European Review for Medical and Pharmacological Sciences 2023; 27: 11673-11690

Bioinformatic analysis identified common pathogenetic processes between epilepsy and COVID-19

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Abstract. – OBJECTIVE: With the ongoing progression of SARS-CoV-2-induced COVID-19, the post-acute COVID-19 syndrome (long COVID) has garnered increasing attention as a novel multisystem disorder. Long COVID-19 has been shown to impact the nervous system, leading to various neurological manifestations, including epilepsy and seizures. Current studies have reported a significant increase in the prevalence and mortality rate of epilepsy in COVID-19 patients. Additionally, COVID-19 exacerbates seizures in patients with epilepsy. However, the mechanisms underlying the impact of COVID-19 on epilepsy remain elusive. This research focused on further identifying and elucidating the molecular mechanisms and biological processes underlying the induction of epilepsy by COVID-19 through bioinformatic methods.

MATERIALS AND METHODS: We retrieved four gene expression datasets related to COVID-19 and epilepsy patients from the GEO and ArrayExpress databases. By crossing the major modules of weighted gene co-expression network analysis (WGCNA), the commonly expressed genes of epilepsy and COVID-19 were identified. By establishing the protein-protein interaction (PPI) network of the common genes, 20 hub genes were recognized through CytoHubba. Furthermore, functional enrichment and immune cell infiltration analyses were conducted to explore the potential mechanisms of COVID-19-related epilepsy.

RESULTS: We identified a total of 373 common genes between the two diseases. The functional enrichment analysis revealed that the common genes were mainly involved in biological processes related to the immune response. Further analysis of the Hub genes revealed the important role of abnormal lipid metabolism in the crosstalk between COVID-19 and epilepsy. LASSO regression identified CD38 and PRKCA as the potential shared diagnostic candidates, which also exhibited excellent diagnostic value in the validation dataset. The immune infiltration analysis showed that activated dendritic cells (DCs) were positively correlated with the phenotypes of both diseases.

CONCLUSIONS: This research revealed the potential mechanisms of COVID-19-related epilepsy, providing novel insights for the prevention, diagnosis, and clinical management strategies of COVID-19-related epilepsy.

Key Words:
COVID-19, Epilepsy, WGCNA, Hub gene, Protein-protein interaction (PPI).

Introduction

Since the initial identification of the SARS-CoV-2 infection in December 2019, its rapid global spread has led to significant consequences. On March 11, 2020, the World Health Organization (WHO) declared the pandemic of COVID-19, marking it as the most significant public health crisis of the 21st century[1]. COVID-19 typically presents with extrapulmonary symptoms at the beginning[2]. However, as the pandemic progressed, a considerable number of COVID-19 recoveries began experiencing persistent complications, collectively referred to as long COVID[3,4]. Long-COVID is commonly defined as the "post-COVID-19 condition", which is observed in people with SARS-CoV-2 infection, typically manifesting three months after the onset of COVID-19 symptoms and lasting for over two months without an alternative explanation for the symptoms[5]. The clinical presentations of long COVID exhibit

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considerable variation in terms of symptoms, severity, and duration. The condition typically involves impairment of multiple systems, such as respiratory, digestive, nervous, cardiovascular, and hematological[6].

Epilepsy is one of the most common neurological disorders with complex etiologies, such as genetic, infectious, metabolic, and immune, as well as unknown etiologies[7,8]. Currently, there are approximately 70 million patients worldwide with epilepsy, with over 30% of them experiencing inadequate seizure control despite drug treatment[9,10]. Patients with intractable epilepsy usually undergo surgery to mitigate their mortality risks[11]. Existing literatures have identified the correlation between COVID-19 and epilepsy, as well as seizures. A case report[12] in May 2020 described the occurrence of focal status epilepticus as the initial manifestation of a patient with SARS-CoV-2 infection. Several cohort studies focusing on patients with epilepsy (PWE) revealed a significant increase in seizure frequency among PWE following COVID-19 infection compared to their pre-infection state[13,14]. Another meta-analysis on neurological disorders in COVID-19 patients demonstrated that the prevalence and mortality rates of epilepsy among COVID-19 patients were significantly increased, surpassing those of stroke[15]. A comprehensive analysis[16] conducted in October 2022 based on a retrospective cohort encompassing approximately 1.5 million patients diagnosed with COVID-19 revealed an increased risk of new epilepsy or seizure diagnosis during the first six months as well as two years following the initial COVID-19 diagnosis when compared to adequately matched patients with other respiratory infections. These compelling clinical and epidemiological findings all revealed the association between epilepsy and COVID-19.

COVID-19-related epilepsy can arise from a range of pathophysiological mechanisms, including direct neuronal invasion by SARS-CoV-2 or immunity-mediated nervous damage[17]. Seizures commonly manifest as initial symptoms in viral infections of the central nervous system (CNS)[18]. The brain tissues of some individuals with COVID-19 have exhibited neuropathological changes, along with observed SARS-CoV-2 RNA[19]. Current evidence[20,21] indicates that SARS-CoV-2 can access the CNS *via* two possible routes: the olfactory route and the hematogenous pathway. However, it is relatively rare to detect SARS-CoV-2 RNA in the cerebrospinal fluid of COVID-19 patients with neurological symptoms, which suggests that direct neuronal invasion may not be the main mechanism. SARS-CoV-2 infections, even in the absence of neural invasion, can also contribute to seizures. The important role of pro-inflammatory cytokines in the initiation and persistence of epilepsy has previously been widely focused on[22,23]. SARS-CoV-2 infection is typically accompanied by fever and elevated circulating inflammatory cytokines, which can disrupt the blood-brain barrier (BBB) integrity and potentially facilitate the transfer of inflammatory cytokines into the CNS[24]. Systemic inflammatory cytokines could also enter the CNS through the vagal nerve in the gut-brain axis[25]. Furthermore, following SARS-CoV-2 infection, immune cells often become over-activated, leading to increased infiltration of immune cells from the bloodstream into the brain. These immune cells may contribute to neurotoxicity, synaptic dysregulation, and ictogenesis[26,27]. Additionally, fever, hypoxia, and metabolic abnormalities during COVID-19 may also serve as potential mechanisms underlying the condition. Although literature has discussed the possible pathogenesis of COVID-19-related epilepsy, the molecular and biological mechanisms of epilepsy induced by COVID-19 have not yet been fully elucidated.

RNA-seq and microarray sequencing technologies and bioinformatic analysis have been widely utilized to explore the common pathogenesis and interaction between different diseases. WGCNA is an emerging bioinformatic analysis method to identify genes that are highly correlated with the disease phenotype and investigate their biological functions in the pathogenesis of diseases by correlating the gene sequencing data to the clinical phenotype of diseases. This research aimed to identify and elucidate the underlying molecular mechanisms and biological processes underlying COVID-19-related epilepsy through bioinformatic methods and provide new insights into the prevention, early diagnosis, and therapy of COVID-19-related epilepsy.

Materials and Methods

Datasets Collection and Preprocessing

Figure 1 depicts operations for data collection and analysis. The discovery datasets of COVID-19 (GSE196822) and epilepsy (E-MTAB-3123) were obtained from the publicly available GEO (https://www.ncbi.nlm.nih.gov/geo/) and ArrayExpress (https://www.ebi.ac.uk/biostudies/arrayexpress) public databases, respectively. The GSE196822

