Peripheral Blood Circular RNAs as a Biomarker for Major Depressive Disorder and Prediction of Possible Pathways

Dandan Zhang1,2†, Yao Ji1†, Xiongjin Chen1, RunSen Chen3, Yaxue Wei2, Qian Peng2, Juda Lin2, Jingwen Yin2, Hezhan Li4, Lili Cui1, Zhixiong Lin2* and Yujie Cai1*

1 Guangdong Key Laboratory of Age-Related Cardiac and Cerebral Diseases, Affiliated Hospital of Guangdong Medical University, Zhanjiang, China, 2 Department of Psychiatry, Affiliated Hospital of Guangdong Medical University, Zhanjiang, China, 3 Department of Rehabilitation Medicine Guangzhou Red Cross Hospital Affiliated to Jinan University, Guangzhou, China, 4 School of Humanities and Management, Guangdong Medical University, Dongguan, China

Circular RNAs (circRNAs) are highly expressed in the central nervous system and have been reported to be associated with neuropsychiatric diseases, but their potential role in major depressive disorder (MDD) remains unclear. Here, we demonstrated that there was a disorder of circRNAs in the blood of MDD patients. It has been preliminarily proved that hsa_circ_0002473, hsa_circ_0079651, hsa_circ_0137187, hsa_circ_0006010, and hsa_circ_0113010 were highly expressed in MDD patients and can be used as diagnostic markers for MDD. Bioinformatics analysis revealed that hsa_circ_0079651, hsa_circ_0137187, hsa_circ_0006010, and hsa_circ_0113010 may affect the neuroplasticity of MDD through the ceRNA mechanism.

Keywords: major depressive disorder, circular RNA, biomarker, neuroplasticity, bioinformatics analysis

INTRODUCTION

Depressive disorder is a set of diseases characterized by low spirit and there are approximately 264 million people worldwide suffer from it (GBD 2017 Disease and Injury Incidence and Prevalence Collaborators, 2018). According to DSM-5, depressive disorder includes major depressive disorder (MDD), persistent depressive disorder (PDD), premenstrual dysphoric disorder, disruptive mood dysregulation disorder, depressive disorder due to another medical condition, substance/medication-induced depressive disorder, etc. MDD is the most common type of depressive disorder, characterized by low spirits, loss of interest, inability to feel pleasure, poor concentration, etc. Relapse is also common, and to make matters worse, the patient may act or think suicidally. In comparison to men, women are more likely to suffer from major depression. Data from the China Mental Health Survey (CMHS), from 2013 to 2015, showed a lifetime prevalence of 3.4%, 12-month prevalence of 2.1%, and the 12-month prevalence is higher in women than in men (2.5 vs. 1.7%, p = 0.0061) (Huang et al., 2019). The etiology and pathogenesis of MDD remain unclear. Personality traits and environmental stress, as well as genetics, are thought to play a role in depression (Gonda et al., 2019). Some researchers believe that epigenetic gene modification and immune inflammation are linked to the development of MDD (Park et al., 2019; Beurel et al., 2020). The neurogenic theory posits that MDD is associated with neurogenesis defects, and postmortem investigations indicated that the size and density of neurons in the dorsolateral prefrontal cortex...
(dIPFC) and dentate gyrus (DG) of MDD patients were lower (Rajkowska, 2000; Boldrini et al., 2013; Malhi and Mann, 2018). However, there is currently no objective method to diagnose MDD. The diagnosis of MDD mainly depends on the patient’s subjective expression, which may be affected by the ability of both the patients and the clinicians to interpret symptoms. Further research into the pathogenesis of MDD will help to identify objective and reliable biomarkers of the disease, which can then be used in order to diagnose MDD, identify specific depressions, and select appropriate treatment.

CircRNAs are a class of covalently closed non-coding RNAs without 3’ poly-A tails or 5’ caps, characterized by high stability, wide expression, and tissue/developmental stage-specific expression, and are highly expressed in blood and brain (Rybak-Wolf et al., 2015). Circular RNAs are more stable and have a longer half-life than linear RNAs, and they are resistant to Ribonuclease R (RNase R) degradation, making them suitable as biomarkers for illness (Wen et al., 2020). CircRNAs have been found to have effects on neurological systems, innate immunity, microRNAs, and various disease-related pathways (Mei et al., 2019), and they are also linked to several neurological illnesses, including Alzheimer’s, Parkinson’s, multiple sclerosis, and schizophrenia (Gokool et al., 2020; Mehta et al., 2020). The field of circRNAs research in depression, however, is still in its infancy, with a few studies looking into the association between MDD and circRNAs (Cui et al., 2016; Zhang et al., 2018, 2019; Huang et al., 2020; Shi et al., 2021). Further studies are needed to fully understand the connection.

In this study, we aimed to identify and validate the differentially expressed circular RNAs in MDD patients, assess their utility as biomarkers, and investigate the possible processes of circRNAs in MDD.

