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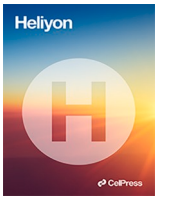

Research article

Analysis and study of the mechanism of narcotic addiction and withdrawal

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ABSTRACT

Narcotic drugs refer to drugs that have anesthetic effects on the central nervous system, and they easily produce physical dependence and mental dependence and can be addictive due to continuous use, abuse or unreasonable use. In this paper, bioinformatics and data analysis and mining techniques were used to analyze the methylation differences in transcriptional and clinical data of narcotic addiction in public databases, to explore the mechanism of narcotic addiction, and to mine some norepinephrine drugs. This study confirmed the possibility of using norepinephrine as an auxiliary drug for drug addiction rehabilitation. In addition, we also conducted a similar analysis on the addiction of three drugs. The results showed that the differences in the body caused by the ingestion of opiates and cocaine were significantly greater than those caused by the ingestion of methamphetamine.

1. Introduction

Anesthetic preparations possess dual attributes as both pharmaceutical and narcotic drugs; their rational and scientific application in strict accordance with medical advice renders them medicinal products with therapeutic effects, while prolonged and excessive abuse results in their addictive qualities as narcotics. Currently, drug abuse and addiction have become social public problems. While narcotic abuse brings pleasure, it poses a wide range of threats to the social population [1]. Narcotic drugs can be broadly classified into opioids, cocaine, and cannabis. Inappropriate intake patterns are directly or indirectly the main vector for the transmission of many serious infectious diseases, especially acquired immunodeficiency syndrome (AIDS), hepatitis and tuberculosis, which are increasingly serious social public problems [2]. The abuse of narcotic drugs has caused a large number of deaths in the population and greatly reduced the social labor force base. From 1999 to 2017, more than 700,000 people died of drug overdose in the United States, far exceeding the number of deaths from HIV, car accidents and shootings [3]. In addition, the prevalence of narcotic drugs leads to frequent social problems, which potentially affect the country's future competitiveness.

Excessive long-term abuse of narcotic drugs produces physical dependence and psychological dependence. How to quit is a

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challenge worldwide. The damage caused by narcotic drug abuse is long-term and irreversible, and people with drug addiction often fall into a situation of "treatment without cure" [4–6]. Opioid receptor antagonists, such as naloxone, methadone, and buprenorphine, are commonly used as withdrawal drugs. The existing methods of smoking cessation have disadvantages such as long withdrawal time, high cost, painful withdrawal process and easy relapse [7]. Researchers are looking forward to finding new drugs and methods for withdrawal treatment. New studies have proposed the use of neurotrophic factors, levodopa, cocaine antibody and levotetrahydropalmetine to replace or assist conventional withdrawal drugs for withdrawal [8–12]. Different withdrawal methods provide new ideas for people with drug addiction to reduce withdrawal reactions and reduce withdrawal costs. Especially in recent years, clinical studies on clonidine and lofexidine have shown that α2 receptor agonists can be used for opium withdrawal [13]. Based on the performance of clonidine and lofexidine in the treatment of opioid addiction, we propose a hypothesis that some norepinephrine drugs can be used in the withdrawal of addiction patients to regulate the binding level of norepinephrine and its receptor, reduce the withdrawal reaction, and help people with drug addiction complete the withdrawal.

Based on the GEO high-throughput data, this paper used the R language to analyze the methylation data to identify the differentially expressed genes at relevant loci, which were mapped into the human protein interaction network. In the network, the key subnetworks of module mining were used to determine the opioid addiction drug targets, therapeutic opioid targets, norepinephrine drug targets, and methylation-related subnetworks. Through the GO function and KEGG pathway analysis of subnetworks, different opioid addiction subnetworks were finally identified. By identifying differential methylation genes in a shared subnet, we elucidate the mechanism of opioid addiction and suggest the potential adjuvant effects of noradrenergic drugs for withdrawal. This study provides further insights into the molecular mechanism of opioid addiction, which will help to improve the accuracy of clinical decision support, improve the rationality of drug use, and promote the scientific design of late treatment plans. Services that help serve a wider range of patients are important. At the same time, this study has high practical significance and promotion value.

2. Materials and methods

2.1. Data collection and group preprocessing of data

The clinical and GEO expression data used in this experiment were obtained from the Gene expression database created and maintained by the National Center for Biotechnology Information. GSE151485 was downloaded for hydrocodone, GSE77056 for crack cocaine, GSE98203 for heroin, GSE154971 for methamphetamine, and GSE54839, GSE174409, and GSE74737 for expression profiling. The data were integrated and stored in the R language.

According to the different detection times, the GSE151485 data were divided into "preoperative" Group C, "short-term withdrawal" Group T, and "long-term withdrawal" Group D. A total of 78 test samples of 26 experimental subjects who participated in the three tests were selected, and the samples of the three groups were compared in pairs to form a paired *t*-test. The cocaine methylation data (GSE77056), which contained a total of 47 peripheral blood samples, were divided into the crack cocaine sample group (23 samples of blood from people with drug addiction) and crack cocaine sample group control1 (24 samples of blood from people without drug addiction). The opioid methylation data (GSE98203), which contained a total of 88 orbitofrontal cortex neuron samples, were divided into the heroin-using sample group (37 samples) and non-heroin-using sample group (control2; 51 samples). The methylation data (GSE154971) of methamphetamine drugs, which contained 24 peripheral blood lymphocyte samples, were divided into the methamphetamine group (16 samples) and control3 group (8 samples).

2.2. DrugBank database and classification of drug target genes

The DrugBank database contains 13,791 drug entries, and each DrugCard entry contains more than 200 data fields, half of which are for drug/chemical data and the other half for drug target or protein data [14]. Through the DrugBank database, 188 drug target genes, 22 drug target genes for the treatment of opioids and opioid antagonists, and some norepinephrine drugs were collected by ATC code. Finally, 45 target genes of partial norepinephrine drugs were obtained. From the perspective of the main components and functional roles, 188 drug target genes are collectively referred to as opioid target genes. Twenty-two therapeutic opioid and opioid antagonist drug target genes were collectively referred to as therapeutic opioid target genes; forty-five partial noradrenergic drug target genes were collectively referred to as noradrenergic drug target genes.

2.3. STRING database and protein interaction network

The STRING database is a database that searches for interactions between known and predicted proteins [15]. Analysis of protein–protein interaction networks can help uncover central regulatory genes.

Protein–protein interaction (PPI) networks are composed of proteins that interact with each other to participate in all aspects of life processes, such as gene expression regulation, energy and substance metabolism, biological signal transduction, and cell cycle regulation [16]. Molecular interactions at the protein or gene level can be used to construct interaction networks in which interacting species are classified based on direct interactions or functional similarities. Interaction data are analyzed, filtered and combined by various computational tools to obtain comprehensive information about biological pathways. The interaction of proteins in biological systems is systematically studied, which is of great significance for understanding the working principle of proteins and the various connections between proteins in organisms.

2.4. GO and KEGG enrichment

The GO (Gene Ontology) database has three categories, namely, biological process (BP), cellular component (CC) and molecular function (MF) [17]. They describe the molecular function that the gene product may perform, the cellular environment in which it is located, and the biological process in which it is involved. KEGG (Kyoto Encyclopedia of Genes and Genomes) is a database that integrates genomic, chemical and systematic functional information [18]. KEGG has powerful graphical abilities. Graphs are used to introduce the numerous metabolic pathways and the relationships among them. The KEGG PATHWAY database mainly stores functional information. From the KEGG PATHWAY database, we extracted the background genes of metabolic pathways related to drug addiction: hsa05030(KEGG PATHWAY: hsa05030 (genome.jp)) had 49 background genes for the cocaine addiction pathway, hsa05031(KEGG PATHWAY: hsa05031 (genome.jp)) had 69 background genes for the amphetamine addiction pathway, hsa05032 (KEGG PATHWAY: hsa05032) had 91 background genes for the morphine addiction pathway, and a total of 162 background genes were identified after duplication elimination. The three drug addiction pathways correspond exactly to the three classes of drugs we discussed: The cocaine addiction pathway corresponds to cocaine drugs, the morphine addiction pathway corresponds to opioids, and the amphetamine addiction pathway corresponds to amphetamines.

2.5. Human protein atlas database and heatmap of brain regions

The HPA (Human Protein Atlas) database uses transcriptome and proteomics technologies to study the expression of proteins in different human tissues and organs at the RNA and protein levels. Based on the RNA-seq data of protein-coding genes in 32 different tissues, the genes were classified. In view of the important role of secreted proteins and membrane-associated proteins in pathophysiology, the proteins were divided into different categories for study. Thus, it can be used to view and analyze the expression of protein-coding genes in different tissues and organs.