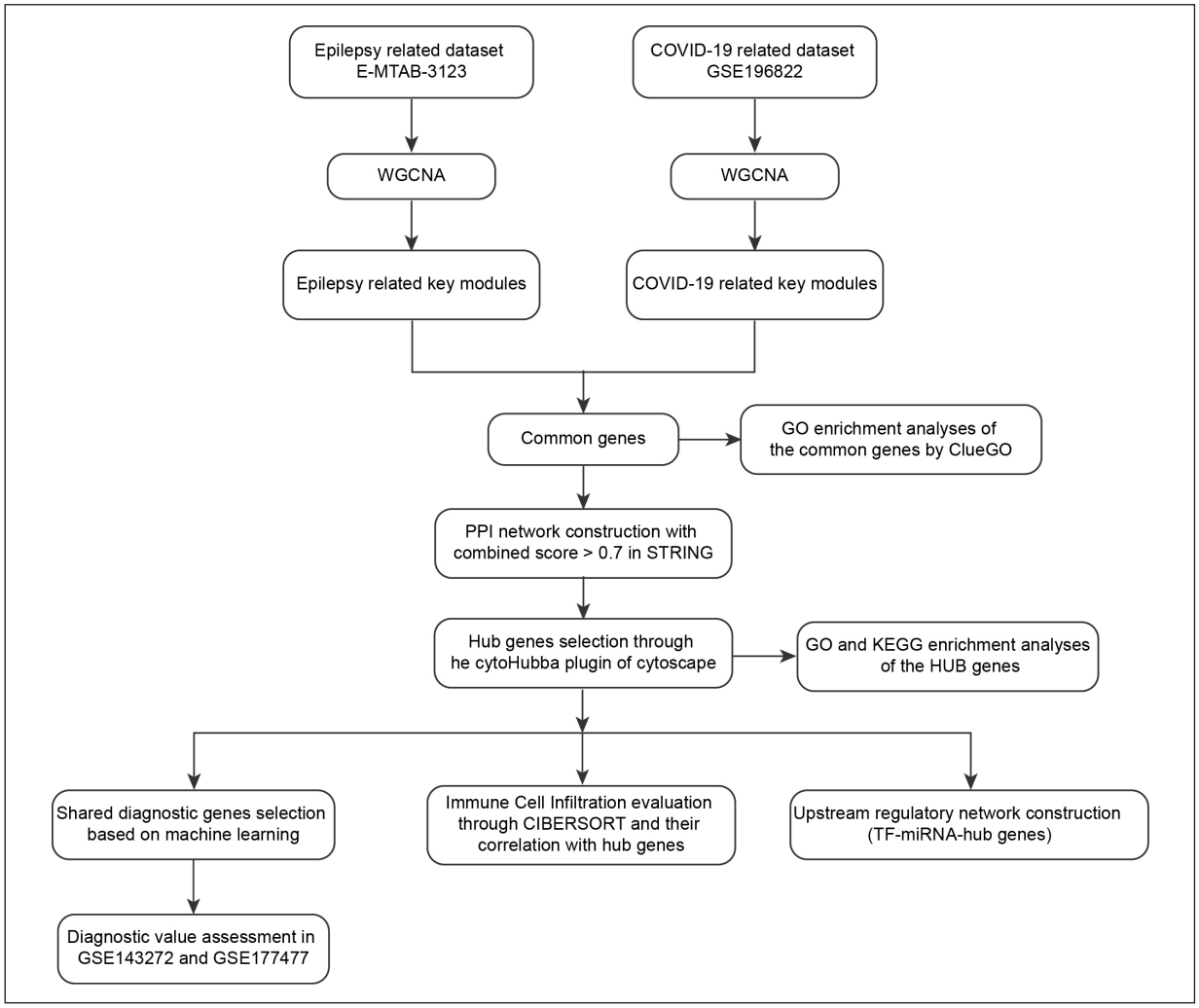

Figure 1. Flow chart for the research design.

dataset comprised the high-throughput sequencing data on mRNA isolated from the whole blood of 34 COVID-19 patients with severity ranging from asymptomatic to severe, six COVID-19 patients with bacterial co-infection, and nine non-COVID-19 healthy controls in South India[28]. The samples were collected from individuals while they were in the hospital or at the clinic. To avoid insignificant transcriptome changes and rule out the effects of bacterial infections on this research, we retained the data of 26 COVID-19 patients with observable symptoms and nine health controls. The dataset was generated using the GPL20301 Illumina HiSeq 4000 (Illumina, San Diego, CA, USA) (Homo sapiens) platform. The normalization of the gene expression count data was carried out by the Voom R function, and then the data was converted into $\log_2$ (CPM).

On the other hand, the E-MTAB-3123 dataset consisted of the microarray sequencing data on RNA isolated from the hippocampal tissue of 24 patients with temporal lobe epilepsy who had undergone therapeutic temporal lobectomy and 23 normal post-mortem controls without epilepsy[29]. The sequencing data were obtained from the Agilent SurePrint G3 Custom 8 × 60 K microarray (Agilent, Santa Clara, CA, USA), which contained all known genes, and the dataset was generated based on the Agilent-039906 custom chip platform (Agilent, Santa Clara, CA, USA). The "read.maimages" function in the limma R package (Bioconductor, Roswell Park Comprehensive Cancer Center, NY, USA) was used to read the raw gene expression data. The background correction and normalization of the data were implemented by the "backgroundCorrect" function in the limma package.

Weighted Gene Co-Expression Network Analysis

The weighted gene co-expression network analysis (WGCNA) is a powerful analytical technique utilized to examine gene expression patterns across multiple samples. This method allows for the identification of gene clusters with analogous expression patterns and enables the exploration of correlations between modules and specific traits or phenotypes[30]. Herein, the WGCNA R package was utilized to construct the gene co-expression modules of COVID-19 and epilepsy. Firstly, the "pickSoftThreshold" function was utilized to define the optimal soft threshold power (β) for constructing the adjacency matrix construction based on the scale-free topology criterion. Subsequently, the adjacency matrix was transformed into a topological overlap matrix through the "TOMsimilarity" algorithm. Hierarchical clustering analysis of the topological overlap matrix, along with the employment of the Dynamic Branch-Cut method, was performed to recognize co-expression modules. Module eigengene (ME) was computed using the "moduleEigengenes" algorithm. Spearman correlation analysis was adopted to determine the link of ME to clinical traits, with $p < 0.05$ and an absolute correlation coefficient (r) > 0.3 identified as statistically significant. Modules with high trait correlation were considered crucial modules, and genes within those modules were selected for further analyses.

Identification of Common and Hub Genes in COVID-19 and Epilepsy

COVID-19-related and epilepsy-relevant modules were intersected to confirm their common genes. In this research, the STRING database (https://cn.string-db.org/) was used to establish a PPI network of the common genes with a score (combined score) > 0.7. This network was subjected to visualization through Cytoscape (version 3.9.1; available at: https://cytoscape.org/). The hub genes (top 20) with the strongest interactions were screened by the Cytohubba plug-in in Cytoscape through the degree topological analysis method.

Functional Enrichment and Pathway Analysis of Hub Genes

To understand the underlying shared pathogenesis between epilepsy and COVID-19, the ClueGO plug-in in Cytoscape and the clusterProfiler R package were adopted for GO and KEGG enrichment analyses on those common genes and hub genes. The GO analysis described the biological functions of the hub genes from biological process (BP), molecular function (MF), and cellular component (CC). Employing ClueGO allowed for the reduction of redundant GO terms while preserving more representative parent or child terms[31]. KEGG analysis elucidated the pathways in which the hub genes were involved. A p-value < 0.05 was considered as the threshold for significant enrichment.

Immune Cell Infiltration Analyses

The characterization of immune cell infiltration in epilepsy and control was accomplished using CIBERSORT (http://CIBERSORT.stanford.edu/). CIBERSORT can transform the expression data of genes into a composition of infiltrating immune cells[32]. By employing the LM22 gene file of CIBERSORT, the components and percentages of 22 infiltrating immune cells in the samples were identified. The Wilcoxon test was performed to identify immune cell populations that exhibited significant differences between disease and control samples. Visualization of the immune cell infiltration results was achieved using the ggplot2 and pheatmap R packages (available at: https://cran.rstudio.com/web/packages/pheatmap/index.html). Additionally, Spearman's rank correlation test with $p < 0.05$ was utilized to explore the link between hub genes and the infiltrating immune cells.

