



## Research article

# Integrated analysis of transcriptional changes in major depressive disorder: Insights from blood and anterior cingulate cortex

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## ABSTRACT

**Background:** Major depressive disorder (MDD) was involved in widely transcriptional changes in central and peripheral tissues. While, previous studies focused on single tissues, making it difficult to represent systemic molecular changes throughout the body. Thus, there is an urgent need to explore the central and peripheral biomarkers with intrinsic correlation.

**Methods:** We systematically retrieved gene expression profiles of blood and anterior cingulate cortex (ACC). 3 blood datasets (84 MDD and 88 controls) and 6 ACC datasets (100 MDD and 100 controls) were obtained. Differential expression analysis, RobustRankAggreg (RRA) analysis, functional enrichment analysis, immune associated analysis and protein-protein interaction networks (PPI) were integrated. Furthermore, the key genes were validated in an independent ACC dataset (12 MDD and 15 controls) and a cohort with 120 MDD and 117 controls.

**Results:** Differential expression analysis identified 2211 and 2021 differential expressed genes (DEGs) in blood and ACC, respectively. RRA identified 45 and 25 robust DEGs in blood and ACC based on DEGs, and all of them were closely associated with immune cells. Functional enrichment results showed both the robust DEGs in blood and ACC were enriched in humoral immune response. Furthermore, PPI identified 8 hub DEGs (CD79A, CD79B, CD19, MS4A1, PLP1, CLDN11, MOG, MAG) in blood and ACC. Independent ACC dataset showed the area under the curve (AUC) based on these hub DEGs was 0.77. Meanwhile, these hub DEGs were validated in the serum of MDD patients, and also showed a promising diagnostic power.

**Conclusions:** The biomarker panel based on hub DEGs yield a promising diagnostic efficacy, and all of these hub DEGs were strongly correlated with immunity. Humoral immune response may be the key link between the brain and blood in MDD, and our results may provide further understanding for MDD.

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## 1. Introduction

Major depressive disorder (MDD) is a serious psychiatric disorder characterized by persistent sadness and loss of interest, affecting more than 300 million individuals worldwide [1], and causing a heavy economic burden on society and families. With the outbreak of the Coronavirus Disease 2019, the global mental health problem was further deteriorated [2]. Meanwhile, the treatment effectiveness of MDD is not ideal. It was estimated the response rate for antidepressant drugs was only 50% [3], and the recurrence rate exceeded 70% [4]. Furthermore, the diagnosis of MDD was mainly relied on clinical evaluation, and it was highly subjective. Owing to the complex and diverse causes, unclear pathological changes and high heterogeneity of clinical manifestations, its molecule mechanism remains unclear. Therefore, there is an urgent need to explore objective biomarkers for MDD.

As a brain region involved in the psychopathology of emotion regulation, anterior cingulate cortex (ACC) has been widely studied in depression [5–7]. Many neuroimaging studies observed the abnormal functional connectivity in ACC of MDD patients [8,9]. For example, a study from Bulgaria found the hyperconnectivity was existed between ACC and superior parietal lobule, lateral occipital cortex in MDD patients [10]. Another study found MDD patients emerged hyper-responses to loss outcomes in the ventral ACC [11]. The cortical thickness of the left anterior cingulate cortex was reduced in treatment-resistant MDD patients [12]. Moreover, an study from Australia confirmed that rostral ACC activity acted as a prognostic biomarker for depression [13]. Moreover, Huang et al. used meta analysis found that there were rsFC specific changes in each ACC subregion, and emotion, sensorimotor and cognition was related to the subgenual ACC, pregenual ACC and dorsal ACC, respectively [14].

Apart from the imaging studies, there were also a lot of other evidences indicating that ACC plays a crucial role in depression. For example, many previous studies showed deep brain stimulation therapy on ACC could alleviated depressive symptoms [15–18]. Furthermore, a study from Australia found kynurenine pathway was obvious altered from the postmortem ACC tissues [19]. Moreover, neuro-metabolites concentration was also observed in the pregenual ACC of MDD patients [20]. In addition, Yoshino et al. found chronic stress can significantly induce transcriptomic changes in ACC using animal models of depression [21–23]. Our previous study also showed increasing Nr4a2 in ACC may improve depression-like behavior by alleviating microglia dysfunction caused by neuro-inflammation [24].