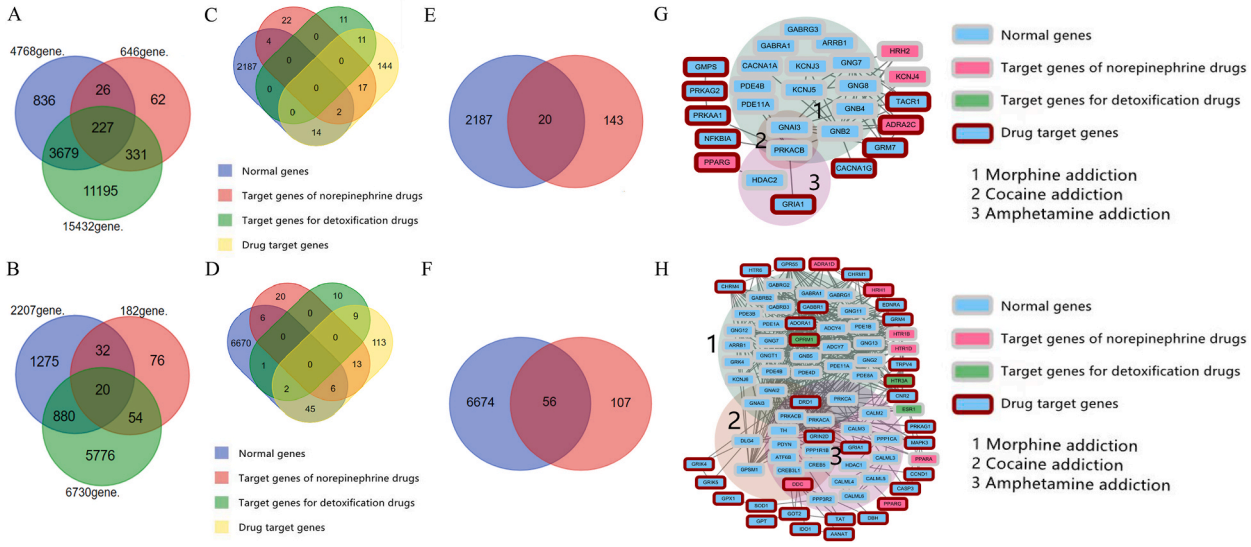


Fig. 1. Differential analysis of methylation between addiction and withdrawal in the hydrocodone group **(A)** Venn diagram of three groups of genes in the hydrocodone group. 4768 genes with a P value < 0.01 in the pretreatment and short-term drug withdrawal group (CT group), 646 genes with a P value < 0.01 in the pretreatment and long-term withdrawal group (CD group), 15,432 genes with a P value < 0.01 in the short-term and long-term withdrawal groups (DT group). **(B)** Venn diagram of three groups of genes in the hydrocodone group at the TSS200 and TSS1500 regions. 2207 genes located in the TSS200 and TSS1500 regions in the CT group, 182 genes located in the TSS200 and TSS1500 regions in the CD group, and 6730 genes located in the TSS200 and TSS1500 regions in the DT group. **(C)** Venn diagram for classification of drug target genes in the hydrocodone CT group. The 2207 genes in the CT group included 6 norepinephrine drug genes, 16 opioid drug genes, and no therapeutic opioid genes. A total of 20 drug target genes were obtained after removing duplicates. **(D)** Venn diagram for classification of drug target genes in the hydrocodone DT group. The 6730 genes in the DT group included 3 therapeutic opioid genes, 12 norepinephrine genes, and 53 opioid genes, and a total of 60 drug target genes were obtained after eliminating duplication. **(E)** Venn diagram of gene classification of the addiction pathway in the hydrocodone CT group. 20 differentially expressed genes among the 2207 genes in the CT group were located in the drug addiction metabolic pathway. **(F)** Venn diagram of the gene classification of the addiction pathway in the hydrocodone DT group. 56 differentially expressed genes in the 6730 genes of the DT group were located in the drug addiction metabolic pathway. **(G)** Diagram and annotation of the protein interaction network of the hydrocodone CT group. **(H)** Diagram and annotation of the protein interaction network of the hydrocodone DT group.

3. Results

3.1. Analysis of methylation differences between addiction and withdrawal in the hydrocodone group

According to the different detection times, the GSE151485 data were divided into "preoperative" Group C, "short-term withdrawal" Group T, and "long-term withdrawal" Group D. A total of 78 test samples of 26 experimental subjects who participated in the three tests were selected, and the samples of the three groups were compared in pairs to form a paired *t*-test. After preliminary CpG alignment, the CpG profiles of the CD, CT, and DT groups were obtained. The differential methylation site information table of DMP in the hydrocodone group was analyzed, and the threshold value was set as a P value < 0.01. The results showed that there were 4768 genes with a P value < 0.01 in the pretreatment and short-term drug withdrawal group (CT group). There were 646 genes with a P value < 0.01 in the pretreatment and long-term withdrawal group (CD group). There were 15,432 genes with a P value < 0.01 in the short-term and long-term withdrawal groups (DT group).

A Venn diagram (Fig. 1A) was drawn for comparison of these three groups of genes. Because the change in gene methylation degree is mainly related to the promoter region, we screened the genes with a P value < 0.01 again and retained only the genes related to the promoter region TSS1500 and core promoter region TSS200. After screening, we obtained 2207 genes located in the TSS200 and TSS1500 regions in the CT group, 180 genes located in the TSS200 and TSS1500 regions in the CD group, and 6730 genes located in the TSS200 and TSS1500 regions in the DT group. For these three groups of genes, a Venn diagram (Fig. 1B) was drawn for comparison.

From the small difference between the premedication group and the long-term withdrawal group (CD group), the small dosage of this experiment (5 mg), and because hydrocodone is a weak opioid, the efficacy of the dose is weak. The duration of long-term drug withdrawal ranged from 29 to 49 days. These 26 individuals could be considered to have completed the withdrawal after long-term drug withdrawal. The predrug group and the short-term drug withdrawal group (CT group) represented the bodily differences caused by taking hydrocodone, that is, the addiction group. The short-term and long-term withdrawal groups, the DT group, represent the differences in the body from the beginning of withdrawal to the completion of withdrawal, that is, the withdrawal group.

Based on the fact that the number of differential genes obtained after screening in the DT group was significantly greater than that obtained in the CT group under the same conditions, we can draw a preliminary conclusion that the body difference produced by the withdrawal period of hydrocodone is significantly greater than that produced by the intake of hydrocodone.

3.2. Genetic analysis addiction and withdrawal in the hydrocodone group

To further test our hypothesis and to reveal the potential role that norepinephrine can play during periods of withdrawal. The 2207 differentially expressed genes in the CT group and 6730 differentially expressed genes in the DT group were compared with 188 opioid target genes, 22 therapeutic opioid target genes and 45 norepinephrine drug target genes collected by ATC code through the DrugBank database by drawing a Venn diagram. The results are shown in Fig. 1C and D. The 2207 genes in the CT group included 6 norepinephrine drug genes, 16 opioid drug genes, and no therapeutic opioid genes. A total of 20 drug target genes were obtained after removing duplicates. The 6730 genes in the DT group included 3 therapeutic opioid genes, 12 norepinephrine genes, and 53 opioid genes, and a total of 60 drug target genes were obtained after eliminating duplication.

The 2207 genes in the CT group were compared with the three background gene sets of drug addiction metabolic pathways obtained through the KEGG PATHWAY database. As shown in Fig. 1E, 20 differentially expressed genes among the 2207 genes in the CT group were located in the drug addiction metabolic pathway. We combined these 20 pathway genes with 20 drug target genes obtained from the previous analysis to obtain 39 genes after duplication elimination. By comparing the 6730 genes of the DT group with the three background gene sets of drug addiction metabolic pathways obtained through the KEGG PATHWAY database, as shown in Fig. 1F, 56 differentially expressed genes in the 6730 genes of the DT group were located in the drug addiction metabolic pathway. These 56 pathway genes were combined with the 60 drug target genes obtained from the previous analysis to obtain 107 genes after duplication elimination.

3.3. Visualization of the cytoscape protein interaction network between addiction and withdrawal in the hydrocodone group

All human protein–protein interaction data were obtained from the STRING database. The interaction pairs with protein interaction confidence greater than 750 were selected as background data, and the key genes screened by the gene classification Venn diagram were used as the core to construct the target gene interaction network. After adjustment and simplification, the protein interaction subnetwork was obtained. Based on the KEGG pathway enrichment information, the gene blocks in the protein interaction subnetwork belonging to the same KEGG pathway were adjusted and aggregated to obtain the genes involved in the final construction of the protein interaction subnetwork in the hydrocodone CT group and the DT group. As shown in Fig. 1G and H, we found from the protein interaction network that the body difference produced by the withdrawal period of hydrocodone was significantly greater than that produced by the taking of hydrocodone in the 26 test subjects under the same conditions. The protein interaction network in both periods involved norepinephrine drug targets, which further confirmed our conclusion—that reducing the withdrawal reaction in people with drug addiction is necessary and that norepinephrine drugs are potential options in the treatment of drug addiction.

3.4. Go and KEGG pathways enrichment analyses of differentially expressed genes between addiction and withdrawal in the hydrocodone group

GO enrichment analysis of BP (biological process), CC (cell composition) and MF (molecular function) was performed on 27 genes involved in the construction of the protein interaction network in the CT group and 83 genes involved in the construction of the protein interaction network in the DT group (http://www.bioinformatics.com.cn/?p=1), and the enrichment results are shown in Fig. 2A and B (P value < 0.05).