Diagnostic Biomarker Selection and Evaluation

The LASSO is a popular regression method that aids in variable selection and enhances predictive accuracy[33]. A glmnet R package (available at: https://glmnet.stanford.edu/) was adopted for performing the LASSO regression, aiming to identify the optimal diagnostic biomarkers of epilepsy and COVID-19 in the hub genes. Afterward, the candidate biomarkers were externally validated based on the receiver operating characteristic (ROC) curve analysis in separate validation datasets of epilepsy and COVID-19. The validation datasets of COVID-19 (GSE177477) and epilepsy (GSE143272) were obtained from the GEO database. The GSE177477 dataset contained the microarray sequencing data on RNA isolated from the whole blood of 11 patients with mild or severe COVID-19 and 18 healthy controls. The dataset was generated from the GPL23159 Affymetrix Clariom S Assay (Human) platform. The GSE143272 dataset comprised the microarray sequencing data on RNA isolated from the whole blood of 34 drug-free epilepsy

patients, 57 epilepsy patients with epileptic drug therapy, and 50 healthy controls. To rule out the effect of the drug on the blood transcriptome, the drug treatment samples were eliminated. The data were obtained from the GPL10558 Illumina HumanHT-12 platform.

Prediction of Transcription Factors (TFs) and MiRNAs

TFs corresponding to hub genes were forecasted by five databases (ChEA, ENCODE, hTFtarget, TRANSFAC, and TRRUST). TFs that displayed interactions in at least three databases were selected for further analysis. Additionally, five bioinformatics tools (miRMap, miRanda, miRDB, TargetScan, and miTarBase) were applied to forecast the interplay between miRNA and hub genes. Only the miRNAs that were forecasted by all five tools were selected. The Cytoscape (version 3.9.1) was used to establish the TF-miRNA-mRNA regulatory network.

Results

Dataset Information

The research procedure is illustrated in Figure 1. Four datasets were selected, and their key information is summarized in Table I. The E-MTAB-3123 and GSE196822 datasets were undertaken as the discovery cohorts for conducting the WGCNA analysis. Subsequently, the GSE143272 and GSE177477 datasets were used to assess the diagnostic value of the shared diagnostic biomarkers.

WGCNA Identifies Key Modules Associated with Epilepsy and COVID-19

WGCNA was utilized to explore the co-expressed gene modules in the epilepsy and COVID-19 datasets. For the E-MTAB-3123 dataset, a soft threshold power (β) of 18 was determined, forming a scale-free topological index (R^2) of 0.815 (Figure 2A). The network exhibited a scale-free topology distribution, in line with the corresponding model and mean connectivity. The association between gene modules and clinical traits (diseases and health states) was calculated based on Spearman correlation analyses (Figures 2C and 2D). Five modules were found to be greatly associated with epilepsy and were designated as epilepsy-related key modules (the dark olive-green module: r = 0.61, p = 5e-6; the dark-green module: r = 0.55, p = 7e-5; the sky-blue: r = -0.52, p = 2e-4; the midnight-blue module: r = -0.84, p = 2e-13; the sienna3 module: r = -0.65, p = 9e-7). Similarly, the β of 28 and R^2 of 0.749 were set in the GSE196822 dataset (Figure 2B). Among the ten identified modules, the steel-blue module (r = -0.63, p = 4e-5), the pale turquoise module (r = -0.58, p = 3e-4), and the black module (r = -0.39, p = 0.02) exhibited strong correlations with COVID-19 (Figures 2E and 2F). A total of 373 common genes were identified by intersecting the epilepsy and COVID-19-related modules, which were considered to be extremely related to the pathogenesis of both epilepsy and COVID-19 (Figure 3A).

To further explore the molecular mechanisms underlying the pathogenesis of COVID-19-related epilepsy and identify the enriched biological functions of the 373 shared genes, we conducted the GO enrichment analysis using ClueGO (Figure 3B). The results revealed that they were mainly enriched in six biological activities, including "T cell activation", "lymphocyte activation", "regulation of cytokine production", "peptide antigen assembly with major histocompatibility complex (MHC) class II protein complex", "cellular response to chemical stress", and "leukocyte mediated cytotoxicity". Notably, T cell activation accounted for about 50% of total GO terms (**Supplementary Figure 1**). These results indicated that the common genes may participate in the activation of immune response-related biological functions or signaling pathways.

Construction of PPI Network and Determination of Hub Genes

To determine the intercorrelations among the common genes, we constructed a PPI network based on the STRING database. This

Table I. Information of datasets containing the epilepsy and COVID-19 patients.

Disease	Dataset	Platform	Case	Control	Group
COVID-19	GSE196822	GPL20301	34	9	Discovery cohort
Epilepsy	E-MTAB-3123	Agilent SurePrint G3	24	23	Discovery cohort
COVID-19	GSE177477	GPL23159	11	18	Validation cohort
Epilepsy	GSE143272	GPL10558	9	50	Validation cohort

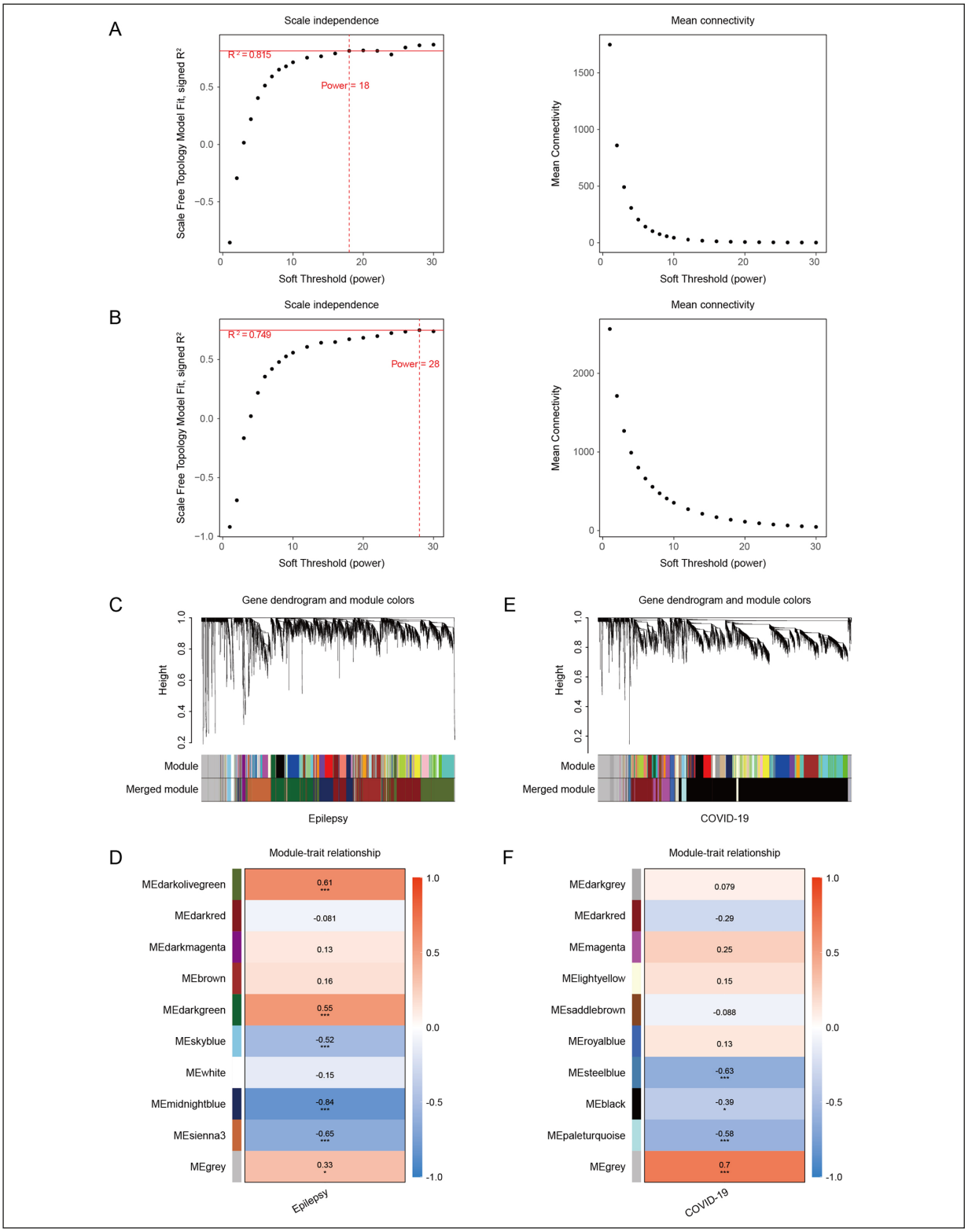

Figure 2. Construction of weighted co-expressed networks in epilepsy and COVID-19 datasets. Selection of soft threshold power (β) in epilepsy (**A**) and COVID-19 (**B**). The left and right results depicted the effect of different β on the scale-free topology fit index and on the mean connectivity, respectively. Cluster dendrogram of genes in epilepsy (**C**) and COVID-19 (**E**), where each color represented one gene module. Heatmaps of module-trait relationships in epilepsy (**D**) and COVID-19 (**F**). (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

