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Preliminary exploration of the co-regulation of Alzheimer's disease pathogenic genes by microRNAs and transcription factors

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Background: Alzheimer's disease (AD) is the most common form of age-related neurodegenerative disease. Unfortunately, due to the complexity of pathological types and clinical heterogeneity of AD, there is a lack of satisfactory treatment for AD. Previous studies have shown that microRNAs and transcription factors can modulate genes associated with AD, but the underlying pathophysiology remains unclear.

Methods: The datasets GSE1297 and GSE5281 were downloaded from the gene expression omnibus (GEO) database and analyzed to obtain the differentially expressed genes (DEGs) through the "R" language "limma" package. The GSE1297 dataset was analyzed by weighted correlation network analysis (WGCNA), and the key gene modules were selected. Next, gene ontology (GO) and Kyoto encyclopedia of genes and genomes (KEGG) pathway enrichment analysis for the key gene modules were performed. Then, the protein-protein interaction (PPI) network was constructed and the hub genes were identified using the STRING database and Cytoscape software. Finally, for the GSE150693 dataset, the "R" package "survival" was used to integrate the data of survival time, AD transformation status and 35 characteristics, and the key microRNAs (miRNAs) were selected by Cox method. We also performed regression analysis using least absolute shrinkage and selection operator (Lasso)-Cox to construct and validate prognostic features associated with the four key genes using different databases. We also tried to find drugs targeting key genes through DrugBank database.

Results: GO and KEGG enrichment analysis showed that DEGs were mainly enriched in pathways regulating chemical synaptic transmission, glutamatergic synapses and Huntington's disease. In addition, 10 hub genes were selected from the PPI network by using the algorithm Between Centrality. Then, four core genes (TBP, CDK7, GRM5, and GRIA1) were selected by correlation with clinical information, and the established model had very good prognosis in different databases. Finally, hsa-miR-425-5p and

hsa-miR-186-5p were determined by COX regression, AD transformation status and aberrant miRNAs.

Conclusion: In conclusion, we tried to construct a network in which miRNAs and transcription factors jointly regulate pathogenic genes, and described the process that abnormal miRNAs and abnormal transcription factors TBP and CDK7 jointly regulate the transcription of AD central genes GRM5 and GRIA1. The insights gained from this study offer the potential AD biomarkers, which may be of assistance to the diagnose and therapy of AD.

KEYWORDS

Alzheimer's disease (AD), differentially expressed genes (DEGs), COX regression, transcription factors, miRNAs

Introduction

Alzheimer's disease (AD) is the most common and complex neurological disease in the world. Neurological conditions such as AD are the leading cause of 70% of dementia worldwide (Mayeux and Stern, 2012). Global Alzheimer's cases are increasing year by year and are expected to reach 78 million by 2030. While the pathogenesis of the disease is not yet known, many believe the accumulation of over-phosphorylated tau and A β plaques in neurofibrillary tangles are the main causes of AD (Andreasen et al., 1999; Shao et al., 2011; Arvanitakis and Bennett, 2019). When extracellular amyloid plaques build up in specific areas of the brain, they can lead to amyloid vascular disease or neurodegenerative diseases (Thanvi and Robinson, 2006; Jäkel et al., 2022). The neurofibrillary tangles (NFTs) are huge paired intracellular helical strands of hyperphosphorylated tau proteins, which induce neuronal and synaptic loss (Moloney et al., 2021). The predominant regions of the pathological process underlying AD in human brain are the association areas of the cerebral cortex and the hippocampus (Wang et al., 2010). Despite these extensive findings on both, there are few effective drugs to improve and treat AD. Worse, many patients have to wait a long time to be diagnosed with the disease. During this period, the A β burden is significant and memory loss has already occurred (Arvanitakis et al., 2019; Long and Holtzman, 2019). Typical AD has gone through a gradual and hidden development process, and there is no specific detection method, which cannot be accurately diagnosed, difficult to cure, difficult to control, and poor prognosis. Therefore, it is urgent to explore new potential biomarkers for early diagnosis and effective treatment of AD.

Alzheimer's disease is the most common neurodegenerative disorder with limited therapeutics, and AD is characterized by the formation of plaques made by protein aggregates. Mounting studies have suggested that targeting transcription factors holds promise for treating neurodegenerative disorders

including AD (Ping Yang et al., 2022). CMV promotor-driven transcription factor EB (TFEB), for example, is injected with adeno-associated viral particles in targeted mice, which are mainly localized in neuronal nuclei and upregulated lysosomes. This resulted in decreased steady state levels of A β in APP proteins and cerebral stromal liquid (Xiao et al., 2015; Song et al., 2020). A growing body of evidence also suggests that the accumulation of misfolding proteins in AD is caused by damage to macromolecular autophagy and autophagy lysosomal pathways, making TFEB, which regulates autophagic lysosomal pathway, a promising target for AD treatment (Zheng et al., 2021). We also found decreased expression of the transcription factor Nrf2 and its NQO1, HO-1, and GCLC driver genes, and changes in related Nrf2 pathways in AD brains. Nrf2 activation may provide cellular protection and prevent an increasing number of diseases including neurodegenerative diseases. These avenues of evidence point to the activation of the Nrf2 transcription factor as a potential new therapeutic scheme for AD (Osama et al., 2020). Additional research shows that ATF6 transcription factor activation can attenuate amyloidosis through BACE1 downregulation, and concomitantly ATF6 overexpression significantly reduces A β 1-42. The results of this study suggest ATF6 may become a potential focus for targeted therapy of AD. Interestingly, TBP and CDK7 also belong to the basal transcription factor family like ATF6 among the 10 key genes selected in our study (Geng et al., 2016; Du et al., 2020).

Recently, progress has begun to clarify the physiological and pathological roles of non-coding RNAs (ncRNAs) in a variety of diseases, including cancer. ncRNAs do not have protein coding functions, but are important regulators of many cellular processes (Esteller, 2011). Of these, miRNAs are the most studied and have become key players in the pathogenesis of AD involved in regulating key growth regulatory pathways (Swarbrick et al., 2019; Toden et al., 2021). miRNAs are small endogenous non-coding RNA molecules that can inhibit or silence the expression of post-transcription genes.

Many miRNAs play an important role in post-transcriptional regulation and are highly conserved (Sand et al., 2009). Previous research has found that numerous miRNAs, including miRNA-126a-3p, MicroRNA-455-3p, miR-501-3p, and miRNA-101a-3p, have a role in the pathogenesis of AD, showing that miRNAs are strongly linked to the development of AD (Hara et al., 2017; Kumar et al., 2017; Kumar and Reddy, 2019; Lin et al., 2022; Xue et al., 2022). However, the specific role and potential mechanisms of miRNA in AD pathogenesis are unclear.

We here established a miRNA-mRNA, TF-mRNAs regulatory network by integrating relevant TFs (transcription factors), miRNAs and mRNAs to try to gain further insight into the potential functions of ncRNAs as well as transcription factors in AD. This study may provide a better understanding of the underlying pathogenesis of AD and potentially new biomarkers for diagnosis and treatment of AD.

Materials and methods

Gene expression profile data collection

The gene expression datasets used in this investigation were collected from the gene expression omnibus (GEO) database.¹ This database generated a total of 340 datasets on human AD. After a careful review, four gene expression profiles (GSE5281, GSE1297, GSE122063, and GSE150693) were selected. Among them, GSE5281 was based on the GPL570 platform ([HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array), GSE1297 was built on GPL96 Affymetrix Human Genome U133A Array. All data were freely accessible online. The GSE5281 expression profile consists of 161 samples and approximately 55,000 transcripts from 74 disease-free patients and 84 patients with AD. There are 31 samples in the GSE1297 dataset, including 9 normal and 22 AD subjects with different severities. The correlation between each gene expression and clinical information of the 31 subjects was verified by the Neurofibrillary Entanglement Score (NFT) and the Minimental Examination (MMSE) (Blalock et al., 2004; Liang et al., 2007). GSE122063 is a gene expression profiling in frontal and temporal cortices from 36 patients with vascular dementia, 56 diseases (AD), and 44 non-demented controls (Control) obtained from the University of Michigan. Brain Bank Mild cognitive impairment (MCI) is a precursor to the development of AD. The GSE150693 data were downloaded from the GEO database from the GPL21263 platform, among them 197 miRNA samples from MCI sera included 83 patients who were converted from MCIs to AD, and 114 patients who did not convert from MCI to AD (Shigemizu et al., 2020).

¹ <https://www.ncbi.nlm.nih.gov/geo/browse/>

Application of weighted correlation network analysis algorithm

First, the GSE1297 sample data obtained from the GEO database was processed by using the log scale robust multi-array analysis (RMA). Then the missing values in the samples were filled by using the K-neighborhood algorithm to improve the accuracy and usability of the data. Next, expression relationships among genes were measured by correlation coefficients to construct weighted co-expression networks in the absence of outlier samples through cluster analysis of gene samples. Based on this, gene modules were identified by using a clustering algorithm as well as a dynamic shear tree algorithm. The calculated correlation coefficients between gene modules and clinical performance traits were used as the association criteria. Using the Pearson correlation test can analyze the relationship between module eigengenes and clinical features, and select the modules for focused mining. Among them, clinical information includes: Age, age at death; NFT, neurofibrillary tangle count; Braak, Braak stage; MMSE, adjusted Minimental Status Exam; PMI, postmortem interval. Finally, the key modules were further mined to find the key core genes.

Functional classification and pathway enrichment of differentially expressed genes

The above differentially expressed genes (DEGs) screened from the weighted correlation network analysis (WGCNA) were submitted to gene ontology (GO) function enrichment analysis, which was consisted of cellular component (CC), biological process (BP), and molecular function (MF), and was analyzed and visualized by using the R language “cluster profile,” “GGplot2,” and “enrichPlot” packages. Kyoto encyclopedia of genes and genomes (KEGG) pathway enrichment analysis of DEGs in this study was performed by the Database for Annotation, Visualization, and Integrated Discovery (DAVID) tools.² KEGG pathway visualization was analyzed by Sangerbox online pathway analysis tool (Shen et al., 2022). Enriched GO terms with adjusted value $P < 0.01$ and gene number > 12 were selected, and $P > 0.05$ of KEGG pathways and gene numbers > 5 were considered statistically significant.

Analyzing differential expression

DEGs are processed using the “R” language “limma” package and calculate adjusted P values and $|\log_2 FC|$. For GSE5281 and GSE97760 gene expression profiles, \log_2 Fold Change (FC) $|\log_2 FC| > 1.0$ and adjusted P-values < 0.05 were selected as cut-off criteria.