MATERIALS AND METHODS

Subjects

We recruited 29 MDD patients and 19 healthy controls from June to September 2020. MDD patients were recruited through the Department of Psychology at the Affiliated Hospital of Guangdong Medical University. Participants or their legal guardians were informed of the trial’s benefits and risks before providing written informed permission. All MDD patients met the following inclusion criteria: (1) met the MDD diagnostic criteria of Manual of Mental Disorders (5th Edition) (DSM-V); (2) the 24-item Hamilton Depression Scale (HAMD-24) score ≥ 8; (3) no history of mental disorder, no significant physical disorder; (7) no history of smoking or alcohol abuse; (8) no history of head trauma. And the following exclusion criteria were applied for the MDD patients: (1) with other mental disorders, e.g., substance abuse, schizophrenia, obsessive-compulsive disorder, etc.; (2) pregnant or lactating female; (3) long-term use of drugs or health care products. The healthy controls were recruited in the community and the inclusion criteria were as follows: (1) the 24-item Hamilton Depression Scale (HAMD-24) score < 8; (2) age 18 to 59 years; (3) no history of mental disorder and no family history of major mental disorder, no significant physical disorder; (6) no history of smoking or alcohol abuse. The following exclusion criteria were applied for the healthy controls: (1) pregnant or lactating female; (2) long-term use of drugs or health care products. All participants were jointly diagnosed by two or more psychiatrists. The demographic data of the patients and controls are shown in Table 1. The differences between MDD patients and healthy controls were not significant in age, gender ratio, or ethnicity, but the HAMD scores were significantly different.

Whole Transcriptome Sequencing

Following an overnight fast, venous blood was taken from each participant. EDTA anticoagulant tubes were used to collect whole blood samples, and whole transcriptome sequencing was done on blood samples from 4 MDD patients and 4 healthy controls. CircRNAs exhibiting \( \log(2\text{(fold change)}) > 1 \) and \( p \)-value < 0.05 were identified as significant. Use the FPKM value to identify the mRNA of each coding gene. Differentially expressed mRNAs were identified based on \( \log(2\text{(fold change)}) \) > 0.58 with a \( p \)-value < 0.05, and at least one sample of the gene FPKM value ≥ 1.

RNA Extraction and Real-Time Quantitative Reverse Transcription PCR

Total RNA was extracted by Trizol (Invitrogen, United States). According to the manufacturer’s protocol affiliated with Eva M-MLV RT Kit with gDNA Clean for qPCR II (Accurate Biology, China), circRNA was reverse transcribed to complementary cDNA and then quantified by SYBR Green Real-time PCR Master Mix (Accurate Biology, China). \( \beta \)-actin was used as the endogenous control, detailed information about these primers was provided in Supplementary Table 1. The expression levels of circRNAs were normalized to \( \beta \)-actin, and the relative expression levels of circRNAs were shown by the value of the \( 2^{-\Delta\Delta C_T} \).

| Table 1 | Clinical characteristics of MDD patients and healthy controls. |
|---------|---------------------------------|
| Variable | HC(n = 19) | MDD(n = 29) | p-value | Statistical analyses |
| Ethnicity | Han | Han | | |
| Age (years) | 22.74 (2.47) | 22.1 (5.27) | 0.586 | Unpaired t-test with Welch’s correction |
| Female | 13 (68.42%) | 21 (72.41%) | > 0.9999 | Fisher’s exact test |
| HAMD-17 score | 0.7778 (1.84) | 16.52 (4.71) | < 0.0001 | Mann whitney test |
| HAMD-24 score | 0.9444 (1.87) | 21.17 (6.84) | < 0.0001 | Mann whitney test |
| HAMA | 0.8889 (1.33) | 16.66 (6.14) | < 0.0001 | Mann whitney test |

Data presented as mean (standard deviation) or number of participants in each group (% of total). MDD, major depressive disorder; HC, healthy control; HAMD-17, the 17-item Hamilton Depression Scale; HAMD-24, the 24-item Hamilton Depression Scale; HAMA, the Hamilton Anxiety Scale.
Enzyme-Linked Immunosorbent Assay (ELISA) Analyses
The plasma was extracted by centrifugation at 3,000 rpm, 27°C for 10 min. By the manufacturer’s protocols, ELISA kits (Multi Sciences, China) were used to detect the plasma concentration of BDNF, GDNF, and β-NGF. The maximum absorption wavelength at 450 nm and the reference wavelength at 570 nm was measured using a microplate reader (BioTek Epoch, United States), then subtracting readings at 570 nm from the readings at 450 nm as calibration optical density (OD) value.

Bioinformatics Analysis
Based on miRanda, we predicted potential miRNA targets for the target circRNAs. The potential mRNA targets of the miRNAs were predicted by TargetScan. Cytoscape was used to delineate the circRNA–miRNA–mRNA network. Using clusterProfiler package to perform gene ontology (GO) analysis to analyze the potential function of the target gene. Kyoko Encyclopedia of Genes and Genomes (KEGG) enrichment analysis analyzed the related pathways of target genes through KOBAS 3.0.