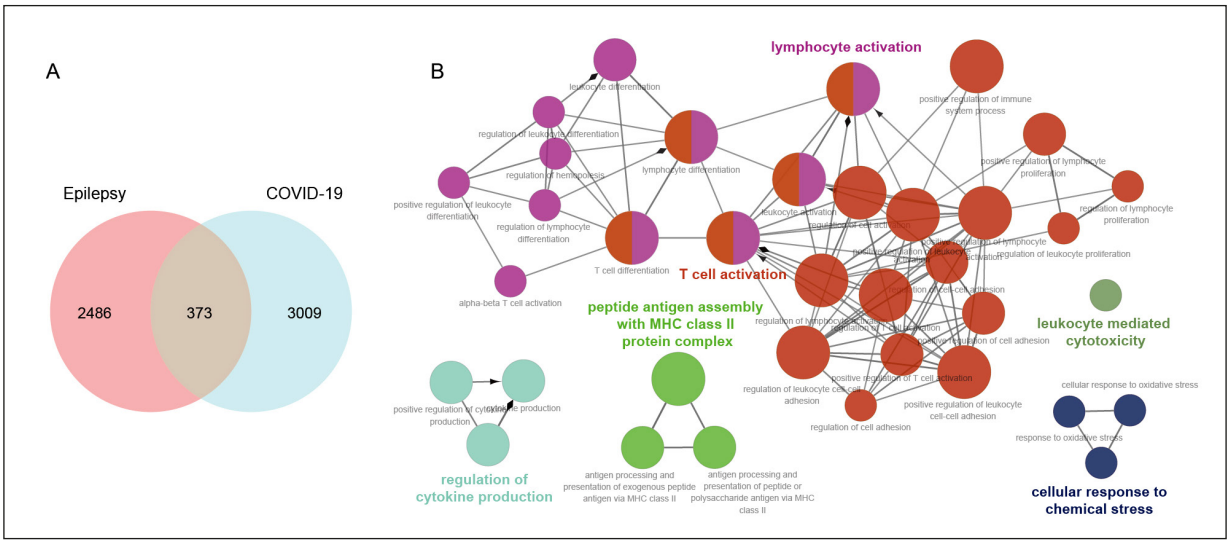

Figure 3. Identification of common genes between epilepsy and COVID-19. **A**, Venn diagram of common genes between the key modules of E-MTAB-3123 and GSE196822 datasets. **B**, GO enrichment analysis of the common genes in ClueGO.

network allowed us to identify the key genes that play essential biological roles[34]. The results revealed that the PPI network contained 185 nodes and 247 interaction pairs (**Supplementary Figure 2**). Next, we analyze the PPI network through the CytoHubba plug-in from Cytoscape and select the hub genes based on the degree algorithms (Figure 4A). The top 20 genes with the strongest interactions were selected as hub genes, which were *PIK3R1*, *HSPA8*, *RBBP7*, *HSPA4*, *NR3C1*, *FCGR3A*, *RPS15A*, *ITPR1*, *DLG1*, *CLTC*, *CD38*, *CCT4*, *PRKCA*, *PIK3C2A*, *ITGAV*, *HLA-DRA*, *HLA-DPB1*, *HLA-DPA1*, *EIF2S1*, and *UBE3A*. The strongest interactions among these genes suggested their importance in the PPI network.

To explore the shared regulatory pathways and biological functions of the hub genes, GO and KEGG analyses were implemented. The GO terms of BPs uncovered that the hub genes were significantly enriched in immune response-activating, antigen processing and presentation, and regulation of cell-cell adhesion. In terms of CCs, these genes were primarily associated with coated vesicles, clathrin-coated vesicles, transport vesicles, endocytic vesicles, and their membranes, as well as lysosomal membranes. For MFs, the genes were predominantly involved in amide binding, peptide binding, phospholipid binding, antigen binding, and binding to MHC proteins (Figure 4B). Notably, "lipid and atherosclerosis" and "Influenza A" are the top ranked KEGG pathway terms, both encompassing six Hub genes (Figures 4C and 4D). Our results imply that the activation of immune response and the abnormalities in lipid metabolism may be present in both diseases.

Immune Cell Infiltration Evaluation and Their Correlation with Hub Genes

In order to provide a more precise characterization of the immune responses in both diseases, we analyzed the components of 22 immune cells in the COVID-19 and epilepsy groups through CIBERSORT. The results revealed that the epilepsy samples were positively correlated with naïve B cells, activated DCs, resting mast cells, resting NK cells, resting CD4 memory T cells, and regulating T cells. While negative links were observed with memory B cells, M2 macrophages, activated mast and NK cells, plasma cells, CD8 T cells, and follicular helper T cells (Figure 5A). For COVID-19 samples, positive correlations were observed with memory B cells, M0 macrophages, activated DCs, neutrophils, and CD8 T cells, while negative relationships were noted with naïve B cells, activated CD4 memory T cells, and CD8 T cells (Figure 5B). Notably, epilepsy and COVID-19 patients exhibited increased activated DCs and reduced CD8 T cells compared to controls. These findings suggested the potential significance of activated DCs and CD8 T cells in the crosstalk between the diseases.