² <https://david.ncifcrf.gov/>

Protein-protein interaction network and module analysis

WGCNA was used to directly set thresholds for gene saliency (GS) and module feature (MM). Based on $|MM| > 0.8$, $|GS| > 0.1$ threshold value for the standard to filter core genes, 269 key genes were selected from 789 genes in the “midnightblue” module. To explore the relationship between these 269 hub genes and MAPT (AD is defined by the presence of extracellular and intracellular neurofibrillary tangles composed of hyperphosphorylated tau proteins) and AD disease (Corbo and Alonso Adel, 2011; Bakota and Brandt, 2016; Chong et al., 2018), the Search Tool was used for the Retrieval of Interacting Genes (STRING version 11.5) for known or predicted, direct (physical) and indirect (functional) PPIs (Szkarczyk et al., 2015). The interaction confidence score of >0.4 was used as the cutoff value for statistical significance. Subsequently, the PPI network was visualized by Cytoscape3.9 software.³ To better search for proteins related to Tau protein (MAPT), the algorithm between Centrality was adopted in this study to find the important nodes [the calculation formula is (1) and (2)].

We used CytoHubba, a plugin in Cytoscape, to compute nodes in the protein network that efficiently convey data. In our study, the top 10 genes were identified as central genes.

$$C_B(N_i) = \sum_{k,j=1}^g sd(k, i, j), (k \neq j) \quad (1)$$

$sd(k, i, j)$ indicates that the shortest path from k to j passes through i , which means, i is on the shortest path from k to j .

$$C'_B(N_i) = \frac{C_B(N_i)}{\sum_{k,j=1}^g (k, j)}, (k \neq j) \quad (2)$$

The formula can be standardized to obtain (2), where the denominator represents the number of paths between two points in the figure, that is, the number of all paths.

Gene set enrichment analysis

For Gene Set Enrichment Analysis (GSEA), we obtained data from GSEA (DOI: 10.1073/pans.0506580102)⁴ website for the GSEA software (version 3.0), the samples were divided into high expression group ($\leq 50\%$) and low expression group ($< 50\%$) according to the expression level of GRM5 and GRIA1. Obtained from the Molecular Signatures Database (DOI: 10.1093/bioinformatics/btr260),⁵ a subset of “c3.mir.v7.4.symbols.gmt” and “c3.tft.v7.4.symbols.gmt” was downloaded to evaluate related pathways and molecular mechanisms, based on gene expression profile and phenotype

grouping. The minimum gene set was 5. The maximum gene set was 5,000, 1,000 resampling, P -value < 0.05 and FDR < 0.25 were considered statistically significant, to verify the binding relationship of miRNAs and transcription factors with the target genes, and to indicate that they are on the relevant biological pathway.

MicroRNAs associated with hub

Genes

The ENCORI (The Encyclopedia of RNA Interacts) database is used to construct Hub genes and target miRNA interactions with them. We then used the “R” software package “Survival” to integrate data on survival, AD transformation status, and 35 features, and evaluated the prognostic significance of these features in 197 samples by Cox and selection of key microRNAs (miRNAs). Finally, these central genes and miRNA networks were mapped using Cytoscape software.

The least absolute shrinkage and selection operator logistic regression algorithm model was constructed

Here, we used the R package “glmnet” to integrate AD status and gene expression data from GSE5281 database for regression analysis using least absolute shrinkage and selection operator (Lasso)-Cox. Tenfold cross validation was performed to obtain the optimal model. We set the Lambda value to 0.00208765767322578 and finally obtained four genes. The model formula is: RiskScore = $-0.00111259701091014 \times TBP-0.00540566228324965 \times CDK7 + 0.0013635456849925 \times GRM5 - 0.000512469864405875 \times GRIA1$ and then, the database GSE122063 was used to verify the model to ensure the rationality of the model.

The key genes obtained from network analysis were verified with FpClass tool

The FpClass for predicting high confidence PPIs on a proteome-wide scale, including proteins with few (low-degree proteins) or no known binding partners (orphans). To identify co-expressed genes with the queried genes, the tool considers two specific scores: the gene expression score and the topological network score. The gene expression score is based on the Pearson correlation of the gene expression pattern. The topology score of the network shows whether genes exist in the training data and the strength of their interaction (Kotlyar et al., 2015; Srireropreddy et al., 2019).

Drug selection of hub gene

Drugs targeting key genes were retrieved from the DrugBank database, which is a web-enabled database.⁶ The database collects more than 7,800 drugs, including interactions

³ www.cytoscape.org/

⁴ <http://software.broadinstitute.org/gsea/index.jsp>

⁵ <http://www.gsea-msigdb.org/gsea/downloads.jsp>

⁶ www.DrugBank.ca

and their targets, drug binding data, drug-drug and drug-food interaction data. It also includes hundreds of drug impact information on metabolite levels, protein expression levels, hundreds of drug clinical trials and drug reuse trials (Wishart et al., 2018).

Results

Gene co-expression modules

To explore the co-expression patterns of mRNA in AD, we performed WGCNA analysis on the GSE1297 dataset. The dataset contained 22 columns of AD samples and 9 columns of normal human samples, with a total of 22,283 genes. The raw data can be downloaded from the GEO database of NCBI.⁷ The read-in data was first preprocessed by the *rma* function in the *Affy* package in R language, which contains background correction, normalization, calculation of expression to normalize the data. Then, for the missing values in the data, we used the K-neighborhood algorithm (KNN) to complement the missing values data. Through this algorithm, genes with high similarity were identified to supplement the null values. The absolute median difference (MAD) is the median of the difference between each data in a set of data and the median of that set of data. To filter out a large number of genes with relatively constant expression in different gene samples, the top 7,200 genes of MAD values were selected for WGCNA analysis in this paper. To explore the co-expression patterns of mRNA in AD, we performed WGCNA analysis on the GSE1297 dataset (Figures 1D,F). To ensure scale-free networks, we chose soft thresholds of $b = 12$ (Figures 1A,B), used WGCNA packages as soft threshold power to generate hierarchical clustering trees (Figure 1C), and then we built co-expression networks of associations between clinical features and these modules. As shown in Figure 2 the “Midnight” module of GSE1297 is significantly related to the clinical features of AD.

Kyoto encyclopedia of genes and genomes pathway and gene ontology analysis of differentially expressed genes

GO analysis of DEGs (including molecular function, biological processes and cell composition analysis) and KEGG pathway analysis, respectively, can provide valuable evidence of processes and pathways in which gene sets may be involved. This type of information is crucial for hypothesis development and for the design of future research. Functional

enrichment analyses of KEGG pathway enrichment and DEGs GO function enrichment analysis for DEGs were performed using the DAVID And R package “cluster profile” (Tables 1, 2). The enriched GO terms were divided into CC, BP, and MF ontologies. The results of GO analysis indicated that DEGs were mainly enriched in BPs, including modulation of chemical synaptic transmission, regulation of trans-synaptic signaling, regulation of supramolecular fiber organization, regulation of membrane potential, vesicle localization, the establishment of organelle localization, protein-containing complex disassembly and regulation of protein-containing complex assembly. MF analysis showed that the DEGs were protein serine/threonine kinase activity, tubulin binding, guanyl nucleotide binding, and geranyl ribonucleotide binding. For the cell components, the DEGs were synaptic membrane, presynapse, mitochondrial matrix, transporter complex, neuron-to-neuron synapse, microtubule, and microtubule. In addition, the results of KEGG pathway analysis showed that DEGs were mainly enriched in pathways in retrograde endocannabinoid signaling, human papillomavirus infection, tight junction, purine metabolism, and dopaminergic synapse (Figures 3, 4B and Supplementary Data 1).

Identification of differentially expressed genes in Alzheimer’s disease

We downloaded the series GSE1297 and GSE5281 datasets about AD from the NCBI GEO database. After using the “limma” package in R software, screening with the threshold of an adjusted p -value < 0.05 and $|\log_2FC| > 1.0$, 959 DEGs (320 upregulated and 639 downregulated) were identified in the GSE5281 dataset and DEGs were identified by comparing AD samples with normal samples. 622 DEGs (408 upregulated and 215 downregulated) were identified in the GSE1297 dataset, the DEGs were identified by comparing “control” with the “Severe” status of AD samples. The volcano plot and heatmap analyses were used to visualize the DEGs of the two data sets shown in Figures 5A,B, 6A,B, respectively. In addition, a Venn diagram analysis was performed to evaluate the common DEGs between GSE5281, WGCNA-hub, and GSE1297. As presented in Figure 6C, Intersecting, with 10 hub genes identified two overlapping DEGs (CDK7 and GRIA1).

Building protein-protein interaction networks and screening 10 key genes

A total of 177 nodes and 343 edges were involved in the final PPI network from 269 proteins using the STRING tool, as presented in Figure 7. The top 10 genes evaluated by the algorithm Between Centrality were adopted in the final PPI network. The results showed that the protein, a

⁷ <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE1297>

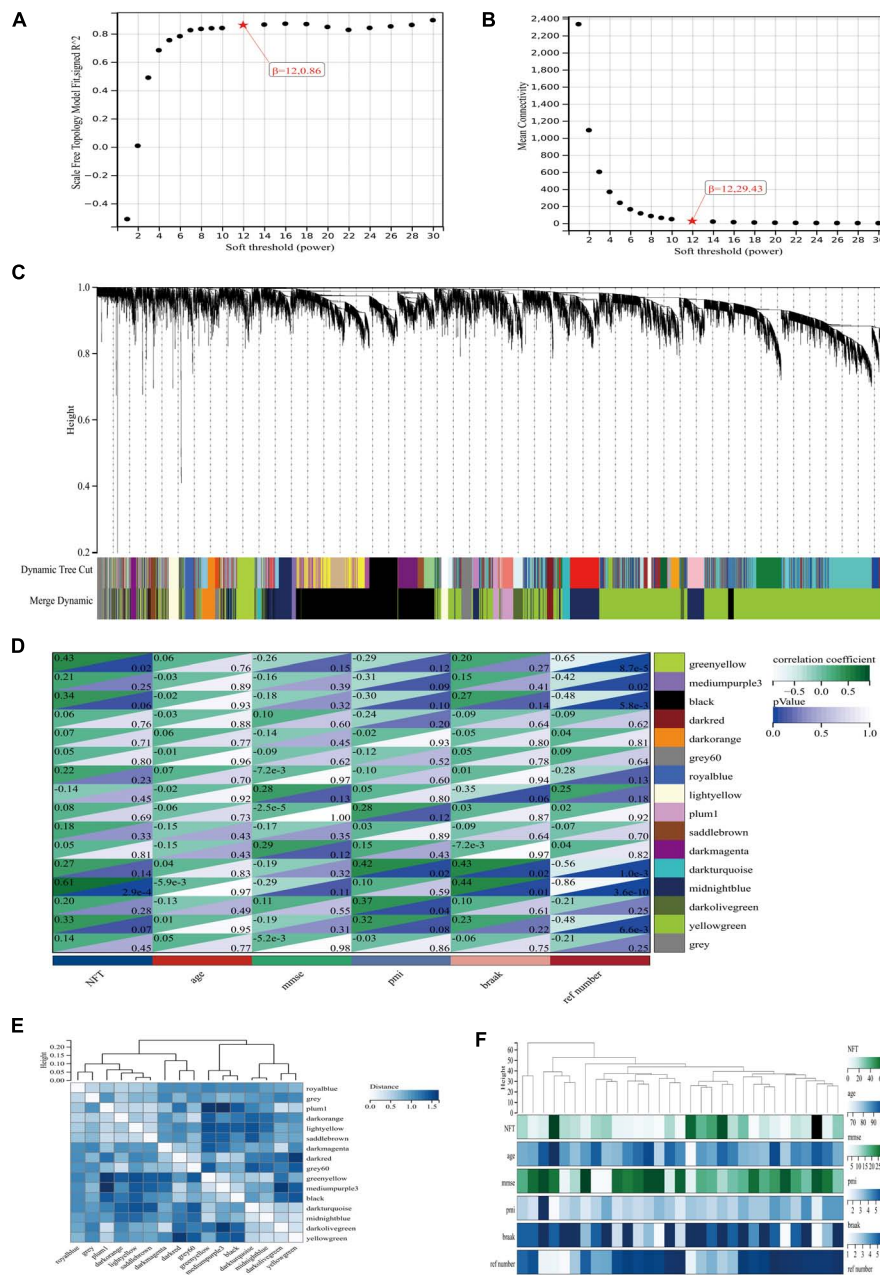


FIGURE 1 Co-expression network of differentially expressed genes in AD. (A,B) Soft threshold power selection. (C) Clustering tree dendrogram of co-expression modules. Different colors represent distinct co-expression modules. (D) Correlation analysis between midnightblue module and clinical condition, each column represents one module, individual rows represent clinical status. (E) Correlation between modules. (F) Specific clinical information of the sample.