Statistical Analysis
The difference of candidate circRNAs expression between MDD patients and healthy controls was evaluated by t-test. Demographic variables were compared between MDD patients and healthy controls with unpaired t-test with Welch’s correction, Fisher’s exact test, or Mann Whitney test. All tests were two-sided and P-value < 0.05 was considered statistically significant. Statistical analysis was performed with GraphPad Prism (GraphPad Software, United States) version 8.42.

RESULTS
CircRNAs Expression Profile in Major Depressive Disorder Patients and Healthy Controls
We used whole transcriptome sequencing to analyze blood samples from four MDD patients and four healthy controls and identified 445 circRNAs expressing differentially (378 downregulated and 67 upregulated) (Figure 1A). The expression profiles of circRNAs in MDD patients and healthy controls exhibited good consistency, as shown in the heat map analysis of the top 100-dysregulated circRNAs (Figure 1B).

Enrichment Analyses of Dysregulated CircRNAs
Gene ontology enrichment analysis and KEGG enrichment analysis based on upregulated circRNAs were shown in Figure 1C. In the GO enrichment results of upregulated circRNAs, negative regulation of apoptotic process (GO: 0043066), protein import into nucleus (GO: 0006606), viral process (GO: 0016032), ubiquitin-dependent protein catabolic process (GO: 0006511), negative regulation of establishment of protein localization to plasma membrane (GO: 0090005) and viral entry into host cell (GO: 0046718) were the mainly enriched biological process terms. In addition, KEGG enrichment analysis (Figure 1C) showed that upregulated circRNAs were enriched in neuron-related pathways: serotonergic synapses (hsa04726) and dopaminergic synapses (hsa04728). GO enrichment analysis and KEGG enrichment analysis based on downregulated circRNAs were shown in Figure 1D. The top five biological process terms of downregulated circRNAs were as follows: protein sumoylation (GO: 0016925), intracellular transport of virus (GO: 0075733), tRNA export from nucleus (GO: 0006409), regulation of glucose transport (GO: 0010827) and regulation of protein localization (GO: 0032880). The first 5 pathways of KEGG enrichment analysis showed that downregulated circRNAs were enriched in RNA transport (hsa03013), thyroid hormone signaling pathway (hsa04919), ubiquitin mediated proteolysis (hsa04120), HTLV-I infection (hsa05166), and endocytosis (hsa04144).

Validation of 20 Dysregulated Candidate CircRNAs
We selected 20 dysregulated circRNAs (the top 10 upregulated and the top 10 downregulated) for further study (Table 2). Heat map analysis of the 10 upregulated circRNAs (Figure 2A) and the 10 downregulated circRNAs (Figure 2B) showed that both could distinguish MDD patients from healthy controls. The 20 dysregulated circular RNAs were further verified by RT-qPCR in 29 MDD patients and 19 healthy controls, subsequently, 5 upregulated circRNAs were consistent with circRNAs sequencing among the 20 dysregulated circRNAs: hsa_circ_0002473, hsa_circ_0079651, hsa_circ_0137187, hsa_circ_0006010 and hsa_circ_0113010 (Figures 2C,D). The receiver operating characteristic curve (ROC) analysis was performed on these five circRNAs to assess their diagnostic capability for MDD (Figure 2E). All of them were able to discriminate between MDD patients and healthy controls, and hsa_circ_0002473 and hsa_circ_0006010 exhibited better predictive capacity of biomarkers (AUC = 0.8619 and AUC = 0.8367, respectively).

Functional Prediction of the ceRNA Network of Verified Candidate CircRNAs
All the sequence information of human miRNA was downloaded from the miRBase database2, and then miRanda software was used to predict the binding relationship between circRNAs and miRNAs. The binding relationship between miRNAs and miRNAs was predicted using the binding relationship between miRNA and mRNA in the TargetScan database. For the miRNAs we used 150 genes that had been detected to be upregulated in whole transcriptome sequencing (Supplementary Table 2). Based on these five circRNAs, we obtained a ceRNA network, including 11 miRNA and 36 mRNA (Figure 3A). Among them, we found hsa_circ_0002473 had no ceRNA relationship.
The probable roles of these 36 mRNAs connected to the ceRNA network were predicted using GO and KEGG enrichment analysis. These mRNAs were linked to the growth and differentiation of neurons, according to biological process items (Figure 3B): hypothalamus cell differentiation (GO: 0021979), regulation of dopaminergic neuron differentiation...
(GO: 1904338), midbrain dopaminergic neuron differentiation (GO: 190494), central nervous system neuron differentiation (GO: 0021884). In terms of cellular components, these mRNAs were enriched in voltage-gated potassium channel complex (GO: 0008076), potassium ion transmembrane transporter activity (GO: 0015079), and G-quadruplex DNA binding (GO: 0018010), etc. Regarding KEGG pathways based on target mRNAs (Figure 3C), it was disclosed that 36 mRNAs were enriched in nitrogen metabolism (hsa00061) and fatty acid biosynthesis (hsa00260), and so on. In addition, these mRNAs were enriched in molecular function such as G-protein-coupled peptide receptor activity (GO: 0008528), peptide receptor activity (GO: 0016535), potassium ion transmembrane transporter activity (GO: 0034705), extrinsic component of the extracellular matrix (GO: 0002959), and so on. A number of these mRNAs showed that the concentration of BDNF was unchanged in MDD patients. In addition, we found that the concentration of β-NF and GDNF were not associated with the relative expression of these 5 circRNAs.