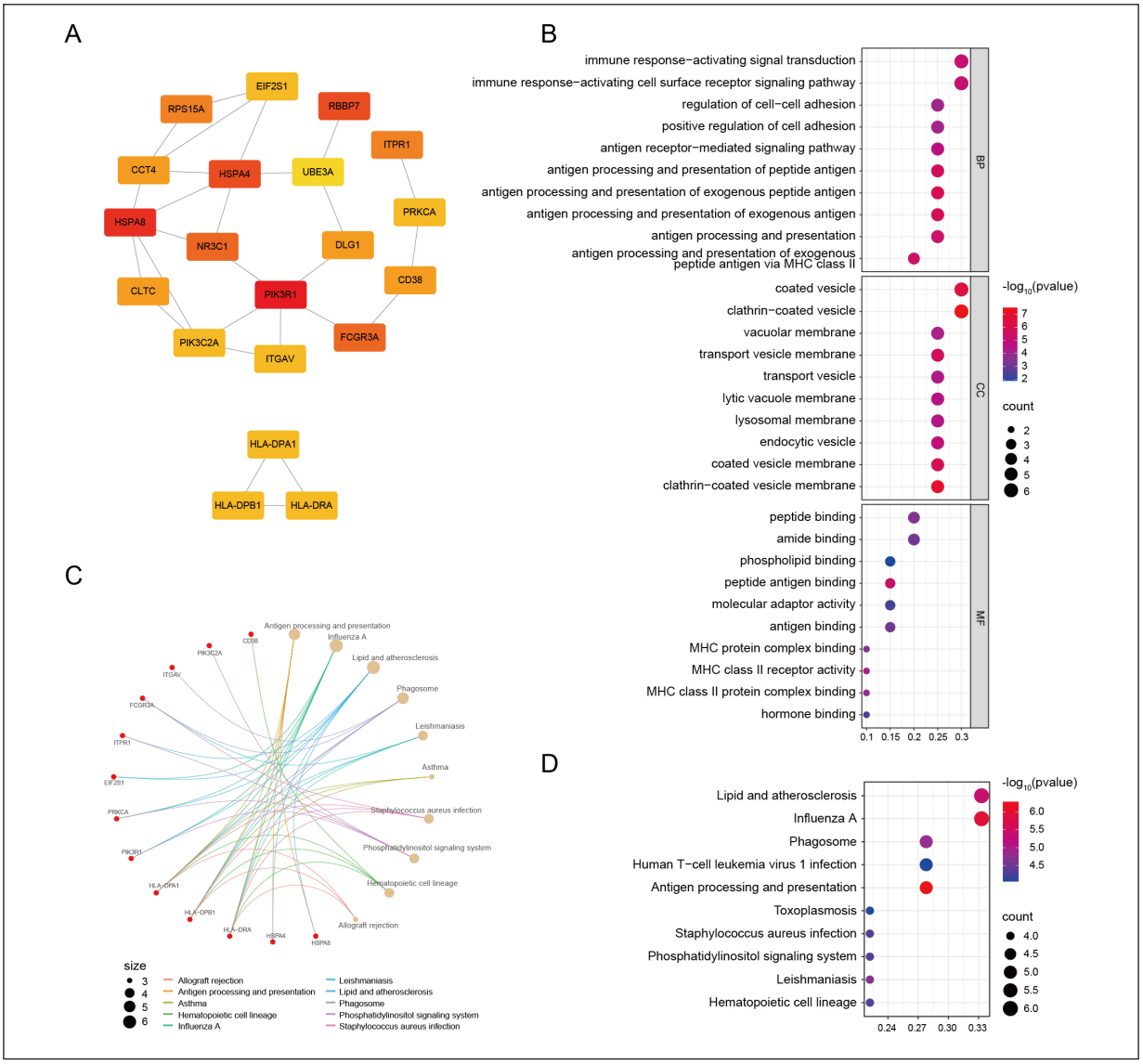

Figure 4. Identification of hub genes and enrichment analysis. **A**, The top 20 hub genes were identified using the degree algorithm using cytoHubba based on the PPI network of the common genes. Bubble plots showed the GO (**B**) and KEGG (**C-D**) enrichment analysis results of the hub genes. Loop graph showed the correlation between the 10 most important KEGG pathways and the enriched hub genes.

Subsequent to that, the association of hub genes with the significantly differential immune cells in epilepsy samples was assessed. The findings demonstrated predominantly positive correlations between most of the hub genes and activated mast cells, activated NK cells, CD8 T cells, and memory B cells. On the other hand, they were negatively linked to resting NK cells, resting CD4 memory T cells, and naïve B cells. (Figure 5C). Our results indicated that these genes may participate in the pathogenesis of epilepsy together with immune cells.

Selection of Potential Shared Diagnostic Biomarkers Based on Machine Learning

Additionally, we constructed the diagnostic models of epilepsy and COVID-19 based on the LASSO algorithm to identify the potential diagnostic biomarkers of both diseases. Nine genes in E-MTAB-3123 and five genes in GSE196822 were identified as potential candidates for epilepsy and COVID-19, respectively (Figures 6A and 6B). Besides, *CD38* and *PRKCA* were found to be overlapping and designated as the common diagnostic biomarkers for epilepsy and COVID-19

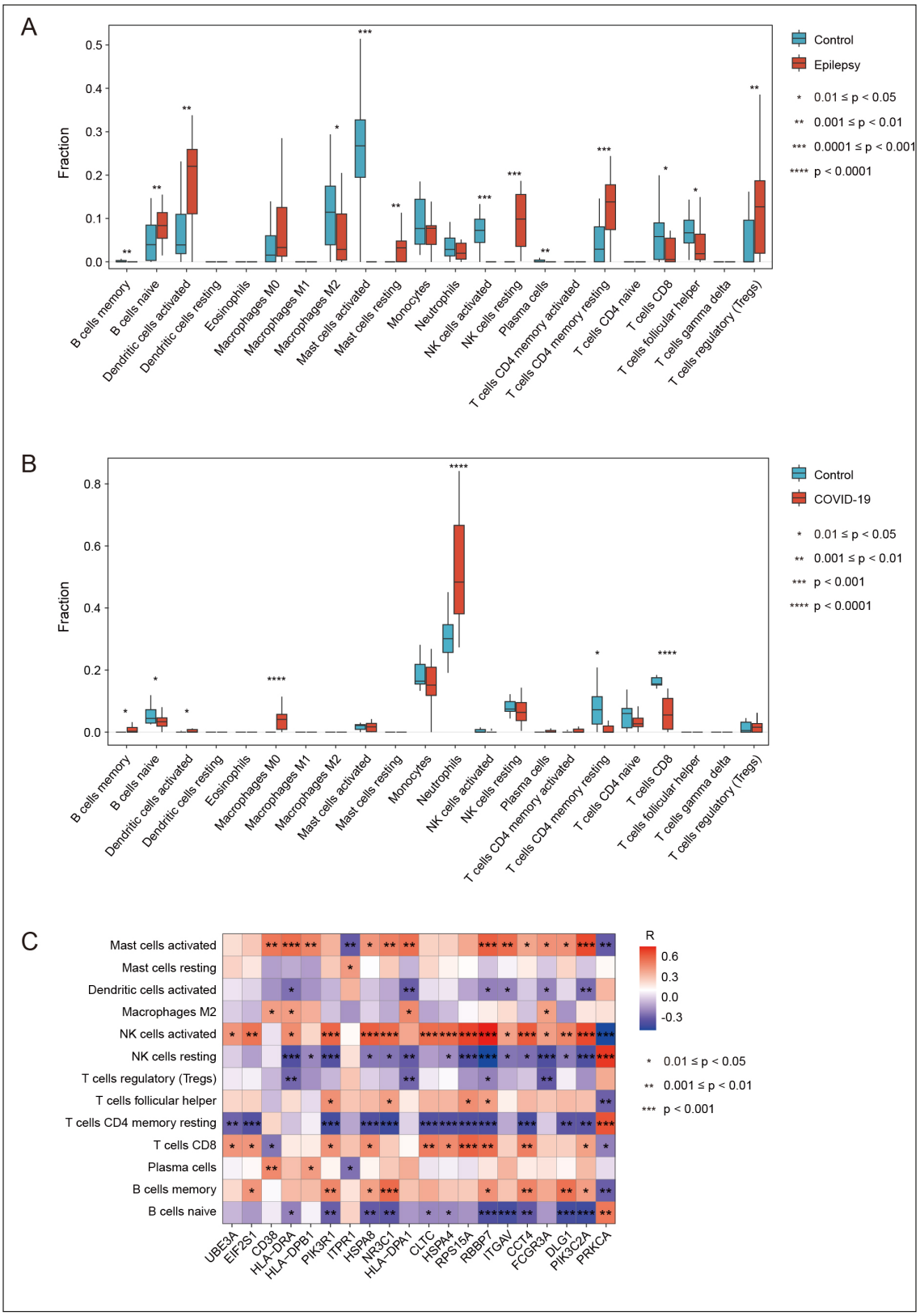

Figure 5. Immune cell infiltration analysis. **A**, The difference for immune cell infiltration between epilepsy and controls in the E-MTAB-3123. **B**, The difference for immune cell infiltration between COVID-19 and controls in the GSE196822. **C,** Spearman correlation analysis between infiltrating immune cells and hub genes in the E-MTAB-3123 dataset.