Due to the characteristics of convenient, economical, easy accessibility and high acceptance rate by patients, blood has long been an ideal vehicle for developing biomarker for diseases [25]. In recent years, increasing studies found there were various molecules disturbed in MDD patients' blood, including some inflammatory factors, such as TNF- $\alpha$ , IL-6, IL-18 [26–28]. Cytokine levels in the blood could also reflect the therapeutic resistance of antidepressants [29]. Korucu et al. found nesfatin-1 may be a state marker in MDD with suicidal ideation [30]. During the past decades, many targets of MDD have been discovered [24,28], while vast of these studies focused on the molecule changes of blood or ACC alone [6,31–33], and these targets lack of intrinsic correlation. Furthermore, these

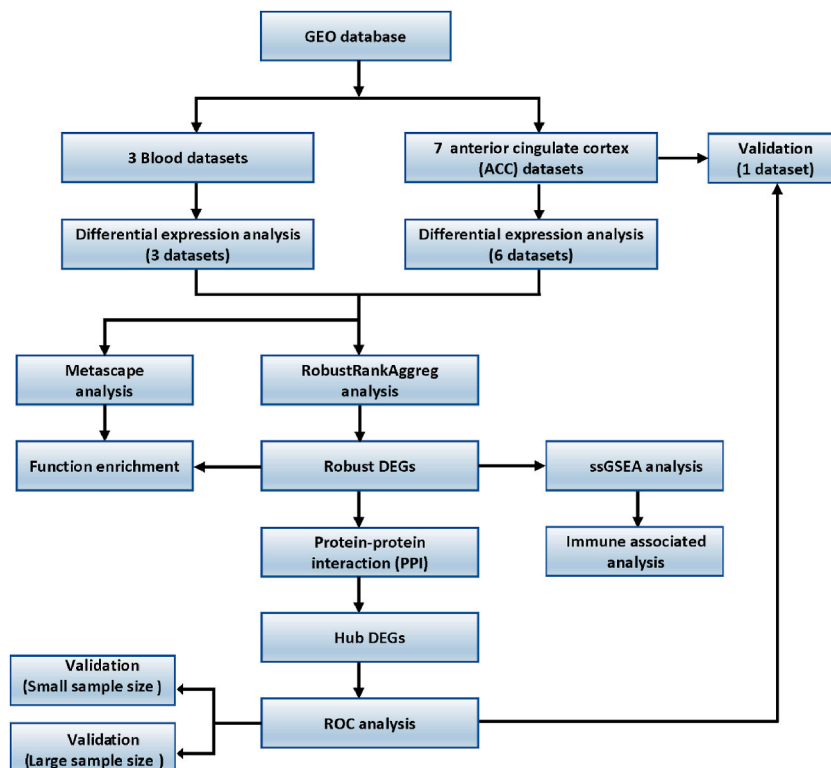


Fig. 1. The flowchart of this study.

studies also raised some common features, such as the limited sample size; unrealistic to use brain tissue as vehicle for diagnostic markers; the results were inconsistent and even contradictory.

Therefore, an integrated analysis is urgently needed to explore the intrinsic correlation between the blood and ACC. Previously, our work showed that blood showed more consistent transcriptional changes with ACC, rather than hippocampus, dorsolateral prefrontal cortex, orbitofrontal cortex, nucleus accumbens (unpublished data). Thus, in the current study, we integrated the transcriptomic profiles of blood and ACC to identify stably expressed genes, explore the intrinsic relationship and construct a blood potential biomarker panel for MDD, which may provide a deeper understanding to the molecule mechanism of MDD.

## 2. Materials and methods

### 2.1. Datasets selection

The whole design of this study was displayed in Fig. 1. By using the keywords of “depres\*”, “anterior cingulate cortex”, “BA 25”, “cingulate gyrus”, and “blood, we systematically searched the high-throughput data from the Gene Expression Omnibus (GEO, <https://www.ncbi.nlm.nih.gov/geo/>) database. The organism was limited to the homo sapiens only. Subsequently, we conducted further screening based on the following criterias: (1) both the MDD patients and healthy controls were enrolled in one study; (2) to avoid the bias of small sample size, the number of MDD patients and healthy controls should be at least 10 [34].

Ultimately, we obtained 10 public datasets in total. Among which, 3 blood datasets and 6 ACC datasets were used for exploratory analysis, and 1 ACC dataset (GSE102556, RNA-sequencing dataset) was used for validation. The datasets information, accession link, tissue extraction and processing et al. was shown in Supplementary Table 1.

### 2.2. Data preprocessing

We downloaded the original data of each dataset and conducted the same data preprocessing pipeline. For microarray data, we performed background correction, quantile normalization and log<sub>2</sub> transformation. Then, we annotated the chip probes into gene symbols by platform files provided by GEO. If a gene corresponded to multiple probes, only the probe owed the largest sum value was retained, and the probe with low level would be discarded. For RNA-sequencing data, we kept the genes with the count value > 5 at least of 80% of sample and annotated them into gene symbols using biomaRt (version:2.50.3) package.