**DISCUSSION**

In this study, we performed whole transcriptome sequencing on the whole blood RNA of MDD patients and healthy controls: 67 upregulated circRNAs and 378 downregulated circRNAs (Figure 1A). In addition, upregulated circRNAs were enriched in neuron-related pathways: serotonergic synapses and dopaminergic synapses (Figure 1C). Downregulated circRNAs were enriched in RNA transport, thyroid hormone signaling pathway, ubiquitin-mediated proteolysis, HTLV-I infection, and so on (Figure 1D). Then, we selected 20 dysregulated circRNAs (the top 10 upregulated and the top 10 downregulated) (Table 2) for further study. Five upregulated circRNAs: hsa_circ_0002473, hsa_circ_0079651, hsa_circ_0137187, hsa_circ_0006010, and hsa_circ_0113010 were specifically expressed in MDD patients (Figure 2C), and could distinguish MDD patients from healthy controls (Figure 2E). We did not validate circRNAs with the same expression trend in the down-regulated circRNAs (Figure 2D). According to the present research, hsa_circ_0002473, hsa_circ_0079651, hsa_circ_0137187, hsa_circ_0006010, and hsa_circ_0113010 might be novel biomarkers for MDD.

---

**TABLE 2** Twenty dysregulated circRNAs in whole transcriptome sequencing were selected.

| CircRNA_id | CircBase_id | hg38_position | Gene | log2Fold-Change | p-value | padj | Trend |
|------------|-------------|---------------|------|----------------|---------|------|-------|
| chr1:112653597-112659779 + | hsa_circ_0000109 | chr1:113196219-113202401+ | CAPZA1 | 2.661389358 | 0.000294984 | 0.469082864 | Up |
| chr:99439768-99465136- | hsa_circ_0077425 | chr6:99888764-99913012- | USP45 | 2.723704441 | 0.000312774 | 0.469082864 | Up |
| chr:13:95723241-95725252 + | hsa_circ_0002473 | chr:13:96375495-96377506+ | DNAJC3 | 2.504480477 | 0.00078066 | 0.469082864 | Up |
| chr:7:26195646-26197732- | hsa_circ_00079651 | chr:7:26236566-26237352- | HNRNPA2B1 | 2.46293726 | 0.01094879 | 0.469082864 | Up |
| chr:15:100564691-100565265− | hsa_circ_0000007 | chr15:101104896-101105470− | LINS1 | 1.995125317 | 0.005230603 | 0.469082864 | Up |
| chr:1:222823883-222835215 + | hsa_circ_0000187 | chr1:222997225-223008557+ | ALZ2 | 2.01109853 | 0.006769898 | 0.469082864 | Up |
| chr:8:17319317-81718224- | hsa_circ_0137187 | chr8:192268826-192304597− | ZFAND1 | 1.991446071 | 0.007293806 | 0.469082864 | Up |
| chr:3:197830769-197839307 + | hsa_circ_0006040 | chr19:197557640-197566288+ | LRCB3 | 1.96759118 | 0.00798366 | 0.469082864 | Up |
| chr:7:32632563-32633965− | hsa_circ_0000610 | chr7:32672154-32679877− | DPY19L1P1 | 1.9489764 | 0.008618962 | 0.469082864 | Up |
| chr:29109335-29115803 + | hsa_circ_0113010 | chr291453847-29442315+ | EPB4I | 1.93827186 | 0.011208077 | 0.469082864 | Up |
| chr:13:21161789-21172681- | hsa_circ_0029696 | chr13:21735928-21746820- | SKA3 | −3.32816757 | 0.00000320 | 0.120960362 | down |
| chr:13:21157921-21172681- | hsa_circ_00007547 | chr13:21732060-21746820- | SKA3 | −3.72104263 | 0.00010838 | 0.120960362 | down |
| chr:12:89466669-89472275- | hsa_circ_0027702 | chr12:89896056-89896052- | PO1C2B | −3.05977156 | 0.00002063 | 0.120960362 | down |
| chr:5:131608916-131709272- | hsa_circ_0000905 | chr5:131034609-131044965- | AC008695.1 | −3.45684584 | 0.00005791 | 0.120960362 | down |
| chr:14:14352767-143530526 + | hsa_circ_0125428 | chr14:14449020-144451679+ | SMARCA5 | −2.965370872 | 0.00020046 | 0.120960362 | down |
| chr:12:89469637-89472275- | hsa_circ_0099436 | chr12:89853414-89866002- | PO1C2B | −2.851444921 | 0.00030462 | 0.120960362 | down |
| chr:17:55410468-55403988+ | hsa_circ_00002015 | chr17:53478829-53481229+ | MMD | −3.10203597 | 0.00037882 | 0.120960362 | down |
| chr:6:149771161-149773189 + | hsa_circ_0006936 | chr6:15009297-150094305+ | PCM1T1 | −2.928561379 | 0.000112222 | 0.120960362 | down |
| chr:5:35646232-53658624 + | hsa_circ_0129114 | chr5:52942062-52954454+ | NDUF4S | −2.769203899 | 0.00025052 | 0.429955668 | down |
| chr:13:37040404-37051583- | hsa_circ_0000475 | chr13:37316541-37625720- | SUPT20H | −2.632288383 | 0.000160359 | 0.295997981 | down |
CircRNAs can act as miRNA sponges, preventing miRNAs from binding and suppressing target mRNA (Chen, 2020). In this study, these five circRNAs were discovered for the first time. For these five circRNAs, we constructed a ceRNA network, and this ceRNA network revealed that 36 mRNA may reflect the function of these circRNAs in MDD.
(Figure 3A). We discovered that hsa_circ_0002473 has no ceRNA relationship, suggesting it does not function as a ceRNA. Biological process items showed that these 36 mRNAs were related to the growth and differentiation of neurons (Figure 3B): regulation of dopaminergic neuron differentiation, midbrain dopaminergic neuron differentiation, central nervous system neuron differentiation, hypothalamus development, neurotransmitter receptor transport, and forebrain neuron development, which was consistent with the KEGG enrichment analysis of the total upregulated circRNAs (Figure 1C). It implies that there may be neuronal changes in MDD patients. Moreover, the size and density of the dorsolateral prefrontal cortex (dlPFC) and dentate gyrus (DG) neurons in MDD patients were reduced (Rajkowska, 2000; Baldrini et al., 2013). There was evidence that neurotrophic factors regulate neurogenesis, and MDD patients often present with neurotrophic factor disorders (Shi et al., 2020). We would like to find out whether neurotrophic factors are affected in this study and if circRNAs are related to neurotrophic factors.