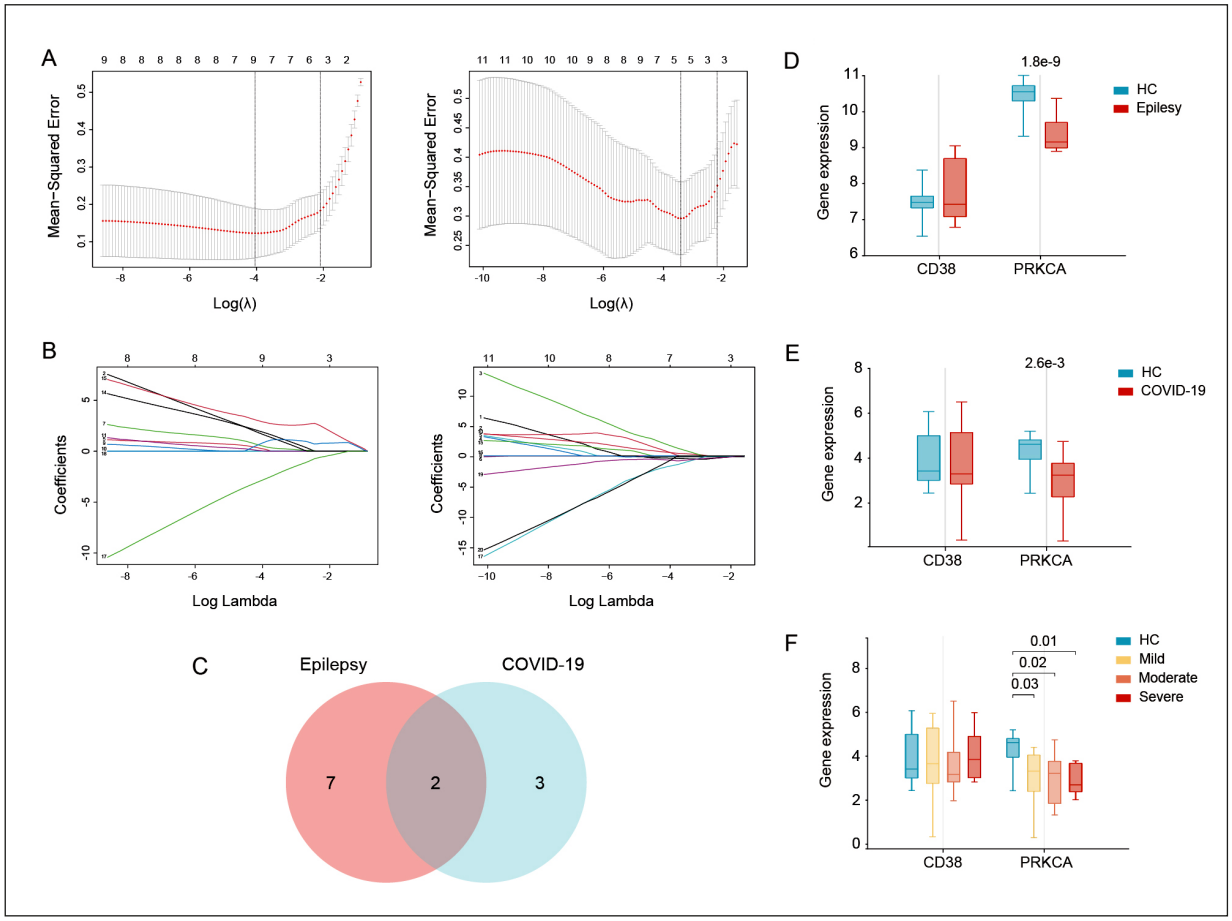

Figure 6. Identification of potential diagnostic biomarkers by the LASSO regression model. Cross-validation selected the optimal lambda value in the E-MTAB-3123 (**A**) and GSE196822 (**B**) datasets, and the LASSO coefficient profiles of the hub genes. **C**, Venn diagram showed two candidate common diagnostic biomarkers in epilepsy and COVID-19. Gene expression of CD38 and PRKCA in E-MTAB-3123 (**D**) and GSE196822 (**E**). **F**, Gene expression in COVID-19 patients with different severities.

(Figure 6C). We then investigated the expression levels of *CD38* and *PRKCA* between disease samples and control samples (Figures 6D and 6E). Compared to the healthy controls, the expression of *CD38* was not significantly different either in epilepsy or COVID-19 samples. However, the expression of PRKCA was significantly downregulated both in epilepsy and COVID-19 samples. We also found that the expression levels of *PRKCA* were correlated with COVID-19 severity. As the COVID-19 severity increased, the expression of *PRKCA* was further decreased (Figure 6F).

Diagnostic Value Assessment of Diagnostic Biomarkers

To assess the diagnostic efficacy of the biomarkers, we established ROC curves to judge the diagnostic specificity and sensitivity of *CD38* and *PRKCA*. In the E-MTAB-3123 dataset, *PRKCA* exhibited a nearly perfect diagnostic value (AUC = 0.951) for epilepsy (Figure 7A). In the GSE196822 dataset, *PRKCA* demonstrated a good diagnostic value (AUC = 0.829) for COVID-19 (Figure 7B). Notably, the combination of *CD38* and *PRKCA* showed the highest diagnostic values in both datasets. To further validate these findings, the diagnostic efficacy was also evaluated using two external datasets. Similar to the previous results, the combination of *CD38* and *PRKCA* demonstrated the highest predictive performance in both the epilepsy dataset GSE143272 and the COVID-19 dataset GSE177477 (Figures 7C and 7D). Similar to the previous results, the combination of *CD38* and *PRKCA* demonstrated the highest predictive performance. Similarly, we checked the expression of the two genes in the

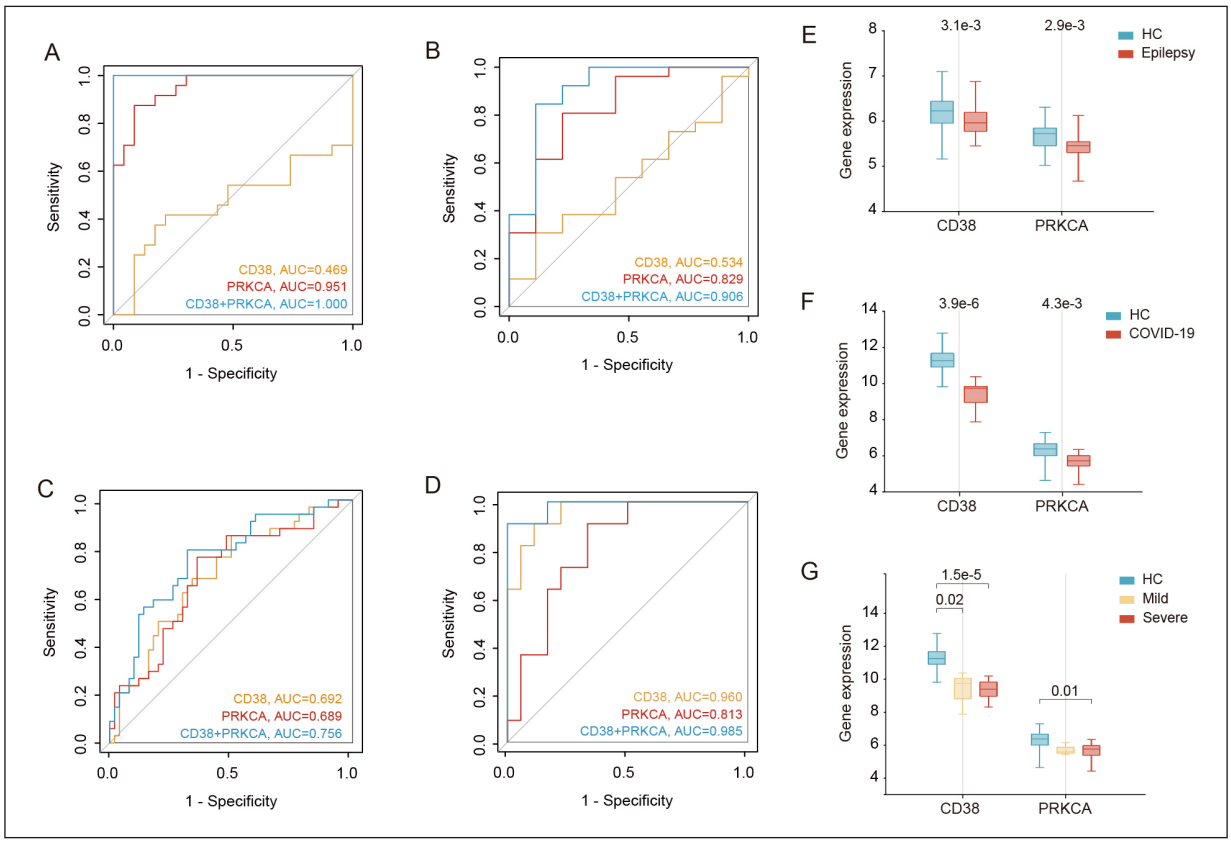

Figure 7. Validation of candidate diagnostic biomarkers. ROC curves of the candidate biomarkers in E-MTAB-3123 (**A**) and GSE196822 (**B**) datasets. ROC curves of the candidate biomarkers in GSE143272 (**C**) and GSE177477 (**D**). Gene expression of CD38 and PRKCA in GSE143272 (**E**) and GSE177477 (**F**). **G**, Gene expression in COVID-19 patients with different severities.

validation cohorts. The results showed that *CD38* and *PRKCA* were significantly downregulated both in epilepsy and COVID-19 samples (Figures 7E and 7F). Moreover, the expression of *PRKCA* in samples with severe COVID-19 was lower than in samples with mild COVID-19 (Figure 7G). Given these results, *CD38* and *PRKCA* may play an important role in the pathogenesis of both diseases.