### 2.3. Differential gene expression analysis

For the array datasets, the limma package (version 3.46.0) was adopted for differential expressed genes (DEGs). For the RNA-sequencing data, the edgeR package (version 3.32.1) was used to screen the DEGs. Only those genes that met the following two thresholds were defined as the DEGs: (1) the gene whose absolute value of logFC exceed “mean (abs (logFC)) + 2 \* sd (abs (logFC))”; (2) the gene whose *P* value < 0.05 [34].

### 2.4. RobustRankAggreg analysis

Owing to the high heterogeneity of MDD, it is necessary to screen the robust DEGs stably expressed. The RobustRankAggreg package (RRA, version: 1.2) can integrate multiple DEGs lists from different platforms by performing a comprehensive reordering and giving new *p*-values to screen DEGs with consistent and prioritized expression changes [35,36]. The top ranked genes in the reordered DEGs list with the lower *p*-values. The *P* value < 0.05 was defined as the threshold of robust DEGs [37].

### 2.5. Functional enrichment analysis

We performed enrichment analysis using Metascape [38](<https://metascape.org/>) to explore biological functions of the robust DEGs. The background database included biological process (GO: BP), molecular function (GO: MF) and cellular components (GO: CC). Those pathways with *P* value < 0.01 were considered to be statistically significant [34].

### 2.6. Immune associated analysis

ssGSEA analysis was used for evaluating the relationships between robust DEGs and immune functions based on GSEA package (version 1.38.2). It included the expression characteristics of 782 genes of 28 immune cells [39]. *P* value < 0.05 was considered to be statistically significant [40].

### 2.7. Protein-protein interaction network analysis

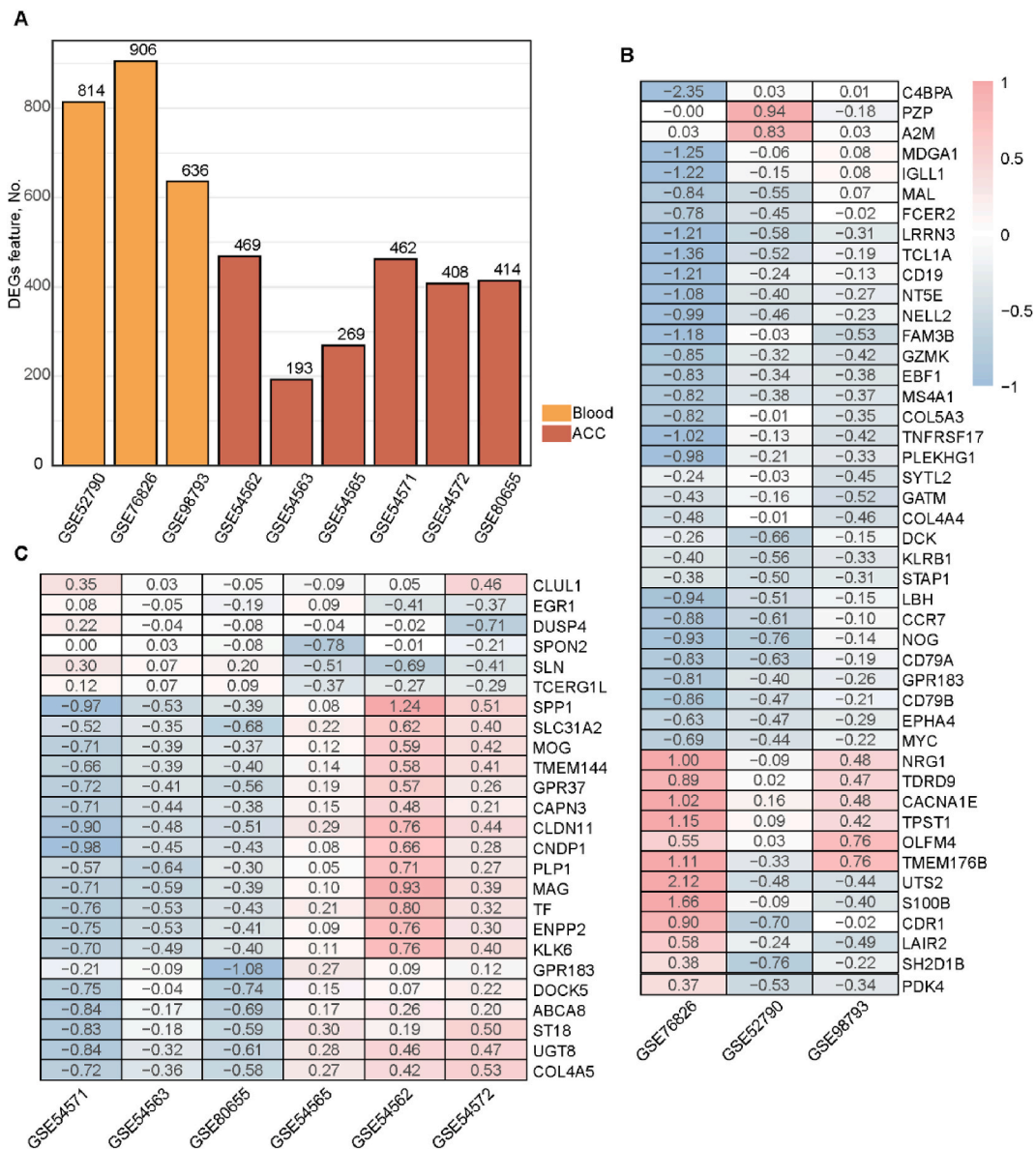
We imported the robust DEGs into STRING database (<https://cn.string-db.org/>, version 11.5) to predict the interactions among these robust DEGs. The protein-protein interaction (PPI) network adopted the default parameters (interaction score ≥ 0.40). Cytoscape software (<https://cytoscape.org/>, version 3.7.2) was used to visualize the PPI network. Furthermore, default parameters of cytoHubba plug-in (top 10 nodes ranked by MCC) and MCODE (degree cutoff 2) in Cytoscape were used to select the hub DEGs.