It is known that neurotrophic factors are able to promote the growth, proliferation, differentiation, and survival of neurons; neurotrophic factors such as BDNF, GDNF, and NGF are dysregulated in MDD patients and are important in antidepressant drug mechanisms (Saavedra et al., 2008; Allen et al., 2013; Song et al., 2017; Sun et al., 2019; Castrén and Monteggia, 2021). In this study, β-NGF and GDNF levels decreased in MDD patients, while BDNF levels were unaffected (Figure 3D). We also found that β-NGF and GDNF concentrations do not affect the relative expression of these five circRNAs. There is still no consensus on how these three neurotrophic factors differ in MDD individuals. The majority of research showed that BDNF expression in MDD decreased (de Azevedo Cardoso et al., 2014; Phillips, 2017; Kojima et al., 2019; Teng et al., 2021). The results of our experiment contradicted this.
TABLE 3 | The potential function of 15 mRNAs in neurogenesis or neuropsychiatric disorders based on previous studies.

| mRNA     | Study ID       | Potential function                                                                 |
|----------|----------------|-------------------------------------------------------------------------------------|
| BAIAp3   | Wojcik et al., 2013 | BAIAp3 was associated with anxiety and changes in response to benzodiazepines.       |
| CCR9     | Liu et al., 2007; Cao et al., 2012 | CCR9 had neuroprotective effects on mouse hippocampal neurons.                        |
| E2F2     | Castillo et al., 2015; Wu et al., 2015 | E2F2 was related to nerve repair after spinal cord injury and repairing neuronal cell DNA damage. |
| ECHDC3   | Tan et al., 2021 | ECHDC3 was related to cranial nerve degeneration.                                     |
| HIP1     | Peng et al., 2017 | HIP1R was involved in the development of neuronal dendrites and the formation of excitatory synapses. |
| KCNE1    | Jamal et al., 2006; Hussain et al., 2010 | KCNE1 was reduced in the entorhinal cortex (EC) of mesial temporal lobe epilepsies (MTLE) patients; the interaction of KCNE1 and Rap2 plays a key role in maintaining the morphological integrity of neuronal dendrites and synaptic transmission. |
| KCNN4    | Skaper, 2011; Sugunan et al., 2016 | KCNN4 channel may be a drug target in neurological diseases.                         |
| KREMEN1  | Wu and Murashov, 2013; Wang et al., 2019 | Decreasing the expression of kremen1 had a protective effect on neurons.            |
| LRG1     | Miyajima et al., 2013; Akiba et al., 2017 | Increased concentration of LRG in cerebrospinal fluid is related to the decline of human cognitive ability; overexpression of hippocampal LRG can lead to synaptic dysfunction and memory impairment. |
| MC1R     | Catania, 2008; Chen et al., 2017 | MC1R had a protective effect on neurons and the nigrostriatal dopaminergic system.  |
| N4BP3    | Schmeisser et al., 2013; Kiem et al., 2017 | N4BP3 had important functions in the development of neurites.                       |
| NLRP6    | Li et al., 2019; Zhang et al., 2020 | NLRP6 regulated the survival of neurons.                                              |
| SFRP2    | Aubert et al., 2002; Amura et al., 2005; Kele et al., 2012 | SFRP2 regulated the development of neurons and embryonic stem cells.                |
| SLC24A3  | Zhou et al., 2011 | SLC24A3 was highly specific in the substantia nigra of the adult rat brain.         |
| UBB      | Jung et al., 2018; Park et al., 2020 | Ubb is related to the differentiation of neural stem cell (NSC).                    |
DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE190518.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of Guangdong Medical University. Written informed consent to participate in this study was provided by the participants’ legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