On the basis of GO enrichment, the data of the CT group and DT group were further enriched by KEGG pathway (http://www.bioinformatics.com.cn/?p=1), and the top 20 result pathways were selected for display as shown in Fig. 2C and D (P value $<$ 0.05). Sankey bubble plot as shown in Fig. S1.Since hydrocodone is a semisynthetic morphine drug, we selected the morphine addiction pathway for visual analysis of KEGG pathway enrichment, and the results are shown in Figs. 3 and 4 (P value < 0.05). The KEGG visualization enrichment results showed that the morphine addiction pathway of the DT group in the withdrawal period was significantly changed compared with that of the CT group. To further verify the potential drug effect of norepinephrine drugs in the withdrawal period, we supplemented the amphetamine addiction pathway and cocaine addiction pathway of the DT group in the withdrawal period. As shown in Fig. S2 (P value < 0.05). In these two pathways, we can clearly observe the target genes of norepinephrine drugs, which are located upstream of the two pathways and indirectly regulate the expression of downstream differentially expressed genes.

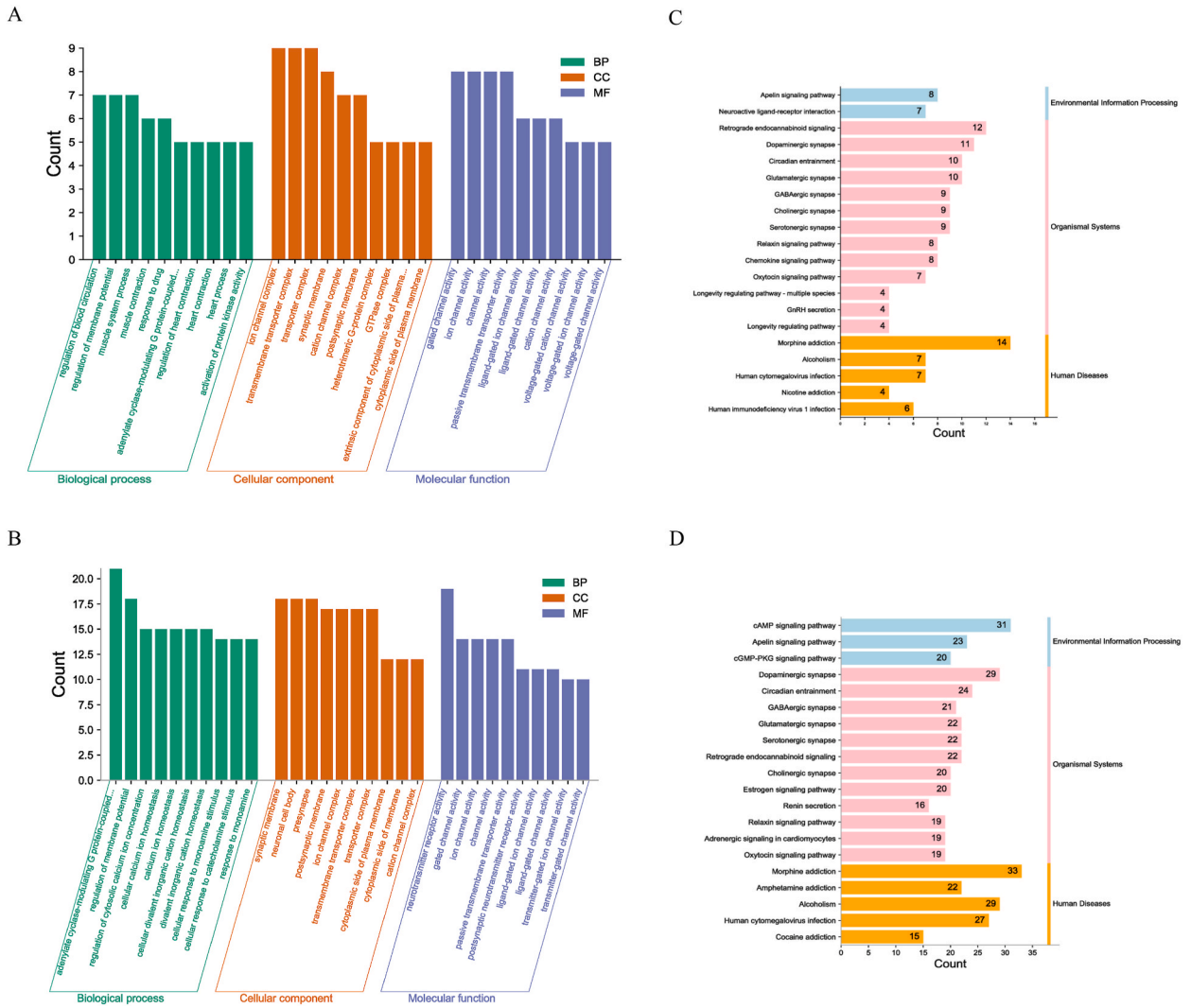
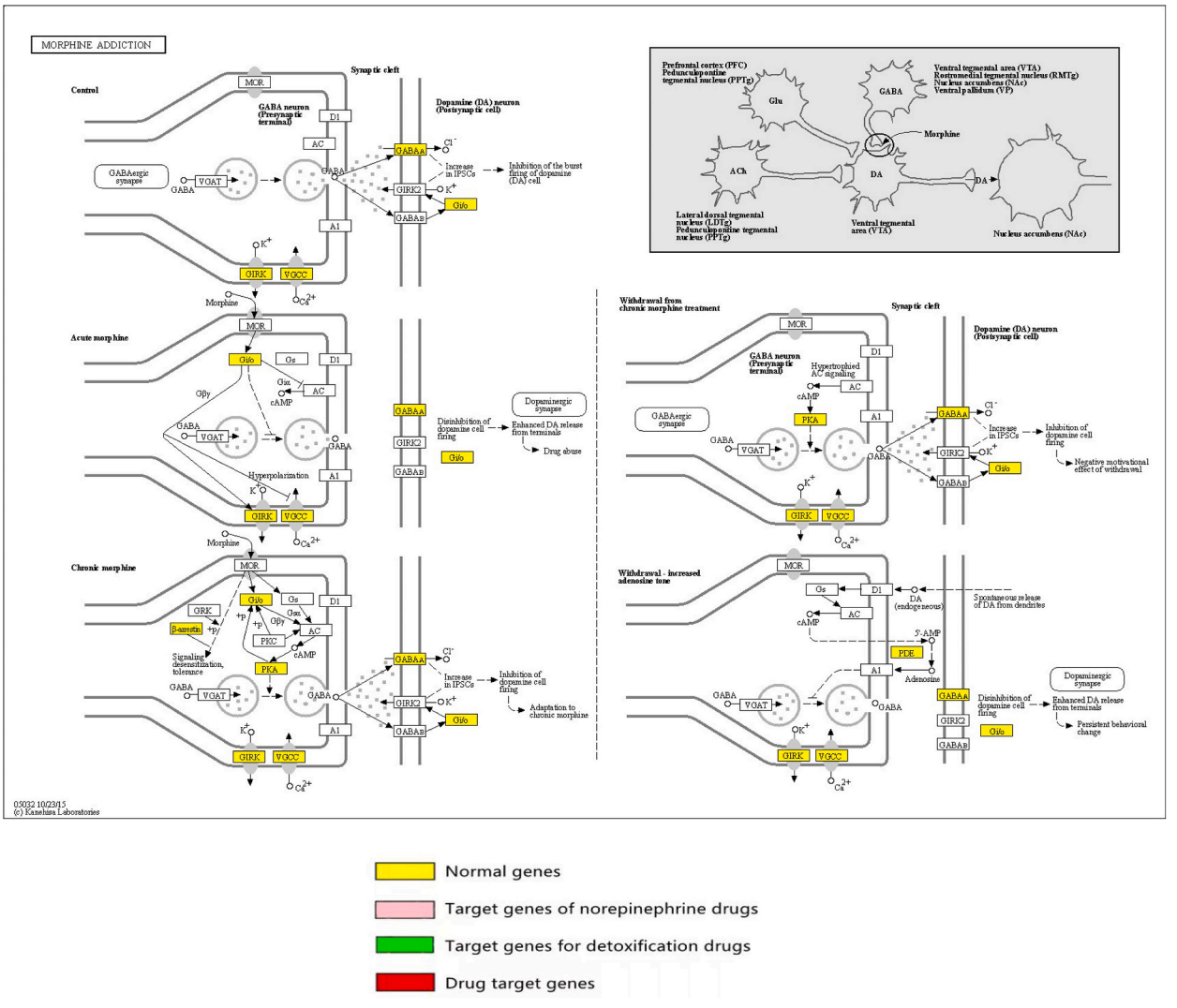


Fig. 2. GO and KEGG enrichment analysis of differentially expressed genes between addiction and withdrawal in the hydrocodone group **(A)** Bar graph of GO enrichment analysis in the hydrocodone CT group. **(B)** Bar graph of GO enrichment analysis in the hydrocodone DT group. **(C)** Classification map of KEGG pathway enrichment results of the hydrocodone CT group. **(D)** Classification map of KEGG pathway enrichment results of the hydrocodone DT group.