regulatory protein involved in mitosis (CCNB1), was the most outstanding one, with a score = 10,430, followed by the predominant excitatory neurotransmitter receptors in the mammalian brain which are activated in a variety of normal neurophysiologic processes (GRIA1; score = 4,243). The TFIID basal transcription factor complex plays a major role in the initiation of RNA polymerase II (Pol II)-dependent

transcription. Other proteins are TATA-binding protein (TBP; score = 4,035), the microtubule-associated protein, tau (MAPT; score = 4,000), Protein Phosphatase 2 Regulatory Subunit Bdelta (PPP2R2D; score = 3,479), Cyclin-Dependent Kinase 7, involved in cell cycle control and RNA polymerase II-mediated RNA transcription (CDK7; score = 3,158), USO1 Vesicle Transport Factor (USO1; score = 2,905), a gene essential for

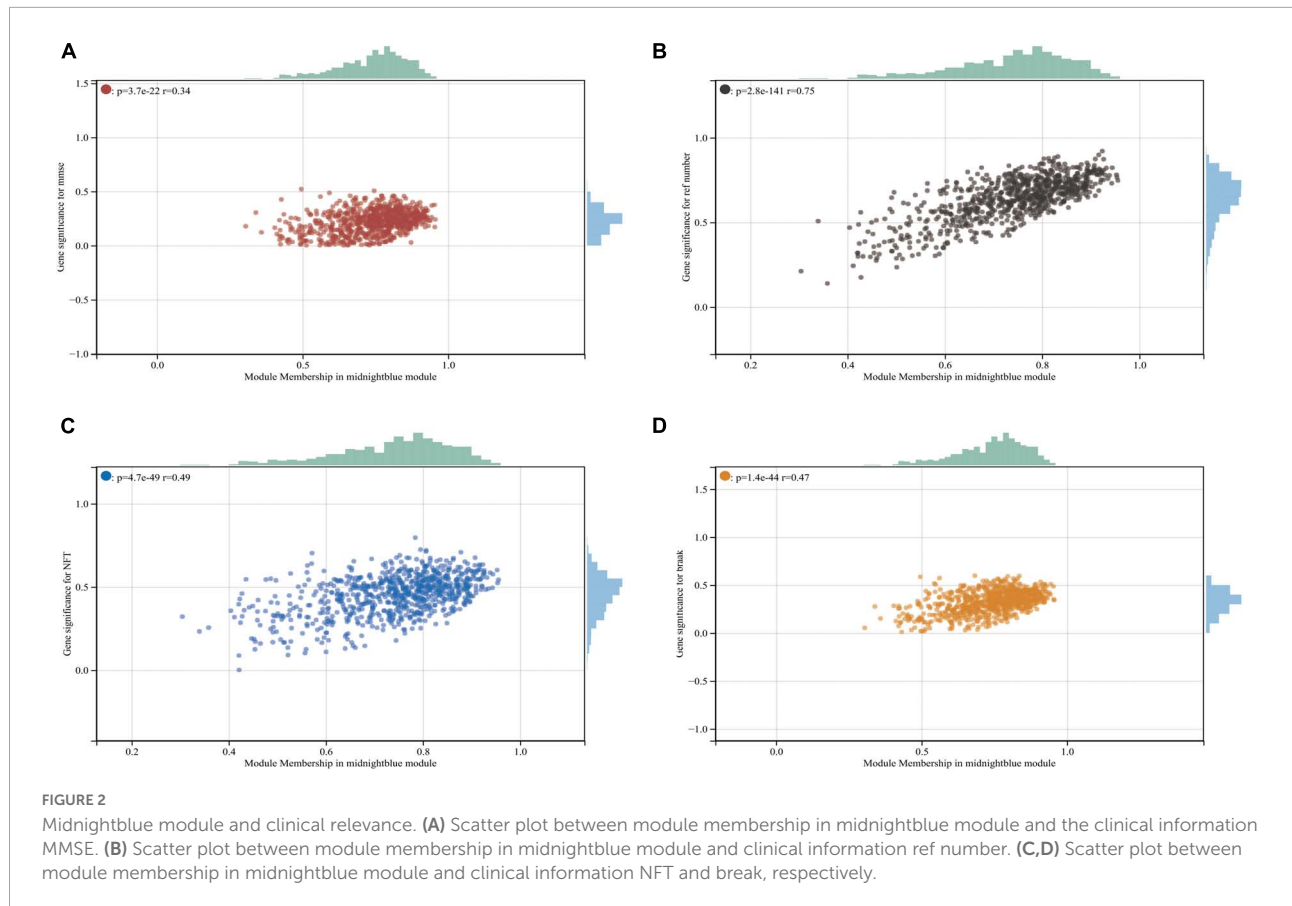


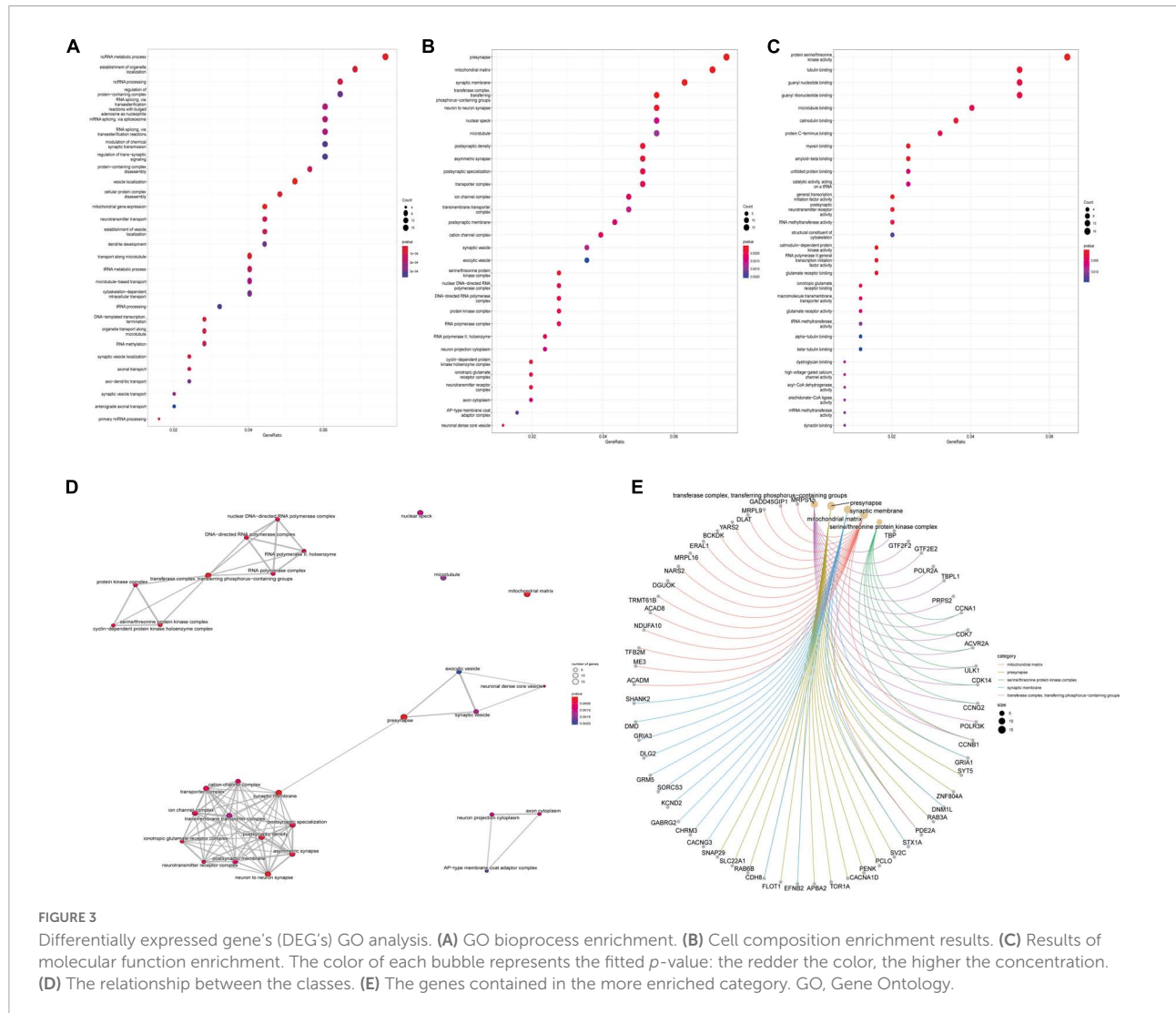
TABLE 1 Significantly enriched gene ontology (GO) terms of differentially expressed genes (DEGs).

Category	Term	Description	P-value	Count
BP term	GO:0051648	Vesicle localization	0.00000495	13
BP term	GO:0051656	Establishment of organelle localization	0.0000306	17
BP term	GO:0032984	Protein-containing complex disassembly	0.0000637	14
BP term	GO:0043254	Regulation of protein-containing complex assembly	0.00015812	16
BP term	GO:0050804	Modulation of chemical synaptic transmission	0.000179862	15
BP term	GO:0099177	Regulation of trans-synaptic signaling	0.000184724	15
BP term	GO:1902903	Regulation of supramolecular fiber organization	0.000237205	14
BP term	GO:0042391	Regulation of membrane potential	0.00034946	15
CC term	GO:0098793	Presynapse	0.0000018	19
CC term	GO:0097060	Synaptic membrane	0.0000146	16
CC term	GO:0005759	Mitochondrial matrix	0.0000279	18
CC term	GO:1990351	Transporter complex	0.000311367	13
CC term	GO:0098984	Neuron to neuron synapse	0.000348414	13
CC term	GO:0005874	Microtubule	0.000822817	14
CC term	GO:0043025	Neuronal cell body	0.002387617	14
MF term	GO:0004674	Protein serine/threonine kinase activity	0.000143327	16
MF term	GO:0015631	Tubulin binding	0.000968776	13
MF term	GO:0019001	Guanyl nucleotide binding	0.001949535	13
MF term	GO:0032561	Geranyl ribonucleotide binding	0.001949535	13

TABLE 2 Significant enrichment of the Kyoto encyclopedia of genes and genomes (KEGG) pathway.