YC and LC designed the study and conceptualization. YC, LC, DZ, and YJ finished the original draft. ZL, DZ, YW, QP, JL, JY, YC and LC designed the study and conceptualization. YC, LC, did the investigation and statistical analysis. YC and LC revised the investigation and methodology. All authors contributed to the article and approved the submitted version.

REFERENCES

Akiba, C., Nakajima, M., Miyajima, M., Ogino, I., Miura, M., Inoue, R., et al. (2017). Leucine-rich α2-glycoprotein overexpression in the brain contributes to memory impairment. Neurobiol. Aging 60, 11–19. doi: 10.1016/j.neurobiolaging.2017.08.014

Allen, S. J., Watson, J. J., Shoemark, D. K., Barua, N. U., and Patel, N. K. (2013). GDNF, NGF and BDNF as therapeutic options for neurodegeneration. Pharmacol. Ther. 138, 155–175. doi: 10.1016/j.pharmthera.2013.01.004

Amura, C. R., Marek, L., Winn, R. A., and Heasley, L. E. (2005). Inhibited neurogenesis in INK1-deficient embryonic stem cells. Mol. Cell. Biol. 24, 10791–10802. doi: 10.1128/MCB.25.24.10791

Aubert, J., Dunstan, H., Chambers, I., and Smith, A. (2002). Functional gene screening in embryonic stem cells implicates Wnt antagonism in neural differentiation. Nat. Biotechnol. 20, 1240–1245. doi: 10.1038/nbt763

Beurel, E., Toups, M., and Nemeroff, C. B. (2020). The bidirectional relationship of depression and inflammation: double trouble. Neurology 107, 234–256. doi: 10.1212/NEU.0000000000007165

Bilgic, A., Celikkol, S. C., Kilinc, I., and Akca, O. F. (2020). Exploring the association between depression, suicidality and serum neurotrophin levels in adolescents. Int. J. Psychiatry Clin. Pract. 24, 143–150. doi: 10.1080/13651501.2020.1723643

Boldrini, M., Santiago, A. N., Hen, R., Dwork, A. J., Rosoklija, G. B., Tamir, H., et al. (2013). Hippocampal granule neuron number and dentate gyrus volume in antidepressant-treated and untreated major depression. Neuropsychopharmacol. 38, 1068–1077. doi: 10.1038/nn.3315

Çakıcı, N., Sutterland, A. L., Penninx, B. W. J. H., Dalm, V. A., de Haan, L., and van Beveren, N. J. M. (2020). Altered peripheral blood compounds in drug-naïve first-episode patients with either schizophrenia or major depressive disorder: a meta-analysis. Brain Behav. Immun. 88, 547–558. doi: 10.1016/j.bbi.2020.04.039

Cao, X., Ma, J., Wu, G., Zhang, C., Wang, L., Dai, S., et al. (2012). Thymus-hippocampus are differentially afflicted in unipolar and bipolar depression: a meta-analysis. Brain Behav. Immun. 26, 163–169. doi: 10.1016/j.bbi.2011.05.005

Castillo, D. S., Campalans, A., Belluscio, L. M., Carcagno, A. L., Radicella, J. P., Canepa, E. T., et al. (2015). EZF2I and EZF2J induction in response to DNA damage preserves genomic stability in neuronal cells. Cell Cycle 14, 1300–1314. doi: 10.4161/cc.2015.14.11.73396

Castrén, E., and Monteggia, L. M. (2021). Brain-Derived Neurotrophic Factor signaling in depression and antidepressant action. Biological Psychiatry 90, 128–136. doi: 10.1016/j.biopsych.2021.05.008