### 2.8. Diagnostic power of hub DEGs

To explore the diagnostic efficacy of the hub DEGs, an independent ACC RNA-sequencing data (GSE102556) was adopted for validation. Furthermore, we used the serum of MDD patients and healthy controls for validation. Briefly, we recruited MDD patients and healthy controls from the department of psychiatry and physical examination center in the First Affiliated Hospital of Chongqing Medical University, respectively. The detail demographic characteristics was provided in the [Supplementary Table 2](#). Enzymelinked immunosorbent assay (ELISA) kit of the hub DEGs were designed by the Jiangsu Meimian Industrial Co., Ltd (<http://www.mmbio.cn/>), and all of the procedure was performed according to the recommend instructions. All participants signed the informed consent, and this study was approved by the ethical committee of Chongqing Medical University (2017013).

### 2.9. Statistical analysis

All of the analysis was performed in R (<https://www.r-project.org/>, version 4.0.5) and SPSS (version 21.0). The mean and standard deviation (SD) was calculated for quantitative data, and the percentage was calculated for qualitative data. Univariate analysis was



**Fig. 2.** The DEGs and robust DEGs in blood and ACC. (A) The number of DEGs in each dataset. (B) The heatmap of the robust DEGs in blood. (C) The heatmap of the robust DEGs in ACC.

performed by Mann-Whitney *U* test or chi-square test. Correlation analysis was conducted based on Hmisc package (version 4.7-0). To explore the diagnostic efficacy of the hub DEGs, we used binary logistics regression to build the biomarker panel prediction model. Hosmer-Lemeshow goodness-of-fit test was used to evaluate the fitting of the model, and the area under the curve (AUC) was used to evaluate the diagnostic efficacy of the model. A *p*-value  $\leq 0.05$  (two tailed) was considered to be statistically significant.

