---|---|---|---|
| Age | 45.83 ± 7.06 | 37.07 ± 8.99 | 19.091 | | < 0.001 |
| Male | 91.7% | 24.1% | | 32.642 | < 0.001 |
| Marriage | | | | | |
| Unmarried | 47.2% | 46.4% | | 6.705 | 0.035 |
| Married | 27.8% | 50.0% | | | |
| Divorced | 25.0% | 3.6% | | | |
| Education (yrs) | 10.75 ± 2.42 | 17.37 ± 3.33 | 83.66 | | < 0.001 |
| Smoking | 100% | 0% | | 60.00 | < 0.001 |
| Alcohol use | 25% | 16.7% | | 0.589 | 0.332 |
| . | | | | | |
| Differences in 5-HTTLPR, STin2, 5-HT2A (rs6311), and TPH2 between heroin-dependent and control subjects | | | | | |
| $\chi 2$ test analysis showed no significant difference over the 5-HTTLPR genotype distribution between the case and control groups ($\chi 2 = 711$, $p = 0.701$). No significant statistical difference in STin2 genotype distribution was observed between the case and control groups ($\chi 2 = 0.804$, $p = 0.370$) but not between the 5-HT2A (rs6311) genotype distribution ($\chi 2 = 2.612$, $p = 0.271$) and TPH2 genotype distribution ($\chi 2 = 2.025$, $p = 0.363$) between the case and control groups. | | | | | |

Table 2
5-HTTLPR, STin2, 5-HT2A (rs6311), and TPH2 genotype distributions between the heroin-dependent and control groups

| | Case group (n = 36) | Control group (n = 24) | χ2 | P-value |
|---|---|---|---|---|
| 5-HTTLPR | | | | |
| SS | 78.8% | 57.7% | 0.711 | 0.701 |
| LS | 18.2% | 37.1% | | |
| LL | 3.0% | 7.1% | | |
| HWE-$\chi2^{\dagger}$ | 0.71 | 2.03 | | |
| STin2 | | | | |
| 10,10 | 0% | 0% | 0.804 | 0.370 |
| 10,12 | 14.3% | 7.1% | | |
| 12,12 | 85.7% | 92.9% | | |
| HWE-$\chi2^{\dagger}$ | 0.21 | 0.04 | | |
| Rs6311 | | | | |
| CC | 0% | 3.6% | 2.612 | 0.271 |
| CT | 60.0% | 71.4% | | |
| TT | 40.0% | 25.0% | | |
| HWE-$\chi2^{\dagger}$ | 6.43 | 6.93 | | |
| TPH2 | | | | |
| AA | 11.4% | 17.9% | 2.025 | 0.363 |
| TA | 42.9% | 53.6% | | |
| TT | 45.7% | 28.6% | | |
| HWE-$\chi2^{\dagger}$ | 0.03 | 0.20 | | |
| SS: short form, short form; LS: long form, short form; LL: long form, long form; | | | | |
| 10,10: Stin2_10, Stin2_10; 10,12: Stin2_10, Stin2_12; 12,12: Stin2_12, Stin2_12; | | | | |
| $^{\dagger}$ = $\chi^2$ in Hardy–Weinberg equilibrium ($\chi^2_{.95} < 5.99$, df = 2). | | | | |
| Differences in CHQ, EPI, PBI, and TAS between heroin-dependence subjects and control group | | | | |

| | Case group (n = 36) | Control group (n = 24) | χ2 | P-value |
|---|---|---|---|---|
| T-test analysis showed a significantly higher CHQ score in the case group than that in the control group (t = 20.944, p < 0.001). Significant differences were also observed in father care (t = 5.031, p = 0.029) and father protection (t = 4.170, p = 0.046) between heroin-dependent and control subjects but not in neuroticism (t = 0.060, p = 0.807), extraversion (t = 0.035, p = 0.853), maternal care (t = 0.049, p = 0.825), mother protection (t = 36.664, p = 0.061), and TAS score (t = 1.028, p = 0.314) between heroin-dependent and control subjects. | | | | |