Category	Term	Description	P-value	Count
KEGG_PATHWAY	hsa04723	Retrograde endocannabinoid signaling	0.004813647	8
KEGG_PATHWAY	hsa05165	Human papillomavirus infection	0.018148818	11
KEGG_PATHWAY	hsa04530	Tight junction	0.031975382	7
KEGG_PATHWAY	hsa00230	Purine metabolism	0.034383145	6
KEGG_PATHWAY	hsa04728	Dopaminergic synapse	0.038482344	6

KEGG, Kyoto Encyclopedia of Genes and Genomes.



cell survival and DNA repair (PRPF19; score = 2,809), The protein is a metabotropic glutamate receptor, whose signaling activates a phosphatidylinositol-calcium second messenger system. This protein may be involved in the regulation of neural network activity and synaptic plasticity (GRM5; score = 2,800) and Mitochondrial Ribosomal Protein S15 (MRPS15; score = 2,510). **Table 3** shows the top 10 hub genes.

Prediction of microRNAs and identification of common differentially expressed miRNAs

In addition, we explored the predicted miRNAs of GRM5 and CRIA1 in AD patients using the ENCORI platform to establish potential miRNA messenger RNA (mRNA) regulatory networks (Wu et al., 2021). We found 53 predicted differentially

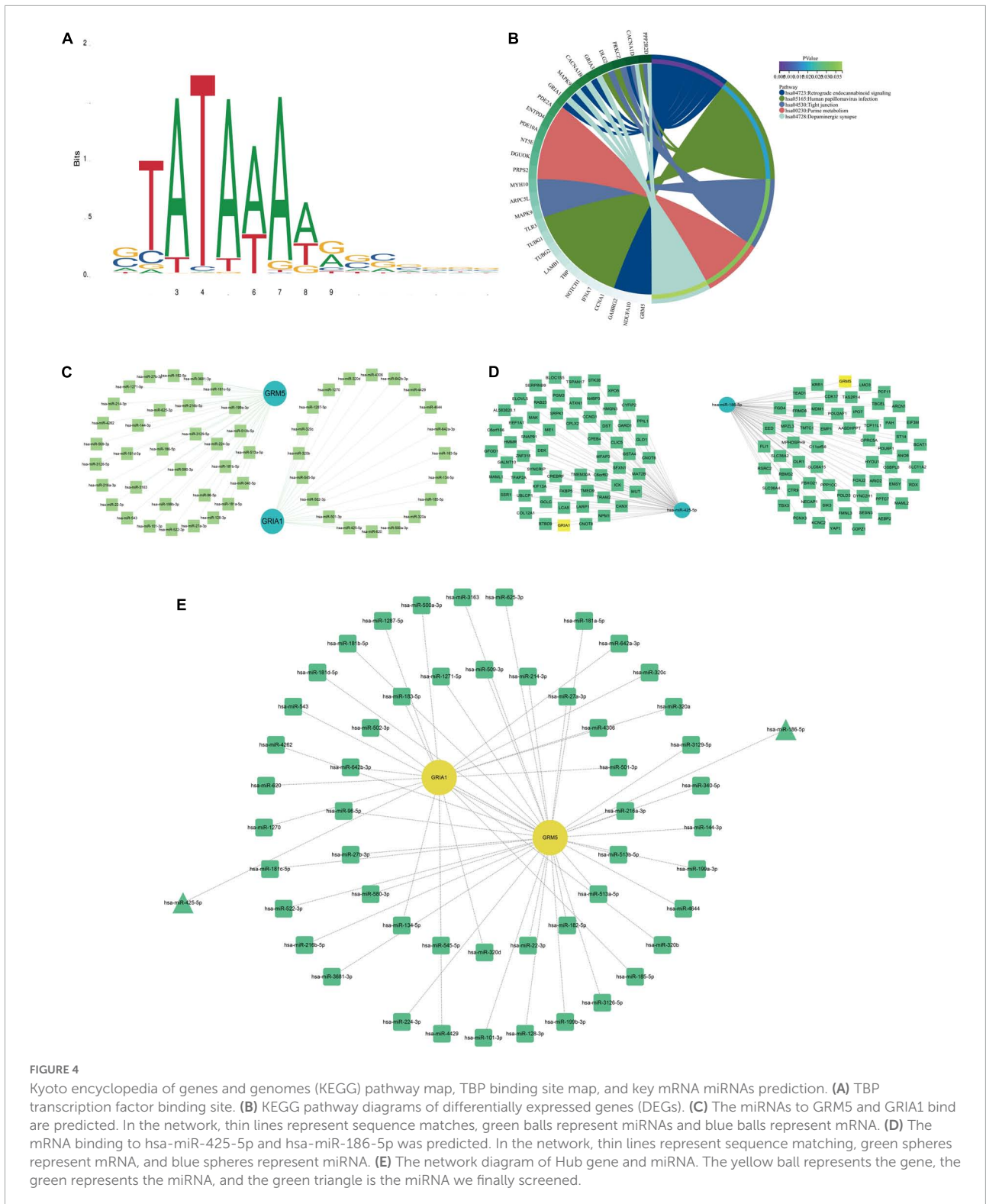
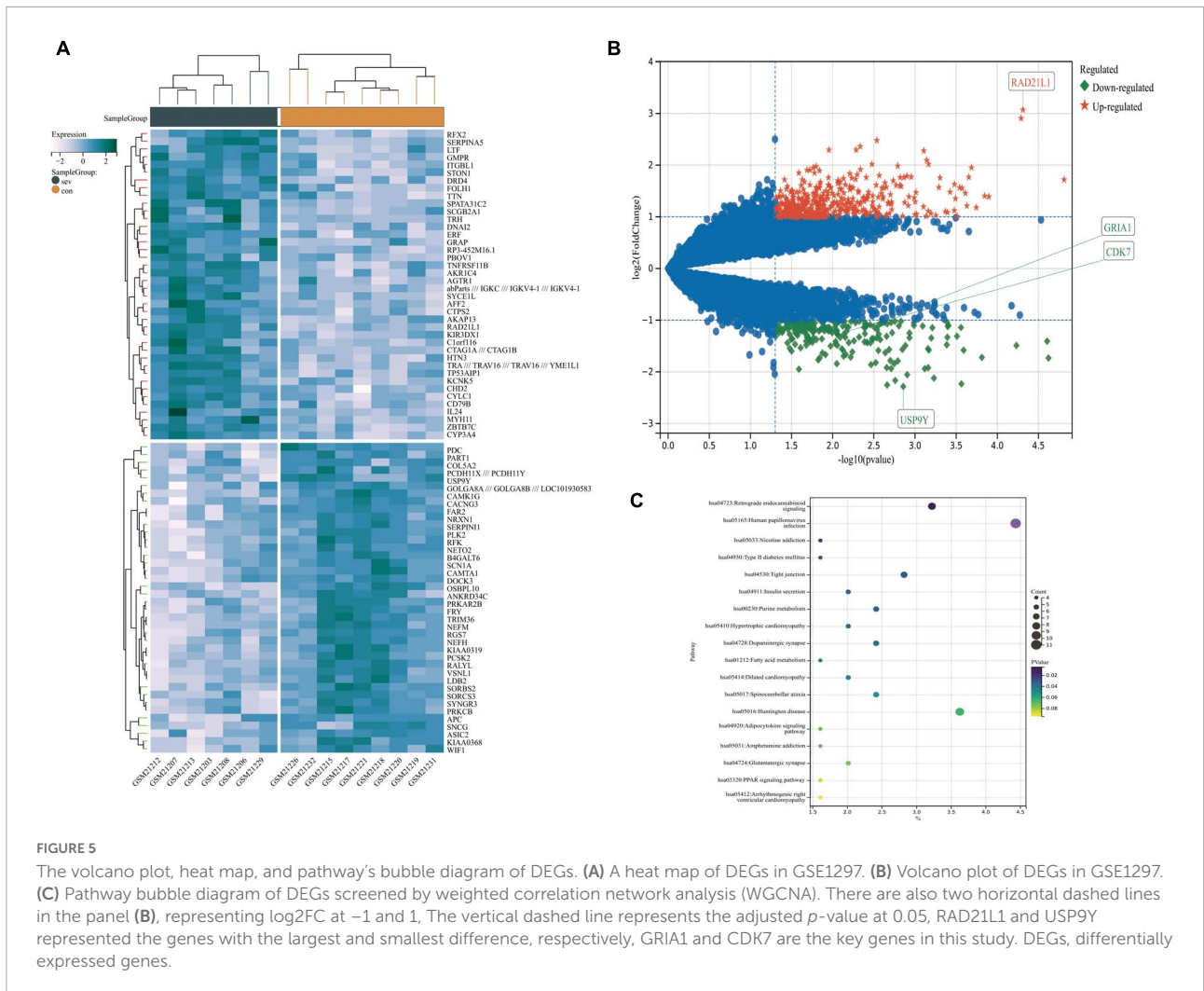


FIGURE 4

Kyoto encyclopedia of genes and genomes (KEGG) pathway map, TBP binding site map, and key mRNA miRNAs prediction. (A) TBP transcription factor binding site. (B) KEGG pathway diagrams of differentially expressed genes (DEGs). (C) The miRNAs to GRM5 and GRIA1 bind are predicted. In the network, thin lines represent sequence matches, green balls represent miRNAs and blue balls represent mRNA. (D) The mRNA binding to hsa-miR-425-5p and hsa-miR-186-5p was predicted. In the network, thin lines represent sequence matching, green spheres represent mRNA, and blue spheres represent miRNA. (E) The network diagram of Hub gene and miRNA. The yellow ball represents the gene, the green represents the miRNA, and the green triangle is the miRNA we finally screened.

expressed microRNAs (Figure 4C). Also, 140 specifically dysregulated differentially expressed miRNAs (DEmiRNAs) (58 downregulated and 82 upregulated DEmiRNAs) in blood samples of patients with AD were identified and used in

our work, according to the report by Leidinger et al. (2013; Supplementary Data 2). The DEmiRNAs from AD were intersected with the 82 upregulated DEmiRNAs from blood samples; the common DEmiRNAs were identified. Respectively,



hsa-miR-186-5p and hsa-miR-425-5p were identified to be potentially involved in the regulation of GRM5 and GRIA1 (Figures 8C,E).