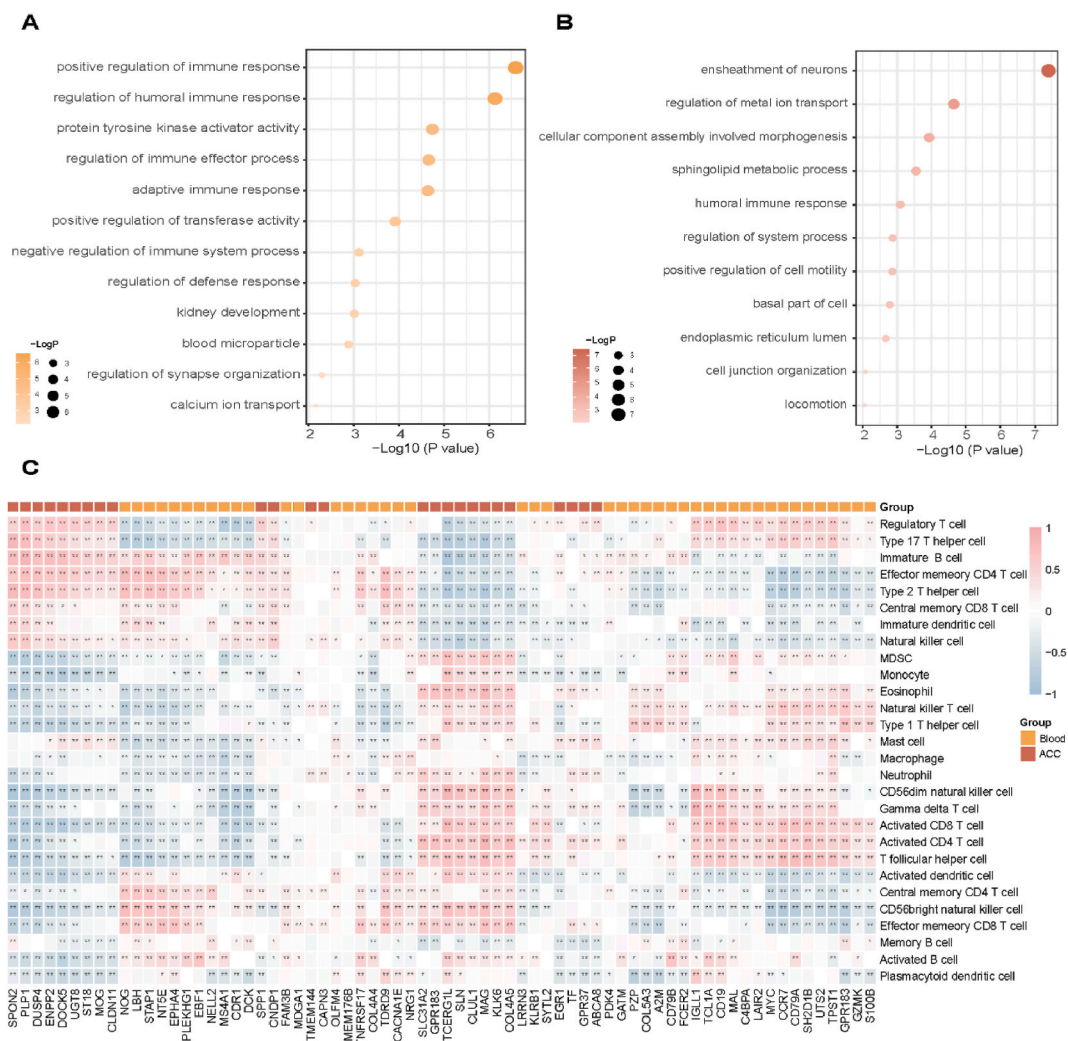
### 3. Results

#### 3.1. DEG analysis

For the blood, the differential gene expression analysis results showed there were 814, 906 and 636 DEGs in GSE52790, GSE76826 and GSE98793, respectively (Fig. 2A), and they were engaged in 735 up-regulated DEGs and 1476 down-regulated DEGs. For the ACC, there were 469, 193, 269, 462, 408 and 414 DEGs in GSE54562, GSE54563, GSE54565, GSE54571, GSE54572 and GSE80655, respectively (Fig. 2A, and they were engaged in 782 up-regulated DEGs and 1239 down-regulated DEGs. The volcano plot and logFC threshold of each dataset was displayed in Supplementary Fig. 1 and Supplementary Table 3. These results indicated that MDD was a highly heterogeneous disease, and involved extensive gene changes in blood and ACC.

#### 3.2. RRA integration analysis

To obtain the robust altered DEGs with priority in blood and ACC, we implemented RRA process for both two tissues. Finally, we



**Fig. 3.** The biological function and immune associated analysis of the robust DEGs. (A) The bubble plot of the robust DEGs in blood. (B) The bubble plot of the robust DEGs in ACC. (C) The heatmap between the robust DEGs and immune cells.

obtained 45 robust DEGs from 3 blood datasets (10 up regulated and 35 down regulated, Fig. 2B) and 25 robust DEGs from 6 ACC datasets (1 up regulated and 24 down regulated, Fig. 2C). The detailed information of the whole 70 robust DEGs were listed in the Supplementary Table 4. Furthermore, we explored the association of robust DEGs between the blood and ACC, results were showed in Supplementary Fig. 2.