**Table 3.** CHQ, EPI, PBI, and TAS distributions between the heroin-dependent

and control groups.

| | Case group (n = 36) | Control group (n = 24) | t-value | P-value |
|---|---|---|---|---|
| CHQ | 7.47 ± 3.359 | 3.76 ± 3.113 | 20.944 | < 0.001 |
| EPI | | | | |
| Neuroticism | 7.31 ± 3.362 | 7.52 ± 3.572 | 0.060 | 0.807 |
| Extraversion | 6.17 ± 3.730 | 6.00 ± 3.381 | 0.035 | 0.853 |
| PBI | | | | |
| FC | 17.77 ± 4.595 | 20.85 ± 5.746 | 5.031 | 0.029 |
| FP | 15.74 ± 4.546 | 12.88 ± 6.009 | 4.170 | 0.046 |
| MC | 20.48 ± 4.638 | 20.81 ± 6.816 | 0.049 | 0.825 |
| MP | 15.79 ± 4.768 | 12.78 ± 7.345 | 3.664 | 0.061 |
| TAS | 60.53 ± 14.700 | 57.10 ± 11.917 | 1.028 | 0.314 |
| CHQ: Chinese Health Questionnaire | | | | |

FC: father care; FP: father protection; MC: mother care; MP: mother protection

TAS: Toronto Alexithymia Scale

PBI: Parental Bonding Instrument

Logistic regression for factors related to heroin dependence

We used logistic regression to analyse the factors related to heroin dependence. Subjects who were older (p = 0.005; odds ratio = 1.176), with higher TAS scores (p = 0.008; odds ratio = 1.179), and of male sex had higher risks of heroin dependence (p < 0001; odds ratio = 0.002).

| Table 4. Logistic regression for factors related to heroin dependence | | | | | | |
|---|---|---|---|---|---|---|
| | B | S.E. | Wald | df | P | Odds ratio |
| Age | 0.162 | 0.058 | 7.724 | 1 | 0.005 | 1.176 |
| TAS | 0.165 | 0.062 | 7.091 | 1 | 0.008 | 1.179 |
| Female | -6.231 | 1.777 | 12.294 | 1 | < 0.001 | 0.002 |
| Constant | -6.997 | 3.271 | 4.575 | 1 | 0.032 | 0.001 |
| Variable(s) entered in step 1: age, TAS, gender. | | | | | | |

Logistic regression for factors related to hepatitis B virus infection among heroin-dependent subjects

We performed logistic regression to analyse the factors related to hepatitis B virus (HBV) infection among heroin-dependent subjects. Heroin-dependent subjects with the TPH2 A allele (p = 0.013; odds ratio = 0.148) had a higher risk of having HBV infection.

Table 5
Logistic regression for factors related to HBV infection among heroin-dependent subjects

| | B | S.E. | Wald | df | P | Odds ratio |
|---|---|---|---|---|---|---|
| TPH2 | -1.913 | 0.768 | 6.204 | 1 | 0.013 | 0.148 |
| Constant | 1.183 | 0.925 | 1.634 | 1 | 0.201 | 3.264 |
| Variable(s) entered on step 1: TPH2 | | | | | | |

# Discussion

There is a consensus regarding the relationship between TPH polymorphism and heroin use disorder in the context of dopamine and serotonin pathways or addiction development mechanisms.

A previous meta-analysis combined available genotype data from case-control and family-based association studies on SLC6A4, 5-HTTLPR, and STin2 polymorphisms and alcohol and drug (heroin, cocaine, and methamphetamine) abuse in European, Asian, African, and Mexican populations [9]. Among the 55 included studies, eight investigated heroin dependence or abuse. The 5-HTTLPR allele frequencies vary significantly in different ethnic populations; thus, differences in sampling methods may cause different results. This discrepancy may also be due to insufficient sample sizes and low statistical power of individual studies.