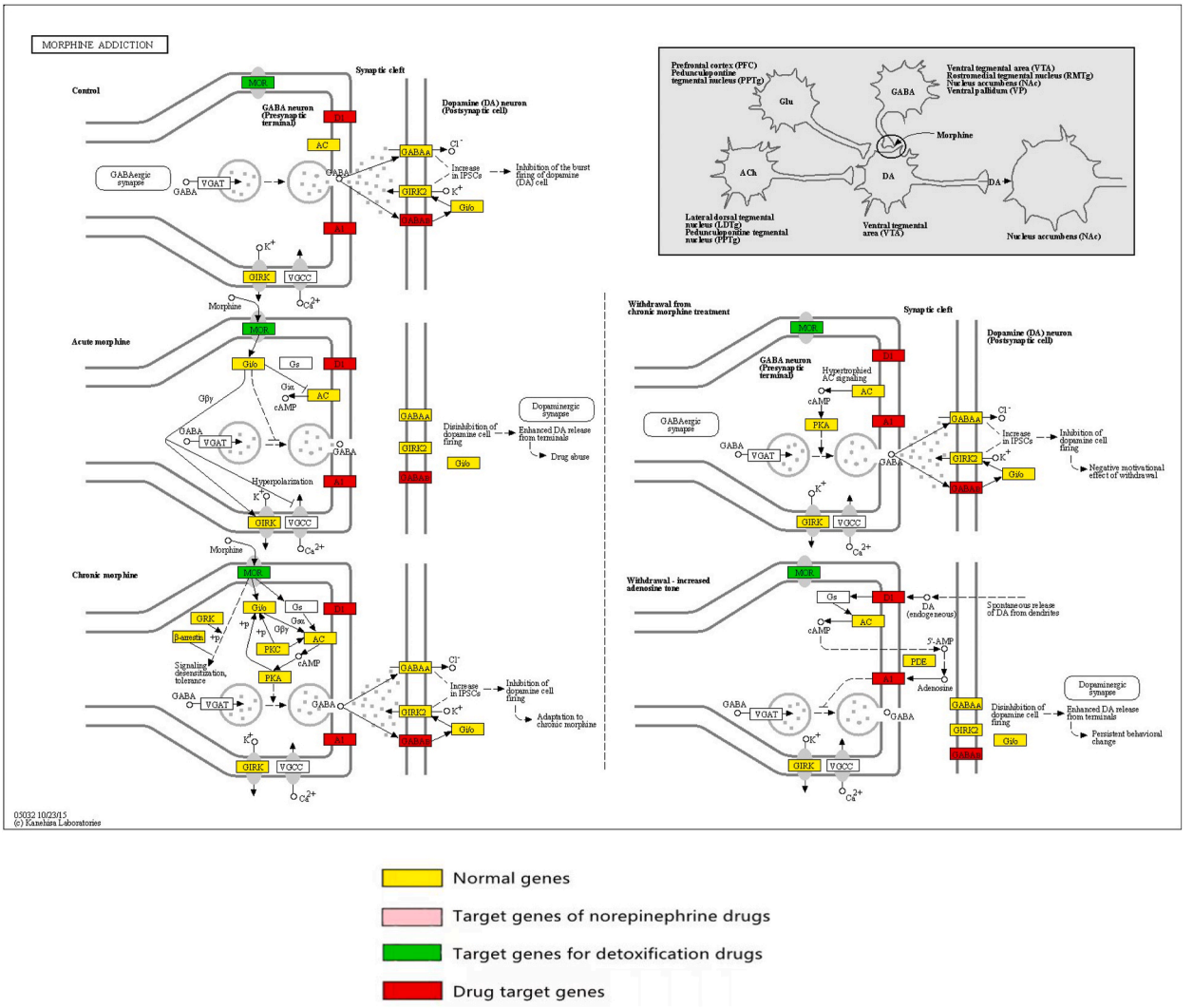
Normal genes
Target genes of norepinephrine drugs
Target genes for detoxification drugs
Drug target genes

Fig. 3. Visualization analysis and annotation of the morphine addiction pathway in the hydrocodone CT group.

3.5. Analysis of the influence of addiction and withdrawal on brain regions and the influence of addiction- and withdrawal-related drugs in the hydrocodone group

The genes involved in the construction of hydrocodone CT and DT protein interaction networks were searched in the Human Protein Atlas database, and the corresponding highly expressed brain regions of these genes were found; that is, these genes have an impact on the highly expressed brain regions during the expression process. It was plotted as a heatmap of the brain region as shown in Fig. 5A and B. We observed that among the 27 genes involved in the construction of the CT histone interaction network, there was a normal gene CACNA1A, which affected the CB cerebellum, which plays a role in motor initiation, planning and coordination in the body. The 83 genes involved in the construction of the hydrocodone-DT group protein interaction network mainly affected the SN substantia nigra region and the BS brainstem region. The substantia nigra is the main nucleus of dopamine synthesis in the brain and an important center for the regulation of movement, while the brainstem has the function of maintaining individual life, including heartbeat and respiration. In 9 genes affecting the substantia nigra and 7 genes affecting the brainstem, we found DDC, the target gene of norepinephrine drugs, which further verified our hypothesis that norepinephrine drugs have potential functions in treating addiction and reducing withdrawal responses.

The alluvial map takes the target gene (target protein) as the link to form the association between the drug and the KEGG enrichment pathway and the GO enrichment pathway, which can visually show which pathways the drug affects the human body through genes. The results are shown in Fig. 6.



Normal genes
Target genes of norepinephrine drugs
Target genes for detoxification drugs
Drug target genes

Fig. 4. Visualization analysis and annotation of the morphine addiction pathway in the hydrocodone DT group.

3.6. Genetic analysis of three drug addictions

The CHAMP package [19] was used to analyze the data of the crack cocaine group (GSE77056) and DMP differential methylation site information table and to set the default threshold of the system p value < 0.05, and a total of 6802 genes were obtained. For the heroin group data (GSE98203), a total of 2498 genes were obtained; for the methamphetamine group data (GSE154971), a total of 537 genes were obtained. A Venn diagram of these three groups was drawn for the first comparison, as shown in Fig. 7A. The 6802 genes of the crack cocaine group were mapped to the STRING database, the interaction pairs with confidence scores greater than or equal to the threshold of 750 were selected as the background of protein association interaction information, and 4488 genes were obtained after repetition. The heroin group contained 1362 genes; a total of 178 genes were obtained in the methamphetamine group. A Venn diagram of these three groups was drawn for secondary comparison, as shown in Fig. 7B. A total of 4488 genes in the crack cocaine group, 1362 genes in the heroin group and 178 genes in the methamphetamine group were compared with 188 opioid target genes, 22 therapeutic opioid target genes and 45 norepinephrine drug target genes collected by ATC code in the DrugBank database. According to Fig. 7C–E the 4488 genes in the crack cocaine group included 4 therapeutic opioid target genes, 16 norepinephrine target genes, and 63 opioid target genes, and the total number of drug target genes was 72 after repeat removal. Among the 1362 genes in the heroin group, there were 5 therapeutic opioid target genes, 8 norepinephrine target genes, 30 opioid target genes, and a total of 34 drug target genes after duplication elimination. Among the 178 genes in the methamphetamine group, only 5 opioid target genes were identified.

3.7. Visualization of the cytoscape protein interaction network of three drug addictions

The 4488 genes in the crack cocaine group, 1362 genes in the heroin group, and 178 genes in the methamphetamine group were