Catania, A. (2008). Neuroprotective actions of melanocortins: a therapeutic opportunity. Trends Neurosci. 31, 353–360. doi: 10.1016/j.tins.2008.04.002

Chen, L. (2020). The expanding regulatory mechanisms and cellular functions of circular RNAs. Nat. Rev. Mol. Cell Biol. 21, 475–490. doi: 10.1038/s41580-020-0243-y

Chen, X., Chen, H., Cai, W., Maguire, M., Ya, B., Zuoo, F., et al. (2017). The melanoma-linked “redhead” MC1R influences dopaminergic neuron survival. Ann. Neurol. 81, 395–406. doi: 10.1002/ana.24852

Chen, Y. W., Lin, P. Y., Tu, K. Y., Cheng, Y. S., Wu, C. K., and Tseng, P. T. (2015). Significantly lower nerve growth factor levels in patients with major depressive disorder than in healthy subjects: a meta-analysis and systematic review. Neuropsychiatr. Dis. Treat. 11, 925–933. doi: 10.2147/NDT.S81432

GBD 2017 Disease and Injury Incidence and Prevalence Collaborators (2018). Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. Global Health Metr. 138, 1798–1859. doi: 10.1016/S0140-6736(13)6379-7

Cui, X., Niu, W., Kong, L., He, M., Jiang, K., Chen, S., et al. (2016). hsa_circRNA_103636: potential novel diagnostic and therapeutic biomarker in Major depressive disorder. Biomark. Med. 10, 943–952. doi: 10.2217/bmm-2016-0130

de Azevedo Cardoso, T., Mondin, T. C., Wiener, C. D., Marques, M. B., Fucolo, B. D. Á, Pinheiro, R. T., et al. (2014). Neurotrophic factors, clinical features and gender differences in depression. Neurochem. Res. 39, 1571–1578. doi: 10.1007/s11064-014-1349-4

Devoto, C., Lai, C., Qu, B., Guedes, V. A., Leete, J., Wilde, E., et al. (2020). Exosomal micrornas in military personnel with mild traumatic brain injury: preliminary results from the chronic effects of neurotrauma consortium biomarker discovery project. J. Neurotraum. 37, 2482–2492. doi: 10.1089/neu.2019.6933

Gokool, A., Loy, C. T., Halliday, G. M., and Voineagu, I. (2020). Circular RNAs the brain transcriptome comes full circle. Trends Neurosci. 43, 752–766. doi: 10.1016/j.tins.2020.07.007