Prediction of transcription factor binding to target genes

We downloaded the TBP's primary structure of the amino acid sequence from the Uniprot database,⁸ and then found the TBP transcription factor binding site features in the JASPAR database⁹ (Figure 4A). Then, 1,000 bp promoter regions of GRM5 and GRIA1 sequences were downloaded from the UCSC database¹⁰ (Supplementary Data 3). Finally, their promoter sequences were entered into the JASPAR database to predict

regulatory transcription factors. Even when the threshold was set as 80%, TBP could be found to be the transcription factor of both. For a more accurate prediction of transcription factors, we used the HumanTFDB database.¹¹ Interestingly, not only TBP is a transcription factor of GRM5 and GRIA1, but CDK7 is also a transcription factor of both (detailed results are provided in the Supplementary Data 4).

Drug selection results

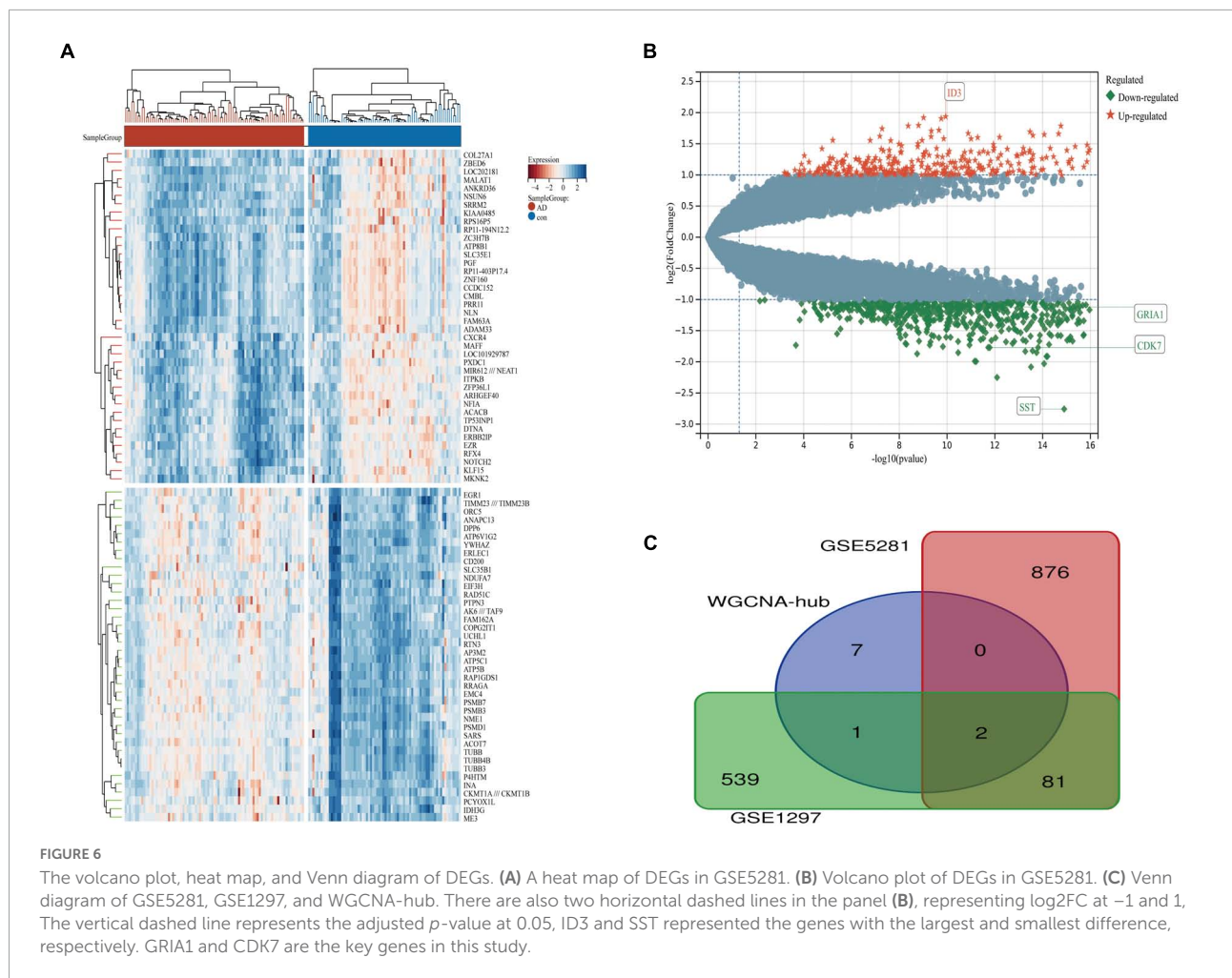
Fifteen drugs targeting the four core genes were selected based on drug and target information contained in DrugBank database. Of these, nine medications have been approved, six are investigational and experimental. All four GRIA1 drugs are primarily responsible for the activity of AMPA glutamate receptors and extracellular glutamate gated ion channels.

8 <https://www.uniprot.org/>

9 <https://jaspar.genereg.net/>

10 <http://genome.ucsc.edu/>

11 <http://bioinfo.life.hust.edu.cn/HumanTFDB#!/>



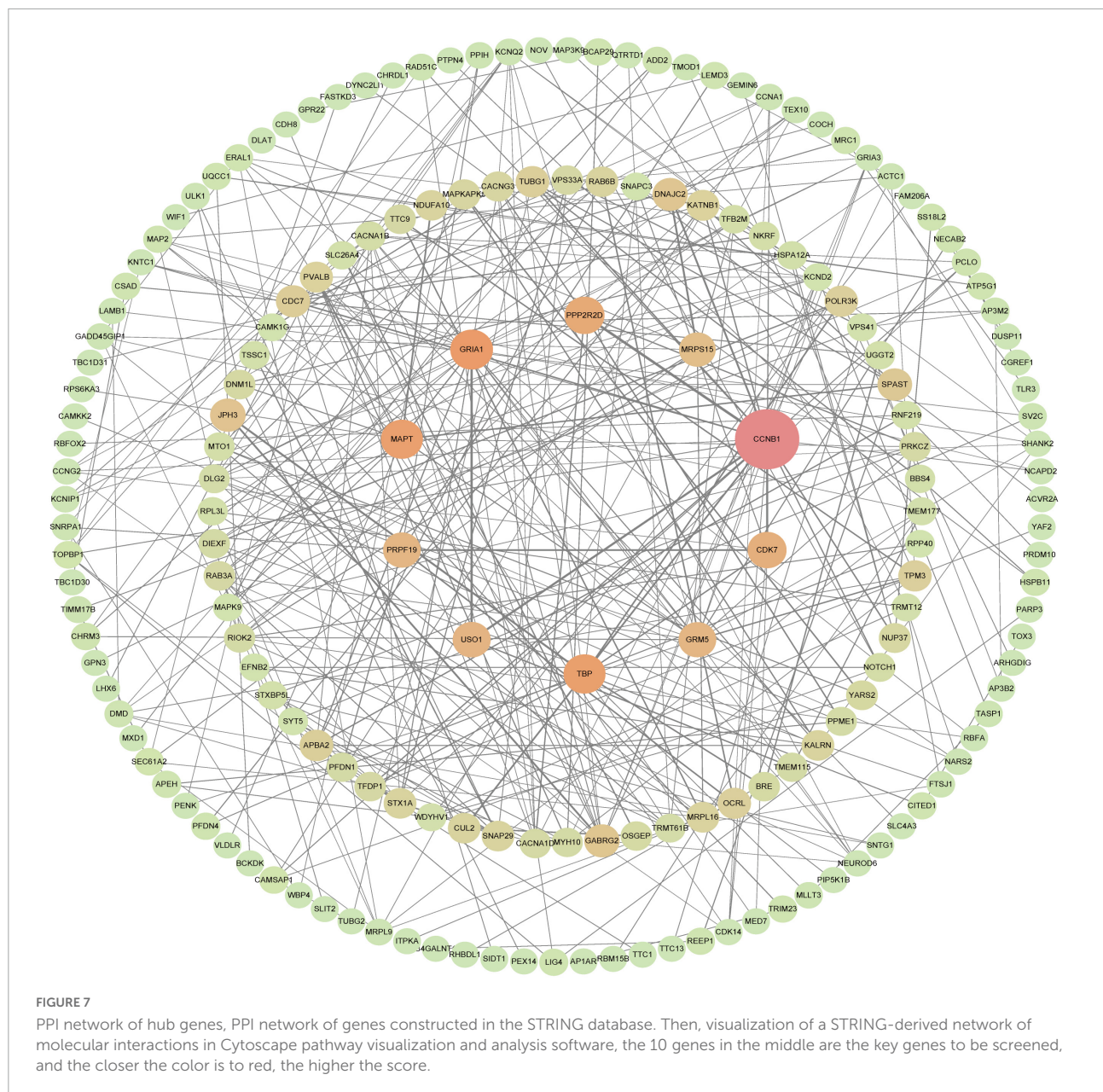
Isoflurane (DB00753), Methodflurane (DB01028), Desflurane (DB01189), Sevoflurane (DB01236) are all known to be an antagonist medication. Isoflurane is a general inhaled cosmetic used in surgery. Methoxyflurane is a general inhalational anesthetic used for the induction and maintenance of general anesthesia. Desflurane is a general inhalational anesthetic for both inpatient and outpatient surgery in adults. Sevoflurane is an inhalational anesthetic agent used for the induction and maintenance of general anesthesia during surgical interventions. Additional information on the features and specific usage of other gene-targeted drugs is presented in **Supplementary Data 6** and in **Table 4**.

Discussion

Due to the heterogeneity of AD pathology, there is a lack of sufficient efficacy in treating AD (Lam et al., 2013; Byun et al., 2015). In the past decades, neurodegenerative therapy for AD has made more and more progress, and drug resistance is often allowed in traditional histology (Nandigam, 2008).

Hence, identifying more appropriate molecular regulation of transcriptional clusters is critical to guiding personalized AD treatment. In this work, the top 7,200 genes based on MAD values were chosen for WGCNA analysis. The "Midnight" module of GSE1297 was chosen as it is significantly associated with clinical AD (Figure 2). Subsequently, according to the cytoHubba plug-in of Cytoscape, we made use of the algorithm Between Centrality to screen 10 key DEGs as hub genes in the PPI network, including CCNB1, GRIA1, TBP, MAPT, PPP2R2D, CDK7, USO1, PRPF19, GRM5, and MRPS15. It's suggesting that these genes may play important role in the mechanism of AD and the specific flow chart is shown in Figure 9G.

Luckily, we discovered that in GO analysis, GRM5 and GRIA1 were predominantly enhanced in the synaptic membrane of CC (cellular component), Neuron to Neuron synapse, postsynaptic membrane, postsynaptic density, and postsynaptic specialization. In BP (biological process), GRM5 and GRIA1 were predominantly enriched in the modulation of chemical synaptic transmission, regulation of trans-synaptic signaling, and regulation of membrane potential. More and more studies have shown that neuron synapse and AD are



closely related, synapse loss and Tau pathology are hallmarks of AD and other tauopathies (Yoshiyama et al., 2007; Skaper et al., 2017; Dejanovic et al., 2018; Kurucu et al., 2022). In KEGG pathway analysis, GRM5 and GRIA1 are co-enriched in glutamatergic synapse and retrograde endocannabinoid signaling pathways. TBP, GRM5, and GRIA1 were co-enriched in Huntington's disease pathway. Both Huntington's disease (HD) and Alzheimer's disease (AD) are similar, albeit clinically distinct, neurodegenerative disorders that share pathologic features related to selective brain injury (Naia et al., 2017; Mukherjee, 2021). TBP and CDK7 are basal transcription factors among them. In addition, through FpClass analysis, we know that TBP and CDK7 have high reliability based on gene

expression and topological network analysis, and GRM5 and GRIA1 also have high scores (Supplementary Data 7). In the verification in the GeneMANIA database, they still have the same relationship network (Supplementary Data 5, Figure A).