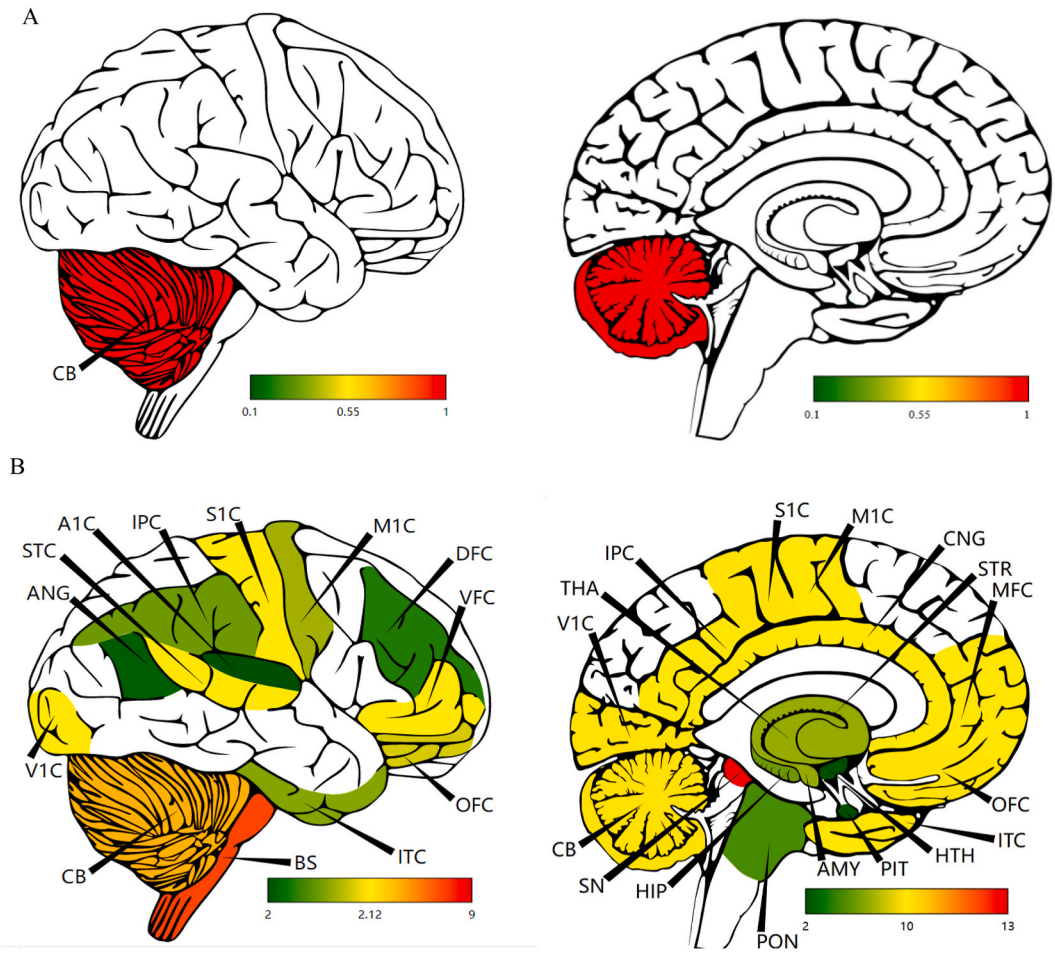
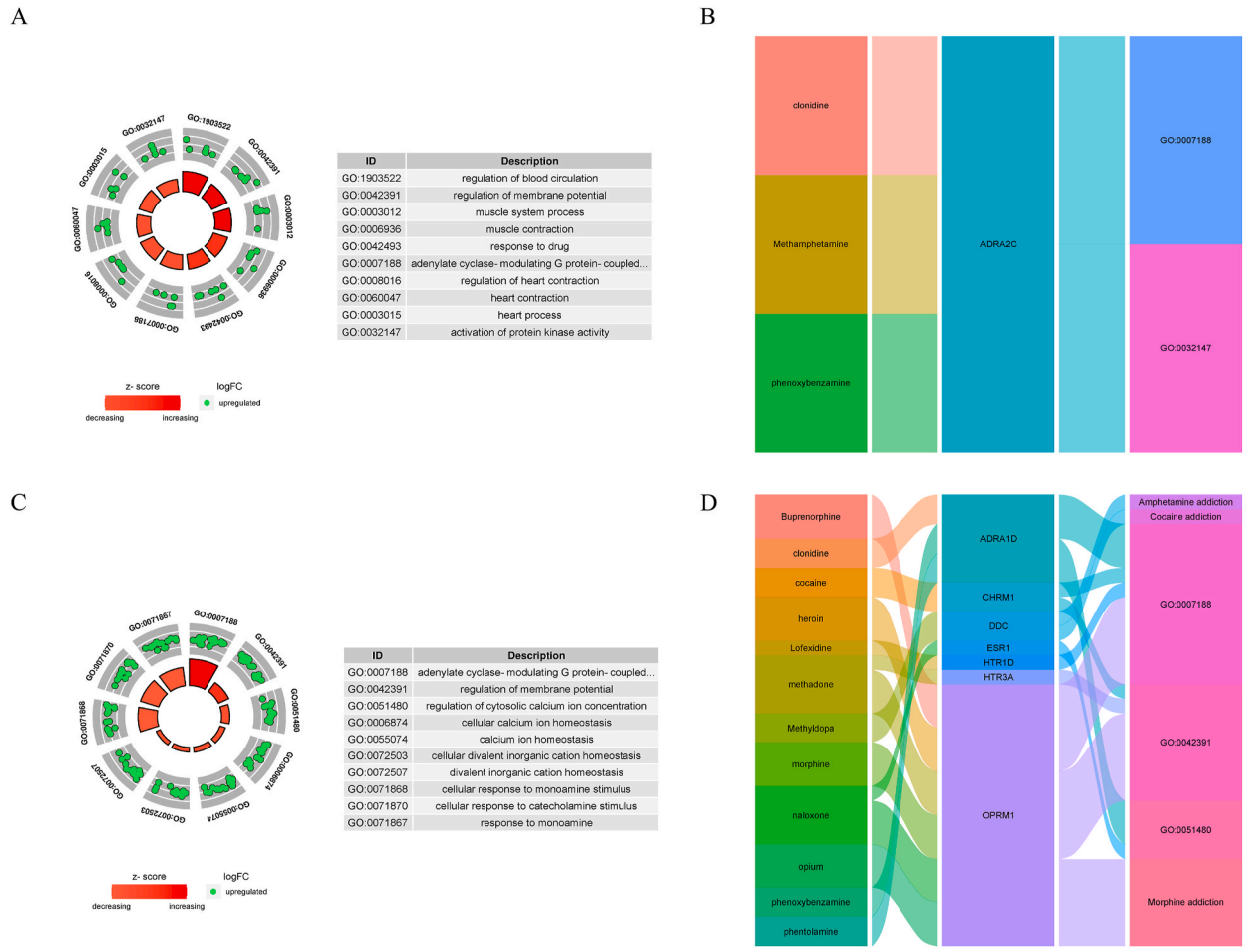


Fig. 5. Analysis of the influence of brain regions on addiction and withdrawal in the hydrocodone group **(A)** Heatmap of brain regions in the hydrocodone CT group. **(B)** Heatmap of brain regions in the hydrocodone DT group.

compared with the background gene sets of three drug addiction metabolic pathways obtained through the KEGG PATHWAY database, as shown in Fig. 7F–H. A total of 66 of 4488 genes in the crack cocaine group, 39 of 1362 genes in the heroin group, and 6 of 178 genes in the methamphetamine group were involved in the drug addiction metabolic pathway. The obtained pathway genes were combined with the previously obtained drug target genes to eliminate duplication: 128 genes in the crack cocaine group, 68 genes in the heroin group, and 9 genes in the methamphetamine group. The resulting genes were used to construct protein–protein interaction networks. Cytoscape was used to visualize the protein interaction network, and then with the enrichment information of the drug addiction pathway as the background, the gene blocks in the protein interaction network belonging to the drug addiction pathway were adjusted and aggregated, and the genes that eventually participated in the construction of the protein interaction network of crack cocaine were obtained as shown in Fig. 7I–K. There were 118 genes in the crack cocaine group, 60 genes in the heroin group, and 5 genes in the methamphetamine group. From the protein interaction network, we found that under the same screening conditions, the body difference of the crack cocaine group and the heroin group was significantly greater than that of the methamphetamine group. Therefore, we propose that the body difference caused by taking cocaine and opioid drugs will be significantly greater than that caused by taking amphetamine drugs. An increase in body difference means an increase in the degree of withdrawal discomfort that needs to be overcome during the withdrawal period, an increase in the difficulty of withdrawal work and an increase in the probability of relapse. In addition, the involvement of norepinephrine drug targets in the protein–protein interaction network was found in both the crack cocaine and heroin groups, which further confirmed our hypothesis that norepinephrine drugs have the potential to treat drug addiction.

3.8. Go and KEGG pathways enrichment analyses of differentially expressed genes of three drug addictions

GO enrichment analysis was performed on 118 genes involved in the construction of the protein–protein interaction network for crack cocaine, 60 genes involved in the construction of the heroin histone interaction network, and 5 genes involved in the construction of the methamphetamine histone interaction network for BP (biological process), CC (cell composition) and MF (molecular



ID	Description
GO:1903522	regulation of blood circulation
GO:0042391	regulation of membrane potential
GO:0003012	muscle system process
GO:0006936	muscle contraction
GO:0042493	response to drug
GO:0007188	adenylate cyclase- modulating G protein- coupled...
GO:0008016	regulation of heart contraction
GO:0060047	heart contraction
GO:0003015	heart process
GO:0032147	activation of protein kinase activity

ID	Description
GO:0007188	adenylate cyclase- modulating G protein- coupled...
GO:0042391	regulation of membrane potential
GO:0051480	regulation of cytosolic calcium ion concentration
GO:0006874	cellular calcium ion homeostasis
GO:0055074	calcium ion homeostasis
GO:0072503	cellular divalent inorganic cation homeostasis
GO:0072507	divalent inorganic cation homeostasis
GO:0071868	cellular response to monoamine stimulus
GO:0071870	cellular response to catecholamine stimulus
GO:0071867	response to monoamine

Fig. 6. Analysis of the effects of drugs related to addiction and withdrawal in the hydrocodone group **(A)** GO Circle diagram of the hydrocodone CT group. **(B)** Impact map of addictive drugs and gene pathways in the hydrocodone group. **(C)** GO Circle plot of the hydrocodone DT group. **(D)** Impact map of withdrawal drug and gene pathways in the hydrocodone group.

function), respectively (http://www.bioinformatics.com.cn/?p=1).The enrichment results are shown in Fig. 8A–C (P value < 0.05). Most of the enrichment pathways were highly related to G protein and nerve cell signal transduction, and relevant literature confirmed that these pathways and functions had a certain role in the process of drug addiction, which was in line with our expected results.

