Genome-wide association meta-analyses identify novel genetic risk loci and polygenic phenotype associations for heroin, methamphetamine and alcohol dependences

Dear Editor,

This work presented a significant correlation between heroin and methamphetamine dependence (HD and MD) which distinguished with alcohol dependence (AD) at the genome-wide level. Three novel risk loci were identified for HD and MD. The shared polygenic risk with cognition and attention-deficit hyperactivity disorder (ADHD) further profiled the similar genetic characteristics between HD and MD compared to AD.

Substance dependencies (SD) are one of the leading public health concerns worldwide. Drug markets are in a constant state of flux. The combined use of different addictive substances, especially illegal drugs, is common among addiction patients. Genetic factors contribute to approximately 40%–70% of the variance in persistent SD. Capturing the shared and specific genetic mechanism of different substance dependences is crucial for coping with the changeable types of addiction.

In this study, genome-wide association meta-analyses (GWMA) were performed independently for HD, MD and AD based on two data sets (DS) of substance-specific dependence patients (1028 HD, 1750 MD, 537 AD and 2862 shared controls for DS1, 980 HD, 701 MD, 224 AD and 1111 shared controls for DS2) (Supplementary Tables S1 and S2, Supplementary Figures S1 and S2, supplementary methods). Consist with our previous study in DS1, One significant locus at chr12 ANKS1B was identified for both HD (peaked in rs140254085, \(p_{\text{meta}} = 6.355\times 10^{-10}\)) and MD (peaked in rs74330628 downstream of NRXN1, \(p_{\text{meta}} = 3.84\times 10^{-8}\)) and chr7 locus (peaked in GTF2IRD1 intron rs76965632, \(p_{\text{meta}} = 1.41\times 10^{-8}\)) were identified (Supplementary Table S4). The functional annotations for the three significant loci are shown in Table 1.

Several regulatory features were located in the loci (Supplementary Figure S4), and eQTL data showed the loci regulated the expression of ANKS1B and NRXN1 in brain tissues (Supplementary Table S5, Figure 2A and Supplementary Figure S5). By comparison, the three significant loci of HD&MD were not associated with AD, while the chr4 ADH and chr12 ALDH loci were not associated with HD and MD or had a different direction in HD and MD with AD (Supplementary Figure S6). These findings are consistent with previous twin study that showed a closer genetic correlation across illicit drug dependences compared to alcohol dependence.

Next, the association with addiction characteristics of the significant loci for HD&MD was examined (Supplementary Table S6 and Figure 2B). The protective allele (A) of rs74330628 in the NRXN1 locus was associated with a lower frequency of heroin usage (\(p_{\text{adj}} = 0.0167\)). The protective allele (C) of rs76965632 in the GTF2IRD1 locus was negatively associated with craving of MA (\(p_{\text{adj}} = 0.0256\)). Using the Human Connectome Project data, the SNPs of ANKS1B was associated with risk of ever used illicit drug (EverDrugs) (Supplementary Table S7), which was associated with the volume of the left and right amygdala (Supplementary Table S8). Mediation analysis showed the ANKS1B locus had an indirect effect on EverDrugs by the mediation of the left amygdala and right amygdala.

Based on the high genetic correlation between HD and MD, the combined GWMA for HD&MD versus controls were analysed, in which HD and MD were grouped as cases and compared with controls (Table 1 and Figure 1). In addition to the ANKS1B locus (peaked in rs140254085, \(p_{\text{meta}} = 6.355\times 10^{-10}\)), another two novel loci located in chr2 locus (peaked in rs74330628 downstream of NRXN1, \(p_{\text{meta}} = 3.84\times 10^{-8}\)) and chr7 locus (peaked in GTF2IRD1 intron rs76965632, \(p_{\text{meta}} = 1.41\times 10^{-8}\)) were identified (Supplementary Table S4). The functional annotations for the three significant loci are shown in Table 1.

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#TABLE 1 Association results for the three significant loci for HD&MD and related functional mapping and annotation

| GWAS Lead SNP | Predicted genes / Genomic coordinate (hg19) / A1:A2 | Substance dependence trait | Beta | SE  | p Value of GWAS meta | #SNPs in LD (r^2 > 0.6) | SNPs in LD with CADD score > 12.37 | SNPsinLDwithRegu-lomeDB scores < 5 | Chromatin state
|---------------|--------------------------------------------------|-----------------------------|------|-----|----------------------|-------------------------|---------------------------|-----------------------------------|---------------------------------|
| rs112706556   | ANKS1B (intronic)/chr12:99839855-99919728/A:G    | AD                          | 0.1489 | 0.03 | 0.3232               | 35                      | rs7313882 (p = 9.334e-9, CADD = 14.22); rs7962904 (p = 1.207e-8, CADD = 12.73) | 3 SNPs with score = 3a, 3 SNPs with score = 4 | Enhancer, strong transcript, heterochromatin |
|               |                                                  | HD                          | 0.6107 | 0.112 | **4.99E-08**         |                         |                           |                                   |                                 |
|               |                                                  | MD                          | 0.4867 | 0.0859 | **1.46E-08**         |                         |                           |                                   |                                 |
|               |                                                  | HD&MD                       | 0.4611 | 0.0801 | **8.70E-09**         |                         |                           |                                   |                                 |
| rs74330628    | RPL7P13 (upstream of TSS), NRXN1 (19 kb downstream)/chr2:50023593-50126480/A:G | AD                          | 0.1684 | 0.1721 | 0.328                | 30                      | rs11675829 (p = 8.201e-6, CADD = 15.82); rs60582193 (p = 5.847e-4, CADD = 16.09) | 1 SNP with score = 3a | Active TSS, enhancer, strong transcript, heterochromatin |
|               |                                                  | HD                          | -0.5038 | 0.1251 | 5.68E-05             |                         |                           |                                   |                                 |
|               |                                                  | MD                          | -0.4834 | 0.0959 | 4.64E-07             |                         |                           |                                   |                                 |
|               |                                                  | HD&MD                       | -0.489  | 0.0889 | **3.84E-08**         |                         |                           |                                   |                                 |
| rs76965632    | GTF2IRD1 (intronic)/chr7:73898830-73938239/C:T  | AD                          | -0.5245 | 0.3531 | 0.1374               | 3                       | NA                        | NA                                | Enhancer, strong transcript, active TSS |
|               |                                                  | HD                          | -1.1865 | 0.2373 | 5.73E-07             |                         |                           |                                   |                                 |
|               |                                                  | MD                          | -1.0607 | 0.2032 | 1.78E-07             |                         |                           |                                   |                                 |
|               |                                                  | HD&MD                       | -1.0578 | 0.1865 | **1.41E-08**         |                         |                           |                                   |                                 |