### 3.3. Functional enrichment analysis of the robust DEGs

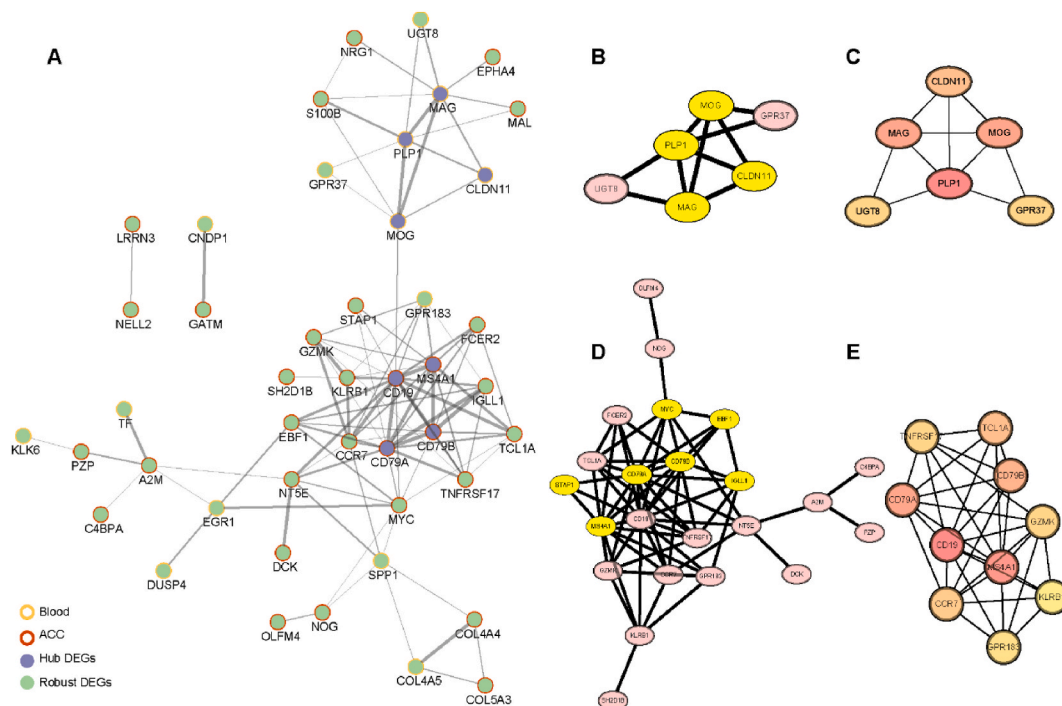
To explore the biological function of the robust DEGs, we conducted GO enrichment analysis. As for blood, 12 pathways were significantly enriched, and they were mainly related to immune processes, such as "positive regulation of immune response", and "regulation of humoral immune response" (Fig. 3A). Meanwhile, 11 pathways were enriched in ACC, including "ensheathment of neurons", "sphingolipid metabolic process", and transmembrane transport associated pathways, such as "regulation of metal ion transport", and "positive regulation of cell mobility" (Fig. 3B). These results were consistent with the enrichment analysis of original DEGs in blood and ACC datasets (Supplementary Fig. 3). Notably, an immune associated pathway was also enriched in ACC, named "humoral immune response". Thus, it suggested that gene changes in blood and ACC may be connected through the immune-related pathway.

### 3.4. Immune associated analysis of the robust DEGs

To further confirm our hypothesis, we explored the association between the robust DEGs and immune cell type by ssGSEA analysis. The ssGSEA results showed the robust DEGs in blood were significantly correlated with immune cells (Fig. 3C). Similarly, we found that the robust DEGs in ACC were also significantly related to immune cells (Fig. 3C). These results suggest that immune associated pathways may be the key links between the blood and ACC in MDD patients.

### 3.5. The hub DEGs identification

For ease of clinical practice, we hope to identify key regulators in a large number of robust DEGs by constructing PPI networks (Fig. 4A). By combining the cytoHubba and MCODE plug-ins to further cluster and segment the networks, we identified an ACC gene module including four membrane protein genes, namely PLP1, CLDN11, MOG and MAG (Fig. 4B and C). We also obtained a blood gene module including three immune-related genes (CD79A, CD79B, CD19) and one transmembrane gene MS4A1 (Fig. 4D and E).



**Fig. 4.** The hub DEGs of blood and ACC. (A) the overview of the hub DEGs. (B) the segment network in ACC. (C) the top ten nodes in ACC. (D) the segment network in blood. (E) the top ten nodes in blood.

### 3.6. Diagnostic power of hub DEGs

To evaluate the diagnostic efficacy of these eight hub DEGs, we constructed a combined diagnostic biomarker panel and performed ROC analysis in each dataset. The average AUC was 0.85, ranging from 0.70 to 1.00 (Fig. 5A, Supplementary Table 5). Subsequently, we used an independent ACC dataset (GSE102556) for further validation, and the AUC of this dataset was 0.77 (Fig. 5B).