Construction of Upstream Regulatory Network

We subsequently constructed a TF-miRNA-hub gene regulatory network to identify the TFs and miRNAs, that regulate the hub genes (Figure 8). Using five databases, including ChEA, ENCODE, hTFtarget, TRANSFAC, and TRRUST, 35 TFs that may regulate the hub genes were identified. Noteworthy, CREB1 and TCF3 emerged as important TFs with the highest number of connections to the hub genes. Similarly, other databases were used and the interactions of 16 miRNAs with the hub genes were found. Five hub miRNAs were detected, including hsa-miR-106b-5p, hsa-miR-17-5p, hsa-miR-20a-5p, hsa-miR-20b-5p, and hsa-miR-93-5p.

Discussion

Since the emergence of COVID-19 in 2019, there have been increasing reports of seizures in COVID-19 patients, which creates additional risks for patients[35,36]. Several theoretical mechanisms have been proposed to explain this correlation, such as fever during infection, neuroinvasiveness of SARS-CoV-2, elevated circulating cytokines, destruction of the BBB, and hyperactivated immune cells[37-41]. However, the intricate molecular mechanisms underlying the interaction between epilepsy and COVID-19 have not been completely elucidated. This research investigated the common genes and shared signatures of epilepsy and COVID-19 using bioinfor-

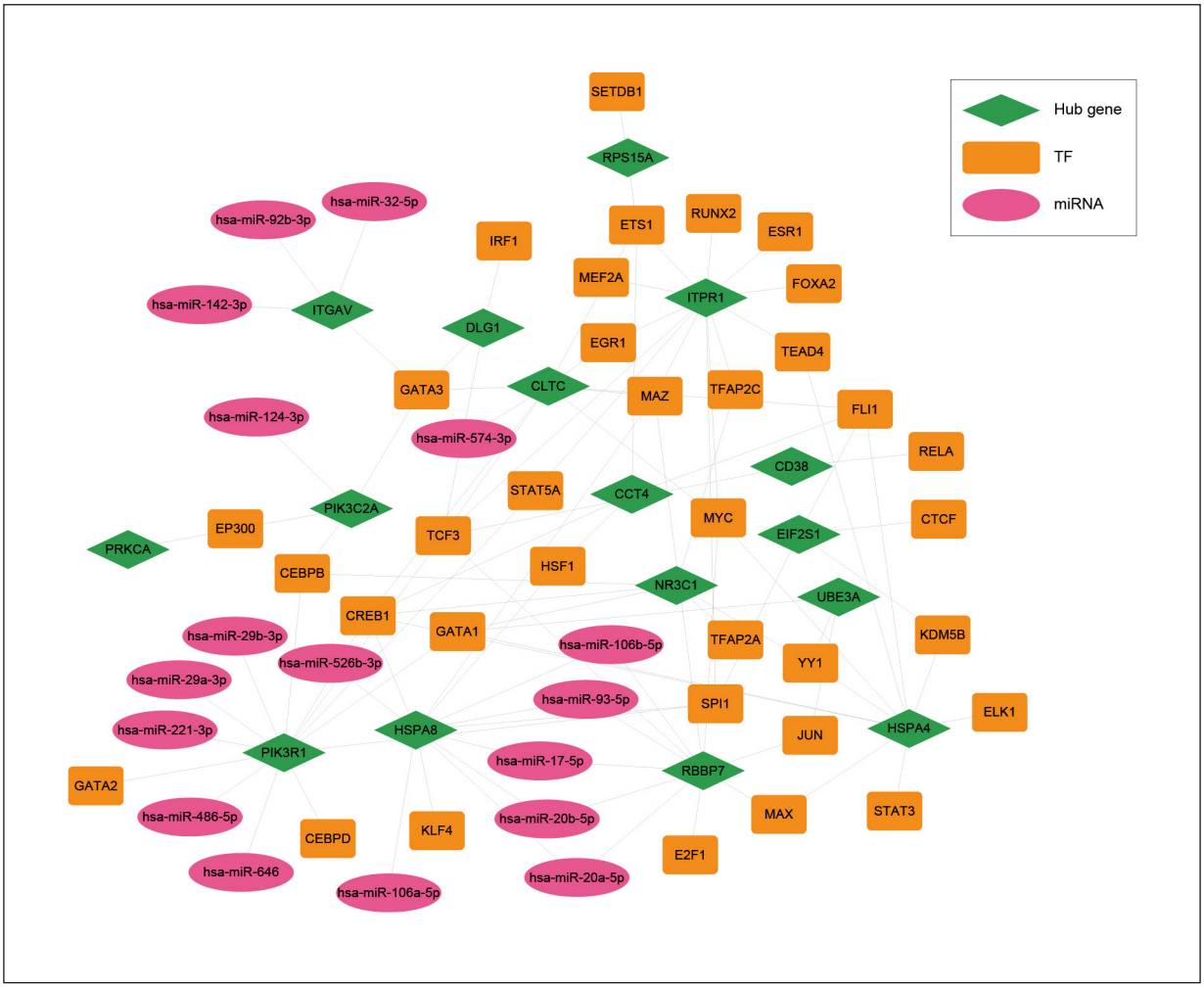

Figure 8. Construction of the TF-miRNA-hub gene regulatory network.

matic methods. We identified 20 hub genes and two diagnostic biomarkers (*CD38* and *PRKCA*) that were significantly related to epilepsy and COVID-19. We also found that the abnormal lipid metabolism pathways and immune responses may be closely related to the common pathogenesis of epilepsy and COVID-19. The hub genes and potential molecular mechanisms identified in this study provide new insights to facilitate early detection and improve therapeutic strategies for COVID-19-related epilepsy.

In this research, we identified a total of 373 common genes between epilepsy and COVID-19. Further analysis indicated that they are mainly enriched in immune response-related biological processes, which suggests the potential importance of dysregulated immune responses in the co-pathogenesis of epilepsy and COVID-19. SARS-CoV-2 infections may induce excessive immune responses and inflammatory responses, especially in COVID-19 patients with severe symptoms. Banerjee et al[28] indicated that the dysregulated immune response in COVID-19 patients may be assumed to be in two stages. In the early stages of SARS-CoV-2 infections, the virus inhibites the immune responses to allow its replication. Subsequently, it enters the second stage, where the viruses that were not cleared in the early stages replicate greatly, resulting in delayed and overactivated immune responses[42]. When the immune responses are overactivated, multiple activated immune cells and excessive amounts of inflammatory cytokines may break the integrity of the BBB, allowing them to cross the damaged brain barrier and then enter the central nervous system, which causes neurotoxicity. Moreover,

inflammatory cytokines and brain inflammation can also lead to the overexcitation of neurons and promote astrogliosis, which enhances epileptogenesis[43].