Gonda, X., Petschnik, P., Eszlari, N., Baksa, D., Edes, A., Antal, P., et al. (2019). Genetic variants in major depressive disorder: from pathophysiology to therapy. Trends Neurosci. 43, 752–766.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnins.2022.844422/full#supplementary-material
postmortem study. J. Psychiatr. Res. 47, 1694–1699. doi: 10.1016/j.jpsychires.2013.07.005
Güleç, E., Iosifescu, D. V., and Tural, U. (2020). Plasma neuronal and glial markers and anterior cingulate metabolite levels in major depressive disorder: a pilot study. Neuropsychobiology 79, 214–221. doi: 10.1159/000505782
Huang, R., Zhang, Y., Bai, Y., Han, B., Ju, M., Chen, B., et al. (2020). N6-methyladenosine modification of fatty acid amide hydrolase messenger RNA in circular RNA STAG1-regulated astrocyte dysfunction and depressive-like behaviors. Biological psychiatry 88, 392–404. doi: 10.1016/j.biopsych.2020.02.018
Huang, Y., Wang, Y., Wang, H., Liu, Z., and Yu, X. (2019). Prevalence of mental disorders in China: a cross-sectional epidemiological study. Lancet Psychiatry 6, 211–224. doi: 10.1016/S2215-0366(18)30511-X
Hussain, N. K., Hsin, H., Huganir, R. L., and Sheng, M. (2010). MINK and TNIK differentially act on rap2-mediated signal transduction to regulate neuronal structure and AMPA receptor function. J. Neurosci. 30, 14786–14794. doi: 10.1523/JNEUROSCI.4124-10.2010
Jamali, S., Bartolomei, F., Robaglia-Schlupp, A., Massacrier, A., Peragut, J. C., Regis, J., et al. (2006). Large-scale expression study of human mesial temporal lobe epilepsy: evidence for dysregulation of the neurotransmission and complement systems in the entorhinal cortex. Brain 129, 625–641. doi: 10.1093/brain/awl001
Jung, B., Park, C., and Ryu, K. (2018). Temporal downregulation of the polyubiquitin gene Ubb affects neuronal differentiation, but not maturation, in cells cultured in vitro. Sci. Rep. 8:1. doi: 10.1038/s41598-018-21032-6
Kele, J., Andersson, E. R., Villaescusa, J. C., Cajanek, L., Parish, C. L., Bonilla, S., Ota, K. T., Liu, R., Voleti, B., Maldonado-Aviles, J. G., Duric, V., Iwata, M., et al. (2021). Implication of cerebral astrocytes in neuroinflammation severity and memory impairments in Alzheimer’s disease. Cell Death Dis. 12, 616. doi: 10.1038/s41419-021-03899-y
Phillips, C. (2017). Brain-derived neurotrophic factor, depression, and physical activity: making the neural plastic connection. Neural Plast. 2017:7260130. doi: 10.1155/2017/7260130
Rajkowska, G. (2000). Postmortem studies in mood disorders indicate altered numbers of neurons and glial cells. Biological Psychiatry 48, 766–777. doi: 10.1016/S0006-3223(00)00950-1
Rybak-Wolf, A., Stottmeister, C., Gläžar, P., Jens, M., Pino, N., Giusti, S., et al. (2015). Circular RNAs in the mammalian brain are highly abundant. Conserved Dynamically Expressed. Mol. Cell 58, 870–885. doi: 10.1016/j.molcel.2015.03.027
Saavedra, A., Baltazar, G., and Duarte, E. P. (2008). Driving GDNF expression: the green and the red traffic lights. Prog. Neurobiol. 86, 186–215. doi: 10.1016/j.pneurobio.2008.09.006
Sala Frigerio, C., Lau, P., Salta, E., Tournoy, J., Bossers, K., Vandenberghe, R., et al. (2013). Reduced expression of hsa-miR-27a-3p in CSF of patients with Alzheimer disease. Neurology 81, 2103–2106. doi: 10.1212/WNL.0000000000003706
Sheng, M. (2010). MINK and TNIK differentially act on rap2-mediated signal transduction to regulate neuronal structure and AMPA receptor function. J. Neurosci. 30, 14786–14794. doi: 10.1523/JNEUROSCI.4124-10.2010
Song, M., Martinowich, K., and Lee, F. S. (2017). BDNF at the synapse: why location matters. Frontiers in Neuroscience | www.frontiersin.org 10 March 2022 | Volume 16 | Article 844422
Wang, H., Lu, B., and Chen, J. (2019). Knockdown of IncRNA SNHG1 attenuated Aβ25-35-induced neuronal injury via regulating KREMEN1 by acting as a ceRNA of miR-137 in neuronal cells. *Biochem. Bioph. Res. Commun.* 518, 438–444. doi: 10.1016/j.bbrc.2019.08.033

Wang, Q., Zhao, G., Yang, Z., Liu, X., and Xie, P. (2018). Downregulation of microRNA1243p suppresses the mTOR signaling pathway by targeting DDIT4 in males with major depressive disorder. *Int. J. Mol. Med.* 41, 493–500. doi: 10.3892/ijmm.2017.3235

Wen, G., Zhou, T., and Gu, W. (2020). The potential of using blood circular RNA as liquid biopsy biomarker for human diseases. *Protein Cell* 12, 911–946. doi: 10.1007/s13238-020-00799-3

Wu, D., and Murashov, A. K. (2013). MicroRNA-431 regulates axon regeneration in mature sensory neurons by targeting the Wnt antagonist Kremen1. *Front. Mol. Neurosci.* 6:35. doi: 10.3389/fnins.2013.00035

Xiao, N., and Le, Q. (2016). Neurotrophic factors and their potential applications in tissue regeneration. *Arch. Immunol. Ther. Exp.* 64, 89–99.

Zhang, L., Verwer, R. W. H., Zhao, J., Huizinga, I., Lucassen, P. J., and Swaab, D. F. (2021). Changes in glial gene expression in the prefrontal cortex in relation to major depressive disorder, suicide and psychotic features. *J. Affect. Disorders* 295, 893–903. doi: 10.1016/j.jad.2021.08.098

Zhang, Y., Du, L., Bai, Y., Han, B., He, C., Gong, L., et al. (2018). CircDYM ameliorates depressive-like behavior by targeting miR-9 to regulate microglial activation via HSP90 ubiquitination. *Mol. Psychiatr.* 25, 1175–1190. doi: 10.1038/s41380-018-0285-0

Zhang, Y., Huang, R., Cheng, M., Wang, L., Chao, J., Li, J., et al. (2019). Gut microbiota from NLRP3-deficient mice ameliorates depressive-like behaviors by regulating astrocyte dysfunction via circHIPK2. *Microbiome* 7:116. doi: 10.1186/s40168-019-0733-3

Zhou, Q., Li, J., Wang, H., Yin, Y., and Zhou, J. (2011). Identification of nigral dopaminergic neuron-enriched genes in adult rats. *Neurobiol. Aging* 32, 313–326. doi: 10.1016/j.neurobiolaging.2009.02.009

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.

Publisher’s Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Copyright © 2022 Zhang, Ji, Chen, Chen, Wei, Peng, Lin, Yin, Li, Cui, Lin and Cai. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.