All three nuclear RNA polymerases have a subunit of the TATA binding protein (TBP) complex required for transcription (Davidson et al., 2004). In all fields of life, transcription regulation by DNA-dependent three-types RNA polymerase is largely achieved at the initial level. Among them, TBP is the most conservative initiation factor, which is crucial to the transcription initiation of all ancient eukaryotes, and is the only initiation factor required for the complete start of RNA polymerase in all eukaryotes (Kramm et al., 2019;

TABLE 3 The hub genes.

Rank	Name	Score
1	CCNB1	10429.51
2	GRIA1	4242.677
3	TBP	4035.28
4	MAPT	4000.436
5	PPP2R2D	3479.117
6	CDK7	3157.932
7	USO1	2904.839
8	PRPF19	2809.138
9	GRM5	2799.903
10	MRPS15	2510.792

Mishal and Luna-Arias, 2022). Back in 2004, they demonstrated significant differences in TBP between the AD group of normal subjects and patients. In addition, their number and distribution are not proportional to the number and the distribution of positive tau or β -amyloid structures, and the TBP accumulates in AD brains, suggesting that TBP might be a contributing factor to AD due to its own entanglements (Reid et al., 2004). Evidence implicating TBP in the molecular mechanism of several neurodegenerative diseases has emerged

in the past few years. TBP may contribute to these diseases through a loss of normal function (likely to be catastrophic to a cell) which affects neurodegenerative diseases, such as AD and Huntington’s disease (van Roon-Mom et al., 2005; Bech et al., 2010).

The transcription cycle of RNA polymerase II is regulated by a group of cyclin dependent kinases (CDK). CDK7 related to TFIIF, a transcription initiation factor, is not only an effector of RNA polymerase II phosphorylation and other targets in the transcription mechanism, but also a CDK activated kinase involved in transcription, and also plays a key role in regulating eukaryotic cell division (Sansó and Fisher, 2013; Fisher, 2019). There is growing evidence that CDK regulates the transcriptional cycle of RNA polymerase II. Specific CDKs are considered as important molecular mechanisms in the transcription cycle, and describe that recently emerged transcriptional CDK can serve as promising drug targets in cancer (Fisher, 2017; Parua and Fisher, 2020). Moreover, some studies show that neuronal activity of CDK7 in hippocampus is related to aging and AD (Zhu et al., 2000). CDK7 is also required for activity-Dependent expression of Neuronal genes, synaptic plasticity at long-term, and memory at long-term (He et al., 2017). It can be hypothesized that CDK7 is a promising drug target for AD.

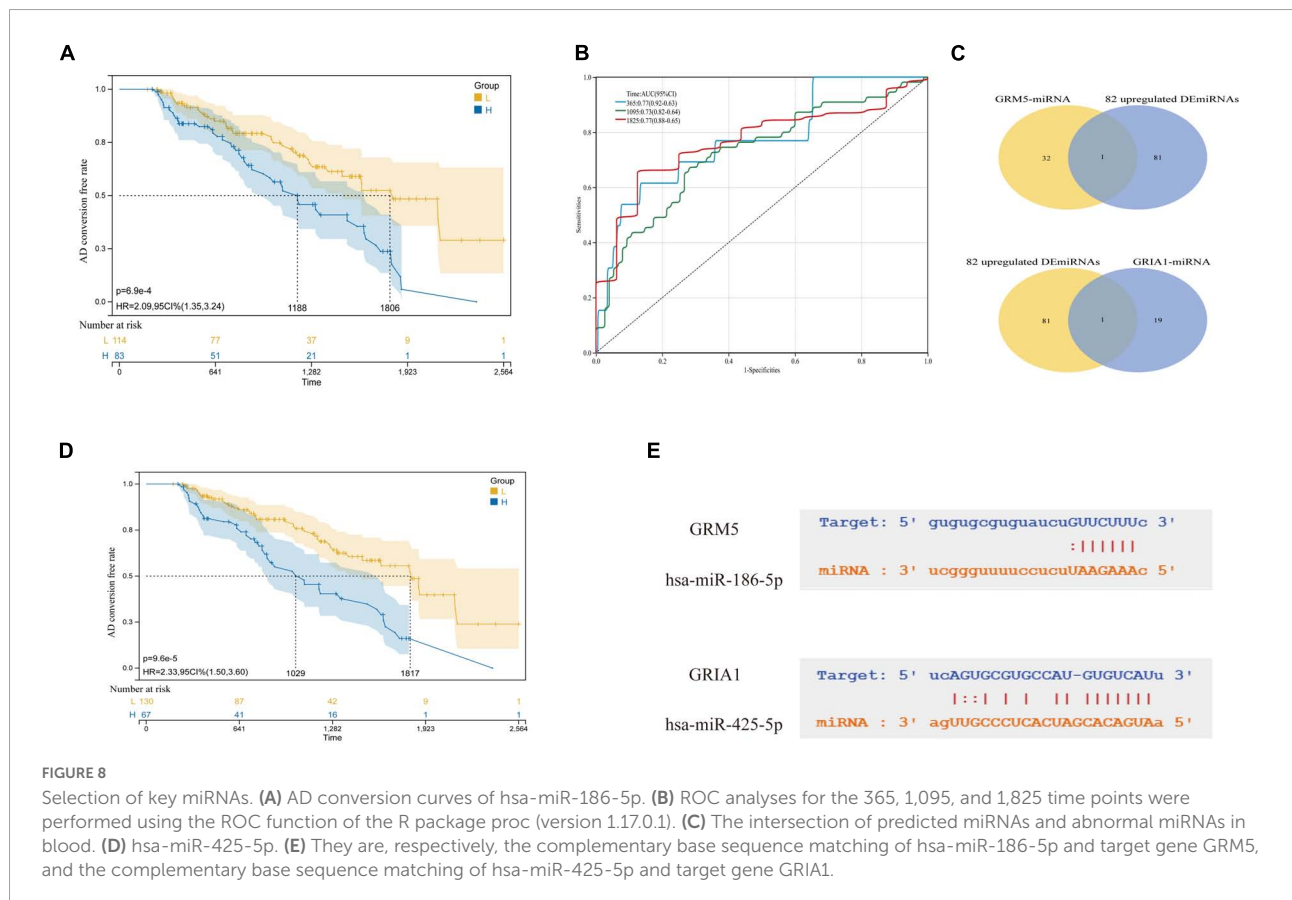
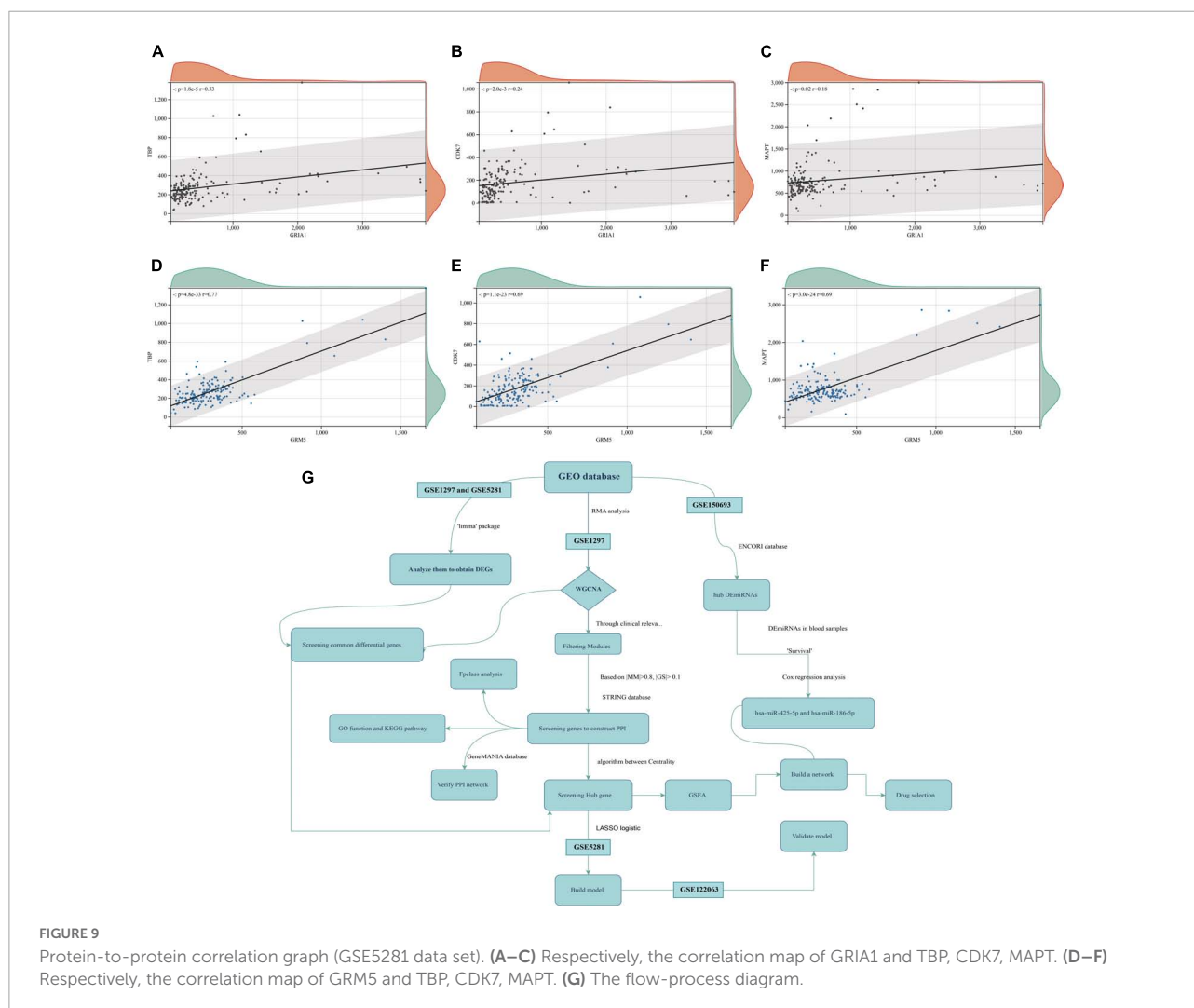
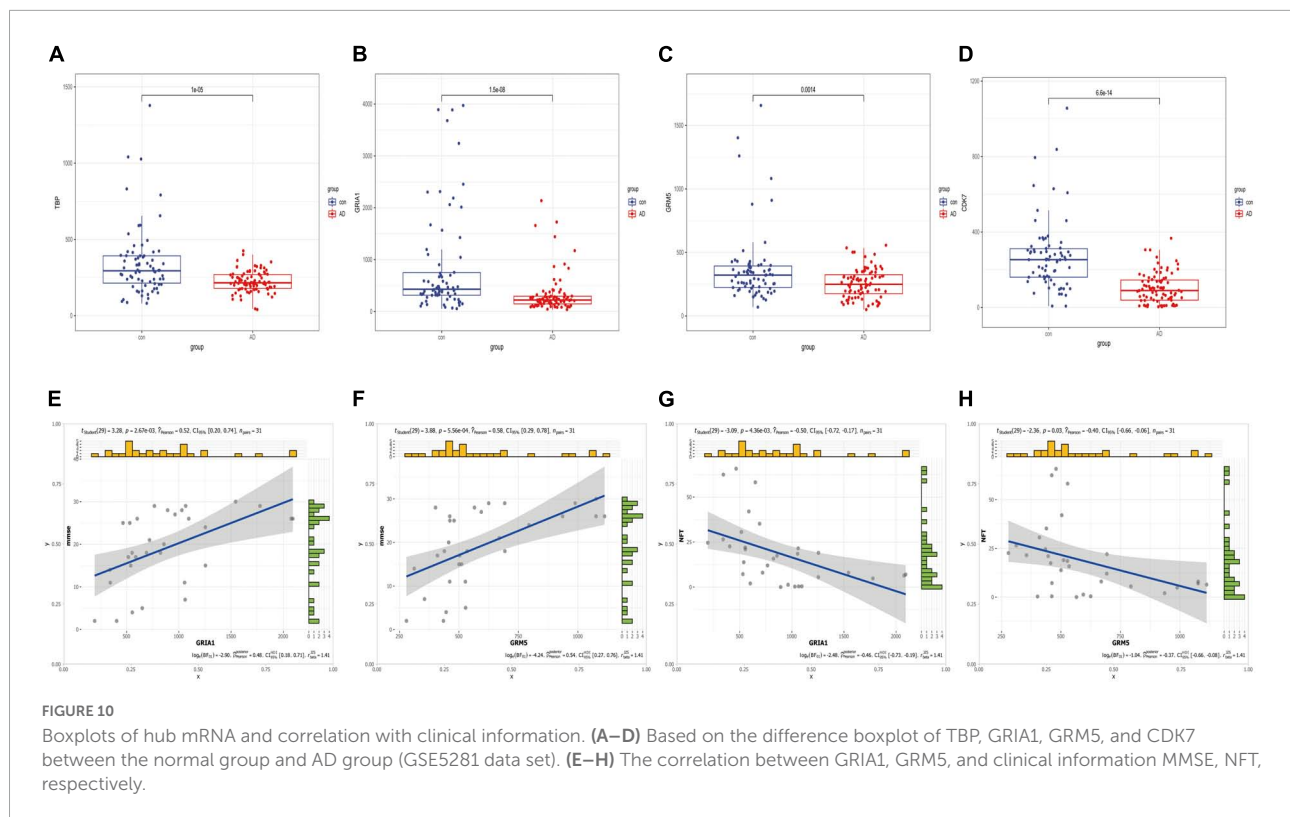