On the basis of GO enrichment, KEGG pathway enrichment was performed on the protein interaction network genes of the crack cocaine group, heroin group and methamphetamine group (http://www.bioinformatics.com.cn/?p=1).The top 20 resulting pathways are shown in Fig. 8D–F (P value < 0.05), and the three drug addiction pathways were visualized by KEGG pathway enrichment analysis and are shown in Fig. 9, Figs. S3–S7 (P value < 0.05). KEGG visualization enrichment results of amphetamine addiction pathways in the crack cocaine group showed that in the addiction pathway, amphetamine, norepinephrine drug target gene CALM1 and opioid drug targets GRIN3A, GRIN3B, GRIN2A, GRIN2B, and GRIN2C were directly connected and directly connected with other normal genes. CALM1 plays an important regulatory role in the middle and late stages of amphetamine addiction, which further validates the potential efficacy of norepinephrine drugs in the treatment of drug addiction. KEGG visualization enrichment results of cocaine addiction and amphetamine addiction in the heroin group showed that the target gene DDC of norepinephrine drugs was upstream of the two pathways and indirectly regulated downstream differential gene expression transmission, which also verified the potential efficacy of norepinephrine drugs in the treatment of drug addiction.

3.9. Analysis of the influence of three drugs on brain regions

The genes involved in the construction of PROTEIN interaction networks in the crack cocaine group, heroin group and methamphetamine group were searched in the Human Protein Atlas database, and the corresponding regional highly expressed brain regions of these genes were found; that is, these genes had an impact on the regional highly expressed brain regions during the expression process. It was drawn into a heatmap of brain regions, as shown in Fig. 10A–C. The genes involved in the protein interaction network of the crack cocaine group mainly affect the SN substantia nigra, which is the main nucleus for the synthesis of dopamine in the brain and

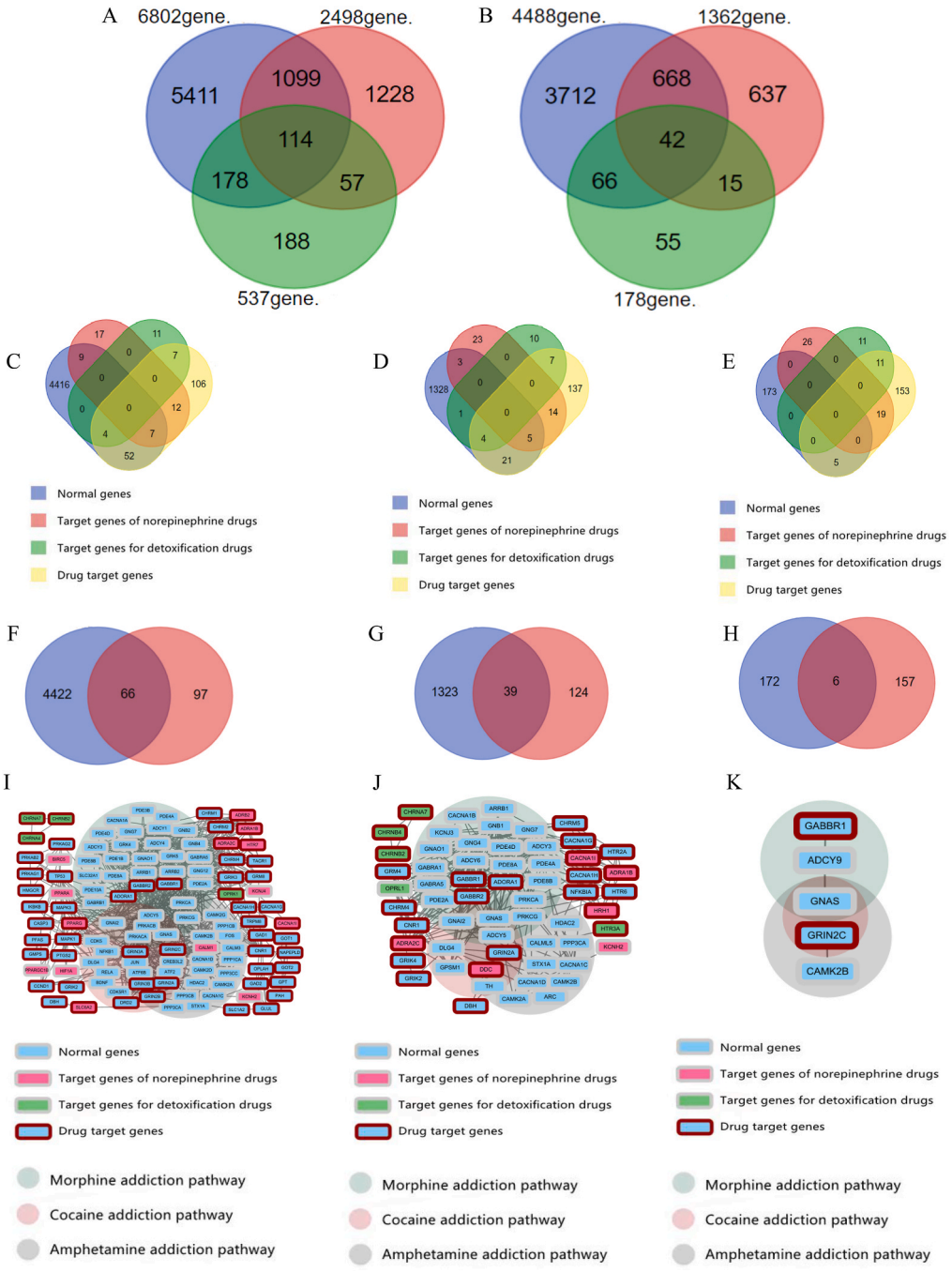


Fig. 7. Genetic analysis of three drug addictions **(A)** Venn diagram of the first comparison of methylation data for three drugs. 6802 genes with a P value < 0.05 in the crack cocaine group, 2498 genes with a P value < 0.05 in the heroin group, 537 genes with a P value < 0.05 in the meth-amphetamine group. **(B)** Venn diagram of secondary comparison of methylation data for three drugs. The 6802 genes of the crack cocaine group were mapped to the STRING database, the interaction pairs with confidence scores greater than or equal to the threshold of 750 were selected as the background of protein association interaction information, and 4488 genes were obtained after repetition. The heroin group contained 1362 genes, a total of 178 genes were obtained in the methamphetamine group. **(C)** Venn diagram of drug target gene classification in the crack cocaine group. The 4488 genes in the crack cocaine group included 4 therapeutic opioid target genes, 16 norepinephrine target genes, and 63 opioid target genes, and the total number of drug target genes was 72 after repeat removal. **(D)** Venn diagram of drug target gene classification in the heroin group. Among the 1362 genes in the heroin group, there were 5 therapeutic opioid target genes, 8 norepinephrine target genes, 30 opioid target genes, and a total of 34 drug target genes after duplication elimination. **(E)** Venn diagram of drug target gene classification in the methamphetamine group. Among the 178 genes in the methamphetamine group, only 5 opioid target genes were identified. **(F)** Venn diagram for the classification of addiction pathway genes in the crack cocaine group. 66 of 4488 genes in the crack cocaine group were involved in the drug addiction metabolic

pathway. **(G)** Venn diagram for the classification of addiction pathway genes in the heroin group. 39 of 1362 genes in the heroin group were involved in the drug addiction metabolic pathway. **(H)** Venn diagram of the classification of addiction pathway genes in the methamphetamine group. 6 of 178 genes in the methamphetamine group were involved in the drug addiction metabolic pathway. **(I)** Diagram and annotation of the protein interaction network in the crack cocaine group. **(J)** Protein interaction network diagram and annotation of the heroin group. **(K)** Diagram and annotation of the protein interaction network in the methamphetamine group.