In our study, the HBV infection rate was higher in the heroin group. This may be due to the increased risk of sharing a needle in this group [38]. Much of the estimated burden of disease attributable to the use of illicit drugs is likely due to blood-borne viral infections through unsafe drug injection [39].

The results showed no statistical significance in the 5-HTTLPR, STin2, 5-HT2A(rs6311), and TPH2 genotype distributions between the heroin-dependent and control groups, which may be due to fewer cases recruited in our study. These cases were not necessarily generalisable to this population. We observed significant differences in sex distribution between the case and control groups (8.3% cases were female in the case group vs. 75.9% cases in the control group), which may be because our control group recruited only 24 cases.

In our study, the CHQ score was significantly higher in the case group under T-test analysis. A similar result was found in another study reporting significant positive correlations between the numbers of lifetime drug dependence diagnoses and lifetime anxiety and affective disorders as well as between the number of current drug dependence diagnoses and the number of current comorbid diagnoses [40]. This result may be attributed to an unhealthy mental status in the CHQ, including increased depression, anxiety, and other poorer presentations in psychiatric function in subjects with heroin dependence. The logistic regression analysis showed that the TAS score had a positive effect on heroin dependence under the control of related factors. Thus, we concluded that alexithymia was also a possible risk factor related to heroin dependence.

Regarding care and protection of parental bonding, fathers showed a larger influence on heroin dependence than mothers in our study. Parenting and upbringing issues in Eastern family cultural background require further exploration and comparisons to those in Western families. A previous study found that maternal and paternal overprotection was reported more commonly by narcotic addicts. Maternal overprotection alone has been implicated in alcoholics. Narcotic addicts seem to have more disturbed parenting than alcoholics, especially paternal parenting [41] Therefore, investigation of the influence of parental bonding on these subjects with substance use warrants further investigation.

# Conclusion

The results of our study showed no association between genetic factors such as 5-HTTLPR, STin2, 5-HT2A (rs6311), and TPH2 and heroin dependence. However, in the demographic data, older males, divorced subjects, and smokers had higher risks of heroin dependence, while subjects with more education had a lower risk of heroin dependence.

The analysis of CHQ, EPI, PBI, and TAS distribution between heroin-dependent and control subjects showed that the heroin-dependent subjects had higher CHQ scores, lower father care, and higher father protection.

The results of the logistic regression analysis for factors related to heroin dependence suggest that older male subjects with higher TAS scores had a higher risk of having heroin dependence. We also analysed the factors related to related disorders such as HBV, hepatitis C virus (HCV), and syphilis among heroin-dependent subjects. However, all heroin-dependent subjects in our study had HCV infection and none had syphilis infection. Therefore, we could not analyse the factors related to HCV and syphilis infection among heroin-dependent subjects. Thus, we analysed only the factors related to HBV among heroin-

dependent subjects. We found that heroin-dependent subjects with the TPH2 A allele (p = 0.013; odds ratio = 0.148) had a higher risk of having HBV infection.

# Declarations


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The funding body had no role in the design of the study beyond organising peer review as part of the process of obtaining funding. The funding body had no role in the data collection, analysis, and interpretation or in writing the manuscript.


### Ethics approval and consent to participate

The study received approval by the Institutional Review Board of Kaohsiung Armed Forces General Hospital. Written, informed consent to participate in the study was obtained from all participants.

### Consent for publication

Not applicable

### Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

### Competing interests

The authors declare that they have no competing interests.


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Not applicable

### Author contributions

The study was conceived and designed by Hung Jiang, Hung-Chi Wu, Jui-Kang Tsai, and Wei-Tsung Kao.

Sample collection and management of patient medical information were performed by Hung Jiang, Hung-Chi Wu, Jui-Kang Tsai, and Wei-Tsung Kao.

All authors participated in the planning of this study.


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