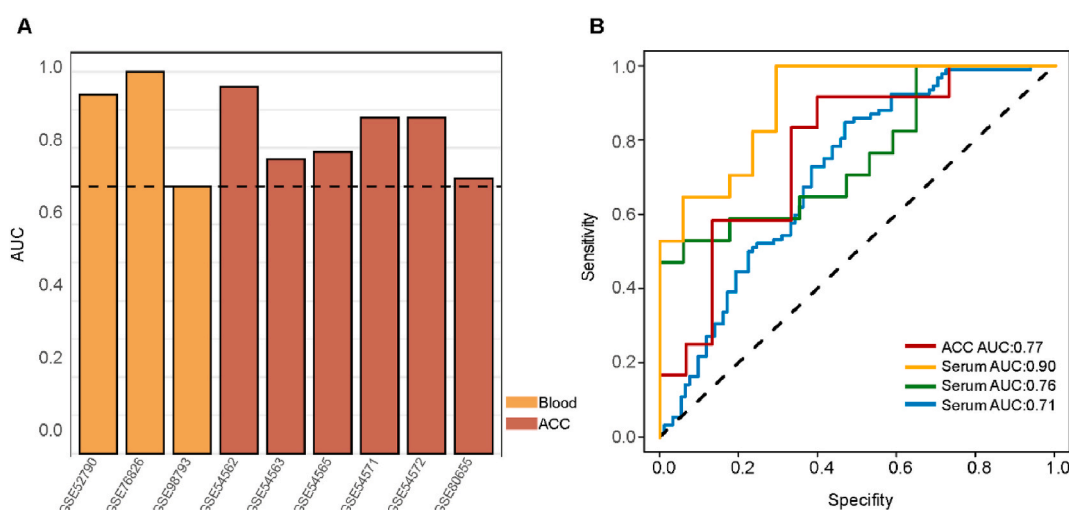
Furthermore, we validated the above hub DEGs in our cohort. Firstly, we used the serum of 17 MDD patients and 17 healthy controls for exploratory ELISA validation, and the results showed the AUC was 0.90 (Fig. 5B). Meanwhile, we found that only CD19, CD79A and MAG showed a significant downward trend in MDD patients (Supplementary Fig. 4), and the AUC of these three hub DEGs was 0.76 (Fig. 5B). Subsequently, we further verified the above three hub DEGs in a larger sample size with 103 MDD patients and 100 healthy controls, and found that CD79A and MAG was still down-regulated in MDD patients (Supplementary Fig. 5), and the AUC was 0.71 based on these two hub DEGs (Fig. 5B). Spearman correlation analysis showed that the correlation coefficients of CD79A, MAG and HAMD score were  $-0.19$  and  $-0.38$ , respectively ( $P < 0.05$ , Supplementary Fig. 4). All of the above models passed the Hosmer-Lemeshow goodness of fit test (Supplementary Table 5, Supplementary Table 6). The above results indicated the biomarker panel based on these hub DEGs displayed a promising prediction ability in MDD.

## 4. Discussion

MDD is a psychiatric disorder that severely disrupts the brain functions. Previous studies have shown there was obvious molecule dysfunction both in the central and peripheral tissues [24,29,30,41,42]. Increasing neuroimaging evidences also showed ACC played a vital role in MDD. For example, a study from China found the functional connectivity between the Distal ACC and left DLPFC was impaired, while, it got normalized after 12-week antidepressant treatment [43]. Jin et al. found aberrant structural connectivity between the rostral ACC and amygdala prospectively predates first onset of MDD [44]. Furthermore, Cheng et al. found a unique orbitofrontal cortex-subgenual ACC circuit abnormality can serve as an important biomarker for identifying refractory MDD patients [45].

In this study, enrichment analysis showed that the robust DEGs of blood were significantly related to immune response. Consistent with our findings, increasing evidences emerged indicated immune mechanism in blood contribute to the pathogenesis of MDD [46]. For example, a large whole-genome transcriptional study from the United Kingdom found genes with abnormal expression in MDD were involved in regulation and implementation of innate immune response [47]. Gwenaël G et al. found the over-expressed genes in MDD were associated with innate immunity, and the under-expressed genes were associated with the adaptive immune response [48]. Previously, we also found multiple interleukin and cytokine levels were disrupted in MDD patients, naturally occurring depression macaca fascicularis and rodent animal models of depression [49]. Furthermore, ssGSEA results in this study also found that robust DEGs in blood was significantly associated with immune cells.