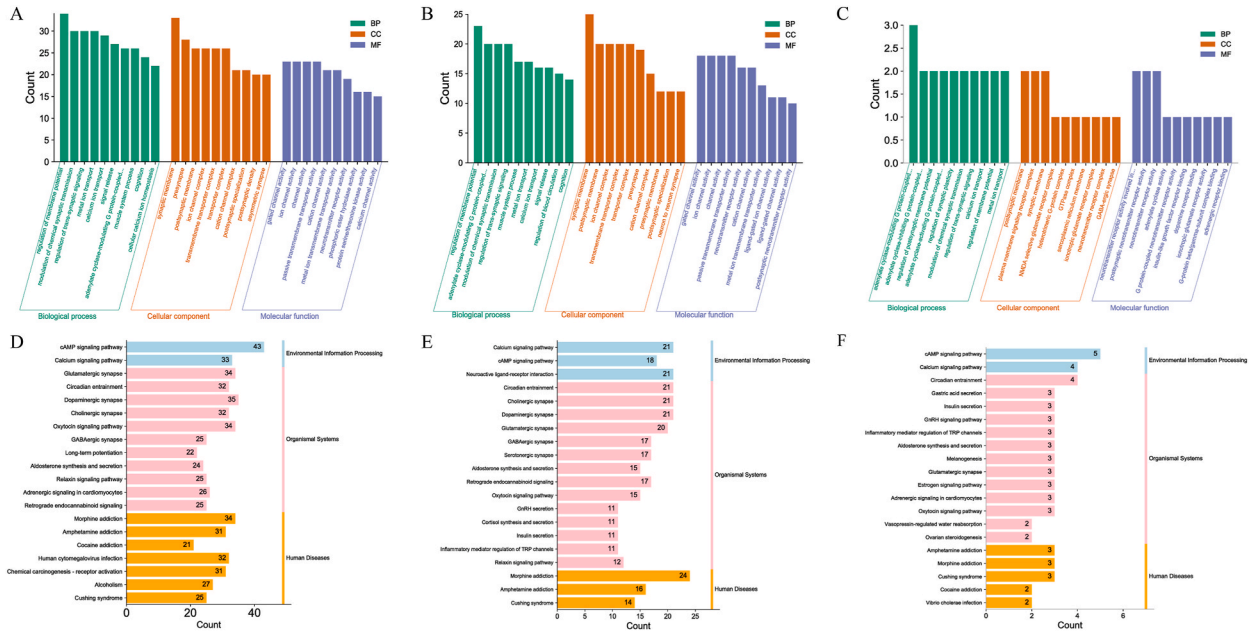


Fig. 8. GO and KEGG enrichment analysis of differentially expressed genes in three types of drug addiction **(A)** Bar graph of GO enrichment analysis for the crack cocaine group. **(B)** Bar graph of GO enrichment analysis for the heroin group. **(C)** Bar graph of GO enrichment analysis for the methamphetamine group. **(D)** Classification map of KEGG pathway enrichment results for the crack cocaine group. **(E)** Classification map of KEGG pathway enrichment results for the heroin group. **(F)** Classification map of KEGG pathway enrichment results for the methamphetamine group.

an important center for the regulation of movement. The norepinephrine drug target gene SLC6A2 was found in 6 genes affecting the SN substantia nigra region and 2 genes affecting the BS brainstem region and PON pons region, while the norepinephrine drug target gene PPARGC1B was found in 3 genes affecting the CB cerebellum region. The brain stem has the function of maintaining individual life, including heartbeat and respiration. The white matter nerve fibers of the pons, which pass to the cerebellar cortex, can transmit nerve impulses from one hemisphere of the cerebellum to the other hemisphere so that it can play a role in coordinating the activities of muscles on both sides of the body. The cerebellum plays a role in the initiation, planning and coordination of movements in the body. All these results further confirmed our hypothesis that norepinephrine drugs have the potential to treat drug addiction and reduce the withdrawal reaction. The test site of heroin group data samples is the orbitofrontal cortex, which is the smallest part of the frontal lobe. Here, the orbitofrontal cortex (OFC) and frontal lobe (FL) are marked in red with the highest degree of influence. In addition to the orbitofrontal cortex and frontal lobe, the remaining genes involved in the protein interaction network of the heroin group mainly affect the SN substantia nigra region of the brain. The norepinephrine drug target gene DDC was found in 5 genes affecting the substantia nigra region and 4 genes affecting the brainstem region of BS. Among the five genes involved in the methamphetamine histone interaction network, one gene, GABBR1, was still significantly expressed in the brain area. The brain areas affected by GABBR1 were the cerebral cortex, HIP, and hippocampus, which are the highest centers that regulate body movement and control the body. The hippocampus functions in short-term memory storage, conversion and orientation.

4. Discussion

Opioid addiction and withdrawal are challenges worldwide. At present, there are relatively few studies on low-cost and effective drugs for the treatment of opioid addiction and withdrawal at home and abroad, and the process from proposing new methods to general promotion requires a large amount of clinical trial data. In this paper, based on the analysis of clinical data and differential gene methylation expression data of opioid addicts, it was found that: firstly, different types of drugs have varying degrees of addictiveness, with cocaine and opioids being more addictive than methylphenidate; secondly, some norepinephrine drugs have the potential to alleviate withdrawal symptoms.

Addiction refers to a type of repetitive compulsive behavior that may be caused by a dysfunctional central nervous system, and the repetition of these behaviors can in turn lead to neurological impairment [20]. In the case of cocaine, which is a reuptake inhibitor of

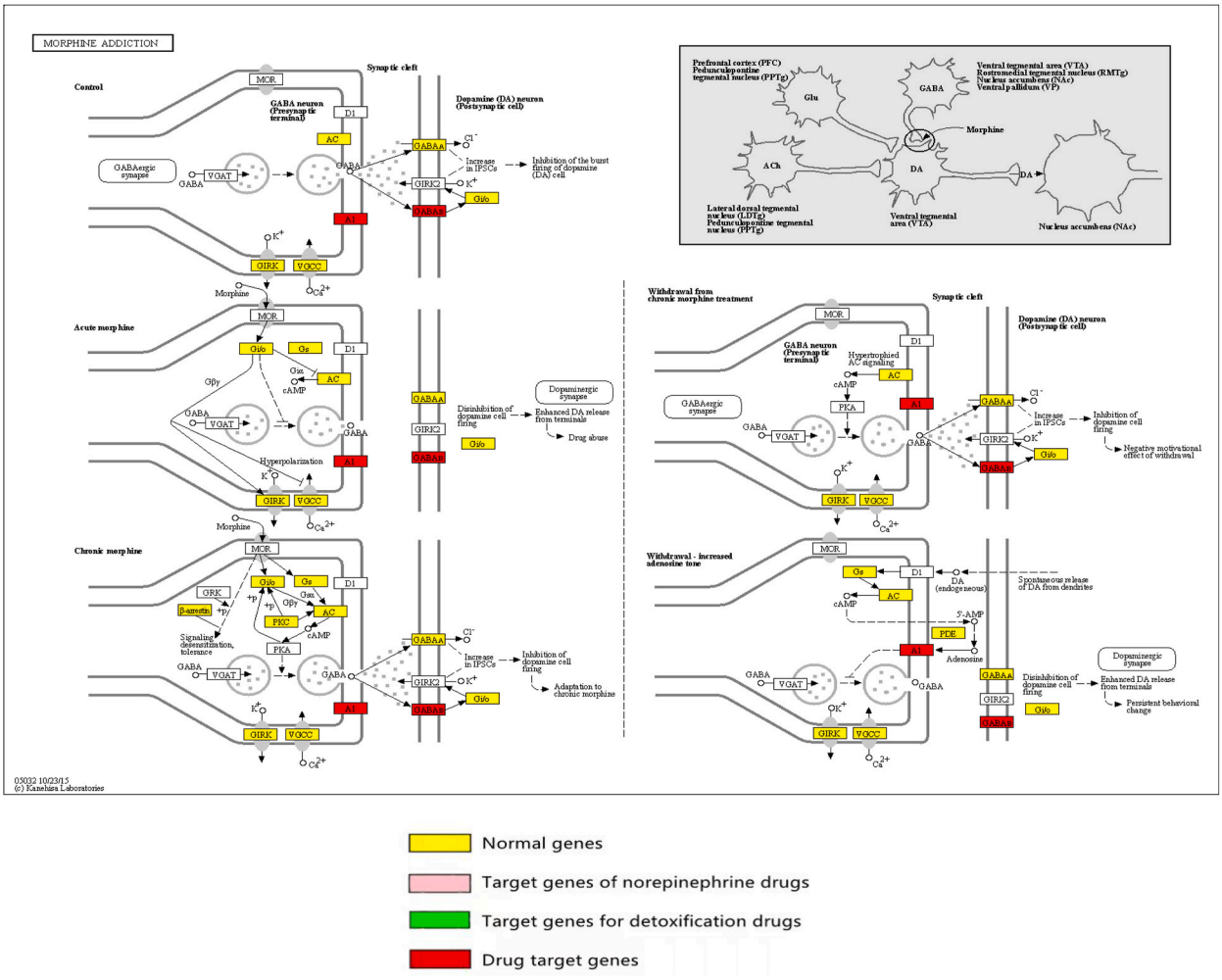


Fig. 9. Visualization analysis and annotation of the morphine addiction pathway in the crack cocaine group.

serotonin, norepinephrine, and dopamine, the increase in brain concentrations of these three neurotransmitters results in a strong euphoric feeling that acts on the reward pathway in the brain and leads to addiction [21]. Methamphetamine can also increase the concentration of neurotransmitters such as dopamine in the brain and is a highly psychologically addictive drug [22]. Heroin is metabolized in the liver to morphine, which acts directly on the central nervous system to alter the body's perception of pain [23]. Both crack cocaine and heroin are more harmful to humans and more addictive than methamphetamine, and our experimental results are consistent with this conclusion.