AD: Alcohol dependence; HD: heroin dependence; MD: Methamphetamine dependence; HD&MD: combined heroin dependence and methamphetamine dependence; NA: not available. The GWAS meta p value < 5.0e-8 is marked with bold.

The p value is the p value of the SNP in the GWAS meta-result. CADD > 12.37 has been suggested as the minimum value for pathogenic SNPs and has been used as a threshold for highly deleterious SNPs.\(^{10}\)

Chromatin state analysis in neuronal cell lines/tissues, including E007, E009, E010, E053, E054, E067, E068, E069, E070, E071, E072, E073, E074, E081, E082, E125 in Roadmap Epigenomics.
FIGURE 1  Manhattan and regional plots for drug dependence traits. (A) Manhattan plot for HD. (B) Manhattan plot for MD. (C) Manhattan plot for HD&MD. The red line denotes the threshold of \( p < 5e-8 \). (D)–(F) The regional plots for the three significant loci of HD&MD in chr12 (D), chr2 (E) and chr7 (F).

(Figure 2C). ANKS1B protein may be involved in the neural plasticity of the amygdala during drug use by affecting glutamatergic neurotransmission.\(^5\) Phenome-wide association analysis based on the GWAS Atlas\(^6\) further validated the association of ANKS1B, NRXN1 and GTF2IRD1 with psychiatric traits (Supplementary Figure S7 and Supplementary Tables S9–S11).

We next explored the associated genes and pathways for the shared risk of HD and MD. ANKS1B, GRM7, RBFOX1 and CDH13 were shared by HD, MD and HD&MD (Supplementary Table S12). Enrichment analysis showed the HD- and MD-related 22 unique genes were significantly enriched in brain-related tissues, GO term ‘modulation of chemical synaptic transmission’ and drug abuse and neurodevelopmental disorder-related diseases (Supplementary Figure S8). These associated genes and pathways provide new candidates and clues for understanding the genetic mechanism of drug dependences.

Additionally, polygenic associations with phenotypes of addiction-related traits, risk behaviour, cognition and psychiatric disorders (Supplementary Table S13) were performed for HD, MD and AD. The three alcohol-related traits were significantly associated with AD, but not with HD and MD (Figure 3A and Supplementary Table S14). Sexual and smoke behaviour risks were positively associated with the MD and HD&MD, but not with AD (Figure 3B and Supplementary Table S15). Cognition performance, education attainment and IQ were negatively associated with HD, MD and HD&MD but not with AD (Figure 3C and Supplementary Table S16). Our findings support that pre-existing cognition disruption could increase the risk of illicit drug dependence.\(^7\) For the eight psychiatric disorders, only the PRS of ADHD was positively associated with HD, MD and HD&MD, but not with AD (Figure 3D and Supplementary Table S17). Mendelian randomisation analysis further highlighted the causal effect of ADHD on HD and HD&MD (Supplementary Table S18). Our previous study also demonstrated that ADHD-relevant childhood behaviour was a risk factor for MA-induced psychosis.\(^8\) First-degree relatives of ADHD probands have an increased risk for drug dependence.\(^9\) This suggests that ADHD and drug dependence have shared genetic factors. Cluster analysis based on the standardised coefficient matrix from the PRS association results showed HD, MD and HD&MD were in one sub-cluster and were different from AD, which further highlighted the closer polygenic correlations between HD and MD compared to AD.
In conclusion, at the genome-wide loci, genes and polygenic levels, we identified significant genetic overlap between HD and MD, which distinguished with AD. Notably, the combined HD&MD GWAS identified three common risk loci, located on ANKS1B, NRXN1 and GTF2IRD1 genes. At the polygenic level, HD and MD were significantly associated with cognition deficiency and ADHD, which distinguished them from AD. Our results would help with the fine-mapping of the common and unique genetic mechanisms underlying drug dependences and alcohol dependence and may provide clues for their prevention.

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Fig. 3  PRS analysis results for (A) six addiction-related traits, (B) five risk behaviours, (C) three cognition traits and (D) eight psychiatric disorders with four SD traits. *denotes the permutation adjusted p was less than .05 in DS1 and replicated in DS2 (p < .05). Heatmap was constructed using the standardised beta coefficient. OUD: opioid use disorder, AUD: alcohol use disorder, PAU: problematic alcohol use, AD-EUR: alcohol dependence of European population, SZ: schizophrenia, ADHD: attention-deficit hyperactivity disorder, OCD: obsessive-compulsive disorder, BIP: bipolar disorder, MDD: major depressive disorder, ASD: autism disorder, AN: anorexia nervosa, TS: Tourette syndrome.

Conflict of Interest
The authors declare no competing interest.
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