TABLE 4 Fifteen drugs targeting key genes screened from DrugBank database.

genes	Drug name and ID	Status	Type
TBP	Quercetin (DB04216)	Experimental, investigational	
TBP	Chloroquine (DB00608)	Approved, investigational, vet_approved	Inhibitor
TBP	Revusiran (DB16309)	Investigational	Regulator
CDK7	Phosphothreonine (DB02482)	Experimental	
CDK7	Alvocidib (DB03496)	Experimental, investigational	
CDK7	SNS-032 (DB05969)	Investigational	
CDK7	Seliciclib (DB06195)	Investigational	
CDK7	Trilaciclib (DB15442)	Approved, investigational	Inhibitor
GRIA1	Isoflurane (DB00753)	Approved, vet_approved	Antagonist
GRIA1	Methoxyflurane (DB01028)	Approved, investigational, vet_approved	Antagonist
GRIA1	Desflurane (DB01189)	Approved	Antagonist
GRIA1	Sevoflurane (DB01236)	Approved, vet_approved	Antagonist
GRM5	Imipramine (DB00458)	Approved	Inhibitor
GRM5	Disopyramide (DB00280)	Approved	Inhibitor
GRM5	Dalfampridine (DB06637)	Approved	Antagonist

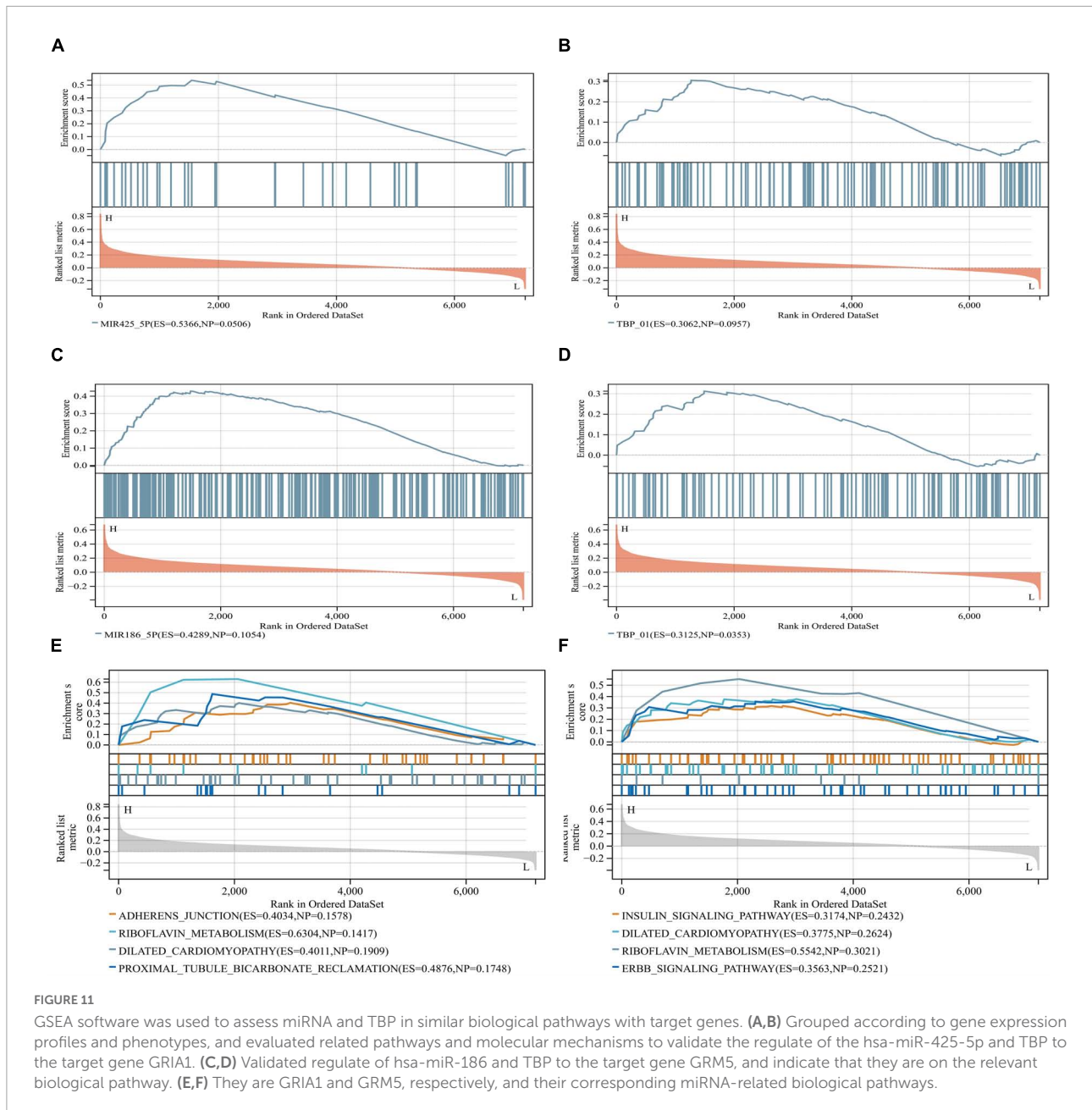




Although GRM5 (coding for metabotropic glutamate receptor 5, mGluR5) is a promising target for treating cognitive deficits in schizophrenia and AD, its association with cognitive and brain phenotypes within this disorder has received little attention. Early researchers found that the common genetic variation of GRM5 in schizophrenic patients would affect cognitive function, hippocampal volume and hippocampal mGluR5 protein level compared with the healthy control group, among them, the metabotropic glutamate receptor subtype 5 (mGluR5) is encoded by GRM5 gene, which is an attractive new drug target for the treatment of schizophrenia (Matosin et al., 2018). mGluR5 is a postsynaptic G-protein coupled glutamate receptor, which is closely related to several key cellular processes destroyed in schizophrenia. In preclinical models of schizophrenia, positive mGluR5 modulators have shown encouraging therapeutic potential, especially in the treatment of cognitive dysfunctions (Matosin et al., 2017). Early in the course of AD inhibition, some have attempted to do so by downregulating cholinergic receptors and glutamate receptors, which, as the disease progresses, play key roles in inflammation and oxidative stress, thereby participating in the neurodegenerative process. Accumulating evidence suggests that perturbation of the excitatory amino acid L-glutamate (L-Glu) in systems may underlie the pathogenesis of hypoxia ischemia, epilepsy, and chronic neurodegenerative diseases (e.g., Huntington's disease and AD) (Hynd et al., 2004; Schaeffer and Gattaz, 2008). It is reported that glutamate excitatory

neurotransmission is an important process in learning and memory, which is seriously damaged in AD, possibly due to β Amyloid peptides (1–42) increase in relation to oxidative stress. The researchers also found that some glutamate receptors are overactivated in AD. This sustained mild activation may lead to neuronal damage and impaired synaptic plasticity (learning). More and more evidence shows that glutamate mediated neurotoxicity is involved in the pathogenesis of AD, and this metabolic glutamate receptor (GRM5), whose signal transduction will activate the second messenger system of calcium phosphatidyl inositol, will also participate in the regulation of neural network activity and synaptic plasticity, which makes us speculate that GRM5 may be a very promising target for exploring treatment and improving AD disease (Danysz and Parsons, 2003; Doraiswamy, 2003; Tanović and Alfaro, 2006; Świetlik et al., 2022).