Further PPI analysis screened 20 hub genes, including *PIK3R1*, *HSPA8*, *RBBP7*, *HSPA4*, *NR3C1*, *FCGR3A*, *RPS15A*, *ITPR1*, *DLG1*, *CLTC*, *CD38*, *CCT4*, *PRKCA*, *PIK3C2A*, *ITGAV*, *HLA-DRA*, *HLA-DPB1*, *HLA-DPA1*, *EIF2S1*, and *UBE3A*. These hub genes are the central nodes of the PPI network, and they are also strongly correlated with the phenotypes of both diseases. Previous studies in literature have shown that some of the hub genes can be involved in the pathogenesis of both diseases separately. Therefore, it is speculated that they may play an important role in the crosstalk between epilepsy and COVID-19. As is well known, *PIK3R1* and *PIK3C2A* are classical members of PI3Ks that activate the PI3K/Akt/mTOR pathway, which is crucial in the regulation of various cell functions. This pathway has been confirmed[44] to be activated in COVID-19 patients, while its inhibitors could exert antiviral effects. The PI3K/Akt/mTOR pathway was also upregulated in the brain tissues of epilepsy patients[45]. Inhibiting the mTOR signaling pathway could effectively reduce seizure frequency[46]. *HAPA4* and *HSPA8* are genes encoding heat-shock proteins; they play protective roles in preventing protein misfolding and cellular metabolic stresses; they are also involved in antigen presentation and act as cytokines that induce the production of proinflammatory cytokines and promote DC maturation[47]. The heat-shock proteins (HSPs) can also exert anti-inflammatory activities and dissipate inflammatory states. The inhibited heat shock responses in severe COVID-19 patients may lead to a poor prognosis. Besides, the imbalanced production of HSPs in the cerebral microenvironment may contribute to various neuropathies. *CLTC* is a component of clathrin, which encodes clathrin heavy chain 1. Clathrin could form a curved envelope and cover the vesicle cytoplasmic face, which was coated by clathrin. These special organelles are linked to intracellular trafficking of receptors and endocytosis of various macromolecules[48]. CTLC plays an important role in neuronal transmission by regulating the vesicular circulation and neurotransmitter release in the brain[49]. A previous study[50] indicated that the *CLTC* variants could constitute different brain disease phenotypes. The disruption of ubiquitin-protein ligase E3A (UBE3A) could result in Angelman syndrome (AS), which is characterized by intellectual disability, delayed development, and seizures[51]. In mouse models of AS, UBE3A has been demonstrated to degrade the ubiquitin-mediated big potassium channels to inhibit neuronal hyperexcitability, thereby improving susceptibility to epilepsy[52]. Notably, we found three hub genes that encode human MHC, also known as human leukocyte antigens, including *HLA-DRA*, *HLA-DPB1*, and *HLA-DPA1*. Previous studies[53] showed that HLA antigens and haplotypes, such as *HLA-DR4*, *HLA-DR7*, and *HLA-DQ2*, are associated with different types of epilepsies. They may play a role in different types of epilepsies. HLA molecules were also critical for antigen presentation to T cells and mediating immune responses during SARS-CoV-2 infection[54]. These findings have shown that some of the hub genes identified in this study had potential correlations with the pathogenesis of COVID-19 and epilepsy. Further and thorough research on these hub genes may help develop effective treatments for epilepsy induced by COVID-19.

Our results showed that most of the hub genes are enriched in the lipid and atherosclerosis pathway, which suggested that the abnormal lipid metabolism, coagulation, and inflammatory response may play a crucial role in the crosstalk between COVID-19 and epilepsy. An observational prospective study revealed the importance of lipids during COVID-19, which was consistent with our results. They indicated that during long-term follow-up, COVID-19 patients with high lipid peroxidation (LPO) at hospital admission showed an atherogenic lipid profile[55]. In the condition of SARS-CoV-2 infection, the virus can directly damage vascular endothelial cells, which leads to a coagulation cascade[56]. The expression of proinflammatory cytokines in the early SARS-CoV-2 infection may also lead to the activation of coagulation pathways and induce further overproduction of inflammatory cytokines[57]. Moreover, the suppressed fibrinolytic system and damaged endothelium could result in the activation and aggregation of platelets, which accelerate microthrombosis and affect the immune system[58]. On the other hand, SARS-CoV-2 could infect tissues containing megalin and interact with angiotensinogen and Ang II through megalin and ACE2, which increase the levels of cholesterol[59]. When the vascular barrier is damaged, the vascular cells are exposed to excessive lipids, such as low-density lipoprotein (LDL), that promote the accumulation of lipids in the intima. Endothelial injury also contributes

to the increased permeability of the endothelium. The circulating monocytes subsequently adhere to the injury endothelia and then differentiate into macrophages, which actively uptake the lipids through phagocytosis[60]. The macrophages release tissue factors, which could bind to coagulation factor VII and then form blood clots. The accumulation of lipids and cholesterol, together with other cellular debris in the arterial wall, results in the formation of plaque. The instability and degradation of the plaques caused their release into the bloodstream, which was subsequently transported into the brain tissues[61].

The CNS is the organ with the highest concentration of lipids except for adipose tissues, which indicates that lipid metabolism is crucial for the stability of the CNS. Abnormal lipid metabolism may lead to damage to the CNS and the occurrence of diseases. The abnormal accumulation of lipids in the epileptogenic focus is one of the main pathological features of drug-resistant epilepsy. The excessive accumulation of lipids induces the transformation from astrocytes to lipid-accumulated reactive astrocytes (LARAs), which exert an ability to be proepileptic through adenosine A2A receptors[62]. Moreover, the expression of apolipoprotein E (ApoE) is significantly upregulated in LARAs. ApoE could increase the expression of βAPP and the release of sAPP, which promotes the activation of glia and the overexcitation of neurons[63]. Therefore, it was speculated that the dysregulated lipid metabolism and blood coagulation during COVID-19 may play a crucial role in the pathogenesis of COVID-19-related epilepsy.

Our results identified the combination of *CD38* and *PRKCA* as potential diagnostic biomarkers of epilepsy and COVID-19. This result was obtained in two validated cohorts. The deficiency of nicotinamide adenine dinucleotide (NAD^+) is an important pathological factor in various diseases related to the CNS[64]. *CD38* is a key regulator of degrading NAD^+ in the hippocampus during epileptogenesis. The production of *CD38* has been identified[65] to be increased in the hippocampus of epilepsy rats, while *CD38* inhibitors could reduce the tonic-clonic seizure severity and seizure duration. However, another study[66] indicated that *CD38* was essential for nerve cells to resist oxidative damage. The decreased *CD38* expression was found able to exacerbate the death of astrocytes induced by oxidative damage[66].

The direct roles of *PRKCA* in the pathogenesis of neurological diseases have not been fully elucidated yet. We hypothesized that *PRKCA* may impact the CNS by regulating the signaling pathways that it is involved in, such as the MAPK signaling pathway, PI3K/Akt/mTOR pathway, NF-κB, and PI3K-Akt signaling pathway.

Our results also showed that activated DCs were positively associated with both diseases, suggesting an important role of dendritic cells in the pathogenesis of epilepsy and COVID-19. DCs are the most efficient antigen-presenting cells. They also play a crucial role in activating immune responses and establishing connections between innate and adaptive immune responses[67]. During SARS-CoV-2 infection, when the DCs were activated by the pathogen, they secreted proinflammatory cytokines and expressed surface molecules, such as MHC and costimulatory molecules, and induced innate and adaptive immune responses. The number of activated DCs was found[68] to be positively correlated with COVID-19 severities. DCs are also present in the brain and respond to pathogens such as viruses or bacteria and to interferons or cytokines that were produced during inflammation or injury[69,70]. Activated DCs mobilized effector T-cells to provide protection. However, the T cells that enter the CNS could also cause tissue damage under certain conditions[71].

Limitations

This research aimed to investigate the common pathogenesis between epilepsy and COVID-19. Nevertheless, it is vital to recognize the limitations of our study. First, the specificity of the epilepsy patients prevented us from including more samples in our work. Second, the diagnostic value of the combination of *CD38* and *PRKCA* needs to be further verified in future clinical trials.

Conclusions

In conclusion, this research proposes the potential common pathogenetic mechanisms between epilepsy and COVID-19. We identified the common genes and biological processes of these two diseases, which helped to better understand their connections. The hub genes, especially *CD38* and *PRKCA*, may be promising prevention and diagnostic targets for COVID-19-related epilepsy.

Conflict of Interest

The authors declare that they have no conflict of interests.

Acknowledgements
The authors thank all the contributors of the gene expression profiling datasets used in this study for making it available publicly..

Authors' Contribution
Conceptualization: Y.L. and C.J.; Transcriptomic analysis: Y.L.; Investigation: Y.L.; Methodology: Y.L.; Software: Y.L.; Supervision: L.Y. and C.J.; Writing original draft: Y.L.; Writing - Review and editing: L.Y., T.J. and C.J. All authors have read and agreed to the published version of the manuscript.

Funding
This research was funded by the National Natural Science Fund of China (No. 32070932, 32170944); the Science and Technology Development Plan Project of Jilin Province (20220402044GH); the Science Research Project of the Education Department of Jilin Province (JJKH20211056KJ).

Data Availability
The datasets used and analyzed during the current study are available from the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

Ethics Approval and Informed Consent
Not applicable.

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