The norepinephrine system plays an important role in the development of opioid dependence and in causing withdrawal syndrome. Endorphins bind to opioid receptors and promote the release of dopamine. Dopamine promotes the release of noradrenergic hormones from nerve terminals, but chronic opioid abuse leads to diminished noradrenergic neuronal activity. Compared with endorphins released by the body itself, ingested opioids bind to opioid receptors for a longer time with stronger signals [24], and the body releases more dopamine [25]. After a period of time, the body develops tolerance, the starting dose of dopamine increases [26], and then the drug dose of people with drug addiction increases. The body's norepinephrine release continues to decrease [27], and the body begins to compensate for the regulation. The norepinephrine receptor increases, and the body's sensitivity to norepinephrine increases. When a person with a drug addiction suddenly stops taking narcotic drugs, the normal release of endorphins and high-tolerance opioid receptors in the body of the person is not enough to form sufficient stimulation, the level of dopamine decreases [28], and the abstinent person begins to produce withdrawal reactions such as pain and depression [29]. On the other hand, due to the nerve damage caused by long-term abuse of narcotic drugs, people with drug addiction will have a false evaluation [30,31], so they are prone to relapse in the early stage of withdrawal [32,33]. At the same time, there is no opioid to inhibit the release of norepinephrine, and the body is in a state of high sensitivity to norepinephrine, which will further aggravate the withdrawal reaction and increase the difficulty of successful withdrawal. Based on this, we have the following two ideas. One is to use the principle of competitive blocking, that is, α-blockers, β-blockers, and α, β-blockers, to offset the agonist effect of norepinephrine through competitive inhibition, which is suitable for use in the acute episode of drug addiction in abstinent people. The second is to inhibit the release of norepinephrine, that is, α2

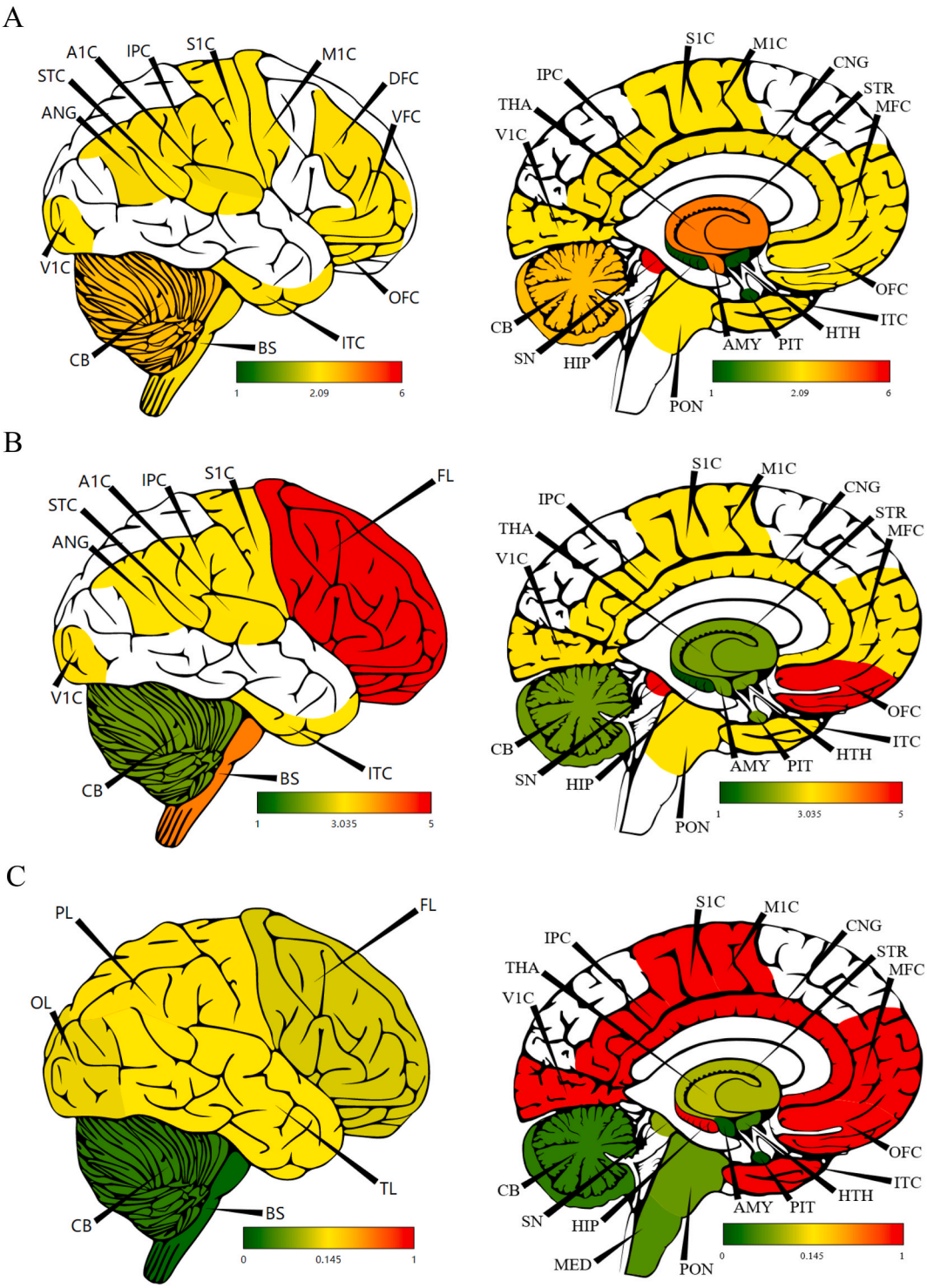


Fig. 10. Analysis of the influence of three drugs on brain regions **(A)** Heatmap of brain regions in the crack cocaine group. **(B)** Heatmap of brain regions in the heroin group. **(C)** Heatmap of brain regions in the methamphetamine group.

receptor agonists represented by clonidine and lofexidine inhibit the release of norepinephrine by binding to the α2 receptor. Both opioids and α2 receptors can tonically inhibit the firing activity of LC neurons and regulate the release and synthesis of norepinephrine. Clonidine and lofaxicidine have been clinically confirmed to reduce the symptoms and signs of opioid addiction after withdrawal and can be used for rapid detoxification treatment of opioid dependence, especially for mild and moderate opioid dependence. It also has

the characteristics of rapid action, nonopioid drugs, not causing euphoria, not causing addiction, and a high success rate of inpatient withdrawal treatment. DDC, a target gene of norepinephrine drugs, was identified multiple times in our experiment and is an α2 receptor agonist, providing us with a theoretical basis for the proposed approach. Second, norepinephrine can not only reduce the withdrawal reaction but also may be used in combination with opioid receptor antagonists. Because the combination of drugs needs to be established on the basis of different drug target receptors, opioid receptor antagonists are mostly μ-receptors, κ-receptors and δ-receptors, and norepinephrine receptors are α-receptors and β-receptors, the difference in receptors provides the feasibility of the combination of the two drugs. Some authors have noted that injection of phentolamine can enhance morphine analgesia, which further demonstrates the possibility of using norepinephrine drugs to assist withdrawal. Phenoxybenzamine, prazosin, as a long-acting drug, can act for 3–4 days each time. Clinical experiments have confirmed that phenoxybenzamine and papaverine have similar functions, and phenoxybenzamine is more effective and less harmful than papaverine in preventing radial artery spasm [34]. To avoid affecting self-regulation in the body of abstinent patients, during the withdrawal stage, phenoxybenzamine, prazosin and other drugs can be used to compete to block the withdrawal reaction caused by norepinephrine, and clonidine, lofexidine, methyldopa and other drugs can be used in the next few days to inhibit the release of norepinephrine to reduce the withdrawal reaction of abstinent patients.

From the perspective of bioinformatics, this experiment verified the feasibility of replacing opioid antagonists with norepinephrine drugs or taking them together with opioid antagonists to participate in the withdrawal treatment of opioid addiction patients and reduce the withdrawal reaction. Comprehensive functional analysis of the cytoCAP network map provided the interaction relationship between proteins of opioid addiction. GO enrichment and KEGG enrichment allowed us to understand the gene-related pathways more intuitively and understand their potential biological effects more comprehensively. Finally, the heatmap of brain regions showed the influence of drug addiction on the human brain.

Admittedly, this study has some limitations. First, only the GEO data of the gene expression database created and maintained by NCBI were used for experimental analysis and verification. If the situation permits, data from more sources can be used for further verification. Second, there have been some studies on the expression and influence of opioid addiction and withdrawal on human brain nerves. According to the existing studies, more in-depth integrated analysis may help us understand the specific role of opioid addiction in the process of human brain nerve changes. Finally, in the future, we should increase the research based on the mechanism of action of opioids and brain regions to strengthen the understanding of the mechanism of action of opioids in human brain regions.

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Data availability statement

All sequencing data applied in this study is freely accessible at the corresponding database described above.

CRediT authorship contribution statement

Yan Wang: Writing – original draft, Validation, Formal analysis. **Jiawei Ke:** Writing – original draft, Validation, Formal analysis. **Shanshan Li:** Software, Methodology. **Qingling Kong:** Software, Methodology. **Mingyue Zhang:** Writing – review & editing, Data curation. **Mingming Li:** Writing – review & editing, Data curation. **Jing Gu:** Supervision, Project administration, Investigation, Conceptualization. **Meng Chi:** Supervision, Project administration, Investigation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e26957.

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