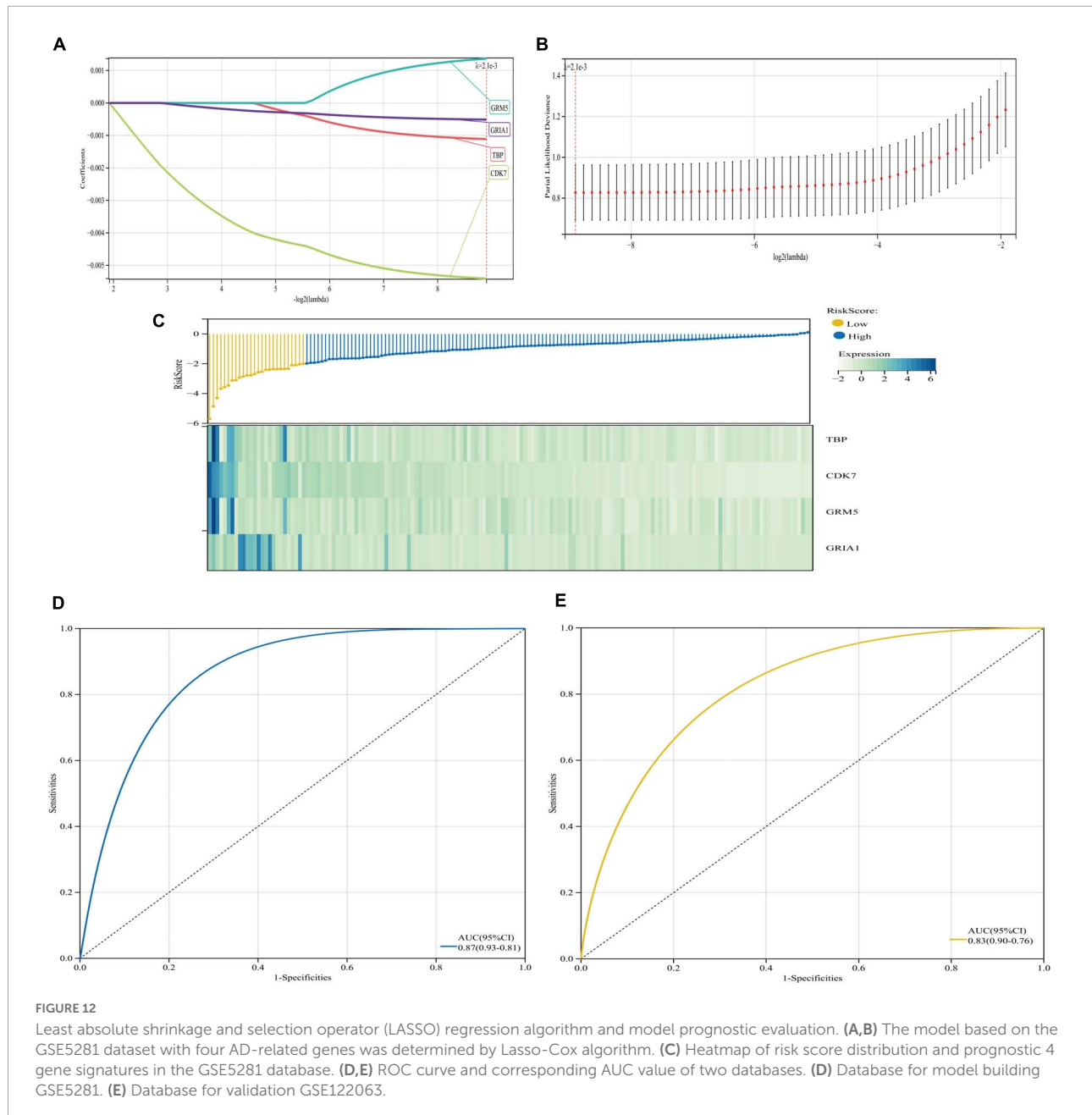
GluA1 (also known as GluRA or GluR1) is a glutamate receptor subunit of AMPA encoded by the GRIA1 gene, there is genome-wide association between GRIA1 and schizophrenia (Barkus et al., 2014; Ang et al., 2018). Hippocampal proteomics study has pointed out that proteins involved in neuronal excitability and synaptic plasticity (e.g., GRIA1, GRM3, and SYN1) were altered in both “normal” aging and AD (Neuner et al., 2017). Similarly, a bioinformatics study identified 464 differentially expressed genes that were modulated by silent transcription factor (REST) between AD patients and controls. REST is strongly associated with glutamatergic



synapses and long-term potentiation, and among them, GRIA1 shows a significant difference in its tendency to change with REST (Xu et al., 2021). From this, we speculate GRIA1 also may be regulated by other transcription factors, thereby affecting the progression of AD disease. From RNA-Seq Expression Data from GTEx (53 Tissues, 570 Donors), the highest median expression of GRM5 and GRIA1 all in Brain—Cerebellum (Supplementary Data 5, Figure B).

A microRNA (miRNA) is a short non-coding RNA with regulatory functions in a variety of biological processes, which has been implicated in many cellular processes

including cell proliferation, apoptosis, gene expression, cellular differentiation, and development (Krol et al., 2010). Many studies have shown that miRNA often binds to target mRNA through complementary sequences and induces translational inhibition or target degradation as a negative regulation of mRNA expression (Towler et al., 2015). We tried to use the ENCORI platform to predict the miRNAs that regulate GRM5 and GRIA1, respectively, and then intersected with 82 up-regulated DEMiRNAs in the blood samples of the previous experiment to determine the hub miRNAs with significant differences. Respectively, hsa-miR-186-5p and hsa-miR-425-5p were identified to be potentially involved in



the regulation of GRM5 and GRIA1. Among them, As shown in **Figures 8A,B,D**, from the AD transformation state diagram and ROC curve, we identified two significantly up-regulated miRNAs, hsa-miR-186-5p and hsa-miR-425-5p may with good prognostic values. Recent studies have found that hsa-miR-425-5p and hsa-miR-186-5p can be used as therapeutic targets for other diseases. For example, hsa-miR-425-5p may promote tumor occurrence and metastasis by activating CTNND1 related pathway (Liu et al., 2020), hsa-miR-186-5p regulates TGF β signaling pathway through expression suppression of SMAD7 and SMAD6 genes in colorectal cancer (Bayat et al., 2021). In our study, GRM5 and GRIA1 were

found to be significantly downregulated at mRNA level and regulated by the up-regulated hsa-miR-186-5p and hsa-miR-425-5p, respectively (**Figures 4D,E**). It has been reported that negatively regulated miRNA-mRNA pairs contribute to the improvement of AD and provide new reliable targets for the treatment of AD (Fu et al., 2019; Qian et al., 2019; Chen et al., 2021).

Anomalous aggregation of microtubule associated tau protein (MAPT) is a prominent pathological feature in various neurodegenerative diseases including AD (Zhou and Wang, 2017; Strang et al., 2019). From in **Figure 9**, we can observe that the correlation coefficients of GRM5 and

GRIA1 with MAPT were 0.69 and 0.18, respectively, with $P < 0.05$. A reliable index of AD-related cognitive state at a particular time point is the MiniMental State Examination (MMSE) (Clark et al., 1999). Furthermore, dementia-NFT of the senile type has been termed tangle-only dementia, NFT-predominant form of SD and limbic NFT dementia (Hyman, 1997). Therefore, MMSE and NFT were chosen as our main markers to quantify AD progression (Schmitt et al., 2000; Yamada, 2003). Since NFT scores increase and MMSE scores decrease with AD severity, genes upregulated with AD could only correlate positively with NFT or negatively with MMSE, whereas genes downregulated with AD could only positively correlate with MMSE or negatively correlated with NTF scores. In our study (Figure 10), the significantly down-regulated gene GRM5 was positively correlated with MMSE with a correlation coefficient of 0.58 and negatively correlated with NFT with a correlation coefficient of -0.4 , GRIA1 and MMSE were also positively correlated with a correlation coefficient of 0.52 and negatively correlated with NFT with a correlation coefficient of -0.5 . At the same time, their $p < 0.05$. As shown in Figures 9A–F, we also can find that the transcription factors TBP and CDK7 are well correlated with their downstream regulated genes. For the transcription factors TBP and CDK7, and the differential expression changes of their regulated target genes GRM5 and GRIA1. It can be seen from Figures 5, 6 that the expression of CDK7 and GRIA1 in AD group is two times lower than that in normal group in two databases. The expression of TBP and GRM5 also decreased significantly compared with the normal group. Grouped according to gene expression profiles and phenotypes, related pathways and molecular mechanisms were evaluated by using GSEA software to validate the regulate of hsa-miR-425-5p, hsa-miR-186, and TBP to target genes GRIA1 and GRM5, respectively (Figure 11).

We also performed regression analysis using Lasso-Cox to construct and validate a prognostic signature associated with the four key genes, which had significant predictive value for AD patients and achieved high AUC values close to 90% in both databases (Figure 12). In addition, we screened 15 drugs targeting key genes from the DrugBank database, most of which are used to relieve pain, systemic anesthetics, inhibit signaling, inhibit cyclin-dependent kinases, inhibit multiple enzyme targets (including CDK7) and change the growth phase of treated cells, treat depression, it is used in potassium channel blockers, ameliorates multiple sclerosis (MS), and so on. We can try to study these drugs, and maybe they may help us to alleviate and treat AD (Supplementary Data 7). In conclusion, based on the bioinformatics analysis in this study, we propose GRM5 and GRIA1 as novel potential prognostic markers of AD and suggest miRNAs and transcription factors that may be related to AD and regulate GRM5 and GRIA1. We hope our findings will inform future research to improve outcomes for patients and try to mitigate and treat AD.

Conclusion

Our research identified a miRNA/TF-gene network that is potentially relevant for AD. The four hub genes (including TBP, CDK7, GRIA1, and GRM5) were markedly downregulated, which may have a critical influence on the pathophysiological mechanism of AD. Two potential target miRNAs (hsa-miR-425-5p and hsa-miR-186-5p) were furthermore forecast. The significantly down-regulated transcription factors TBP and CDK7 may be involved in the transcription of GRM5 and GRIA1, thereby affecting their expression and thus the progression of AD. TBP accumulating in the AD brain, localizing to neurofibrillary tangle structures, may be a contributing factor in AD. CDK7 is necessary for Activity-Dependent Neuronal Gene Expression, Long-Term Synaptic Plasticity, and Long-Term Memory, some studies have demonstrated that CDK7 neuronal activity in the hippocampus is linked to aging and AD. The downregulation of both TBP and CDK7 makes GRM5 and GRIA1 possible to cause neuronal damage and impaired synaptic plasticity in the pathogenesis of AD. As the metabotropic glutamate receptor encoded by GRM5 gene and the AMPA glutamate receptor subunit encoded by GRIA1 participate in the perturbed glutamatergic neurotransmission. Meanwhile, up-regulated hsa-miR-425-5p and hsa-miR-186-5p inhibited the transcription of GRIA1 and GRM5 by binding to them. These findings may contribute to the early diagnostic strategies, treatment targets and prognostic markers of AD disease.

Data availability statement

The original contributions presented in this study are included in the article/Supplementary material, further inquiries can be directed to the corresponding authors.

Author contributions

QZ and PY designed the research study and wrote the original article. QZ, WG, and PY performed the data analysis. QZ, XP, YS, YW, and CP participated in the conception of the study and revision of the manuscript. YW was responsible for guiding the research framework, including concepts and theories. All authors approved the final version to be published.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fnagi.2022.1069606/full#supplementary-material>

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