Interestingly, the robust DEGs of ACC were also significantly enriched to humoral immune response. Furthermore, the robust DEGs in ACC were significantly associated with immune cells. These evidences suggested that the gene changes in ACC may be preserved in the blood, and immune pathway may be the intersection of blood and ACC in MDD. Furthermore, increasing studies supported there was neuroinflammation in brain of depression [50,51]. For example, our previous studies also observed there was widely neuroinflammation in animal models of depression [24,52]. Furthermore, Sha et al. used the integrative omics found the expression in ACC



**Fig. 5.** The AUC based on the hub DEGs. (A) the AUC in each dataset. (B) the AUC of the independent ACC and blood cohort. Red: the AUC of eight hub DEGs in GSE102556. Orange: the AUC of eight hub DEGs in ELISA (17 MDD patients and 17 Healthy Control). Green: the AUC of three hub DEGs in ELISA (17 MDD patients and 17 Healthy Control). Blue: the AUC of two hub DEGs in ELISA (103 MDD patients and 100 Healthy Control). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

was associated with immune response and transmembrane transport [51].

In addition, the robust DEGs in ACC were also significantly enriched in "ensheathment of neurons", "sphingolipid metabolic process", "transmembrane transport", suggesting the functional changes of DEGs were closely related to oligodendrocytes (OL). Previous studies have shown OL have multiple functions, like producing and maintains myelin, promoting neurotransmission [53,54]; secreting neurotrophic proteins [55], including nerve growth factor, brain-derived neurotrophic factor and neuronutrient-3; promoting neuron regeneration after injury [56]. In recent years, a large number of studies reported that OL were closely related to the occurrence and development of neuroinflammation in depression [57–59]. For example, Kokkosis et al. found a novel OL population with immune properties and myelination defects in MDD patients, in which OL line cells expressed immune-related markers [59]. Enache et al. found there was an increase of IL-6 and TNF- $\alpha$  levels brain parenchyma in the context of reduction of OL markers in MDD [58]. Franklin et al. found increasing OL progenitor cells and myelin regeneration were commonly observed in focal areas infiltrated by activated T cells in MDD patients [57,60]. In addition, Zhou et al. found OL lineage cells did play an indirect regulatory role in inflammatory induced mood disorders through multiple signaling pathways [54].

Based on the PPI network, eight hub DEGs (CD79A, CD79B, CD19, MS4A1, PLP1, CLDN11, MOG, MAG) were obtained and formed a biomarker panel. While, only CD79A and MAG were significant down-regulated in our validation cohort. B lymphocyte antigen receptor is a polymeric complex that includes an antigen-specific component, surface immunoglobulin (Ig), and surface Ig non-covalently binds to two other proteins, Ig- $\alpha$  and Ig- $\beta$ , which are essential for the expression and function of B cell antigen receptors. CD79A encodes the Ig- $\alpha$  protein of the B-cell antigen component, and they have been widely used as marker genes for B cell clustering in single-cell analysis [61,62]. Previously, the association between the CD79A and MDD was few explored. Yao et al. found that CD79A could be used as the accurate diagnostic indicators of late-onset MDD [63], while the molecular mechanism deserved further exploration.

As for MAG (myelin associated glycoprotein), it encoded a type I membrane protein, which was thought to be involved in the process of myelination and mediates myelin-neuron cell-cell interactions. Notably, It belonged to a member of the immunoglobulin superfamily, and was closely associated with immune. Previously, many studies found MAG was disrupted in MDD patients, while the direction was inconsistent. For example, Jiang et al. found that the level of MAG in the serum of MDD patients was significantly higher than that of healthy controls [64]. Scifo et al. found that the level of MAG was up-regulated in the subgenual ACC of MDD patients by label-free [65]. However, Al Shweiki et al. found decreased levels of MAG in the cerebrospinal fluid of MDD patients by iTRAQ [66]. By comparing the transcriptional expression profiles of the temporal cortex of MDD patients and healthy controls, Aston et al. found that the levels of MAG in MDD patients were significantly down-regulated [67]. Although the remaining six hub DEGs did not differ significantly in our cohort validation, they have also been shown to be strongly associated with occurrence of MDD in other studies (Supplementary Table 7).

In this study, we integrated multiple dataset from the GEO, and the relatively large sample size ensures the stability of the results. Furthermore, we explored the transcriptional changes of blood and ACC, simultaneously, which may provide further understanding for MDD. Moreover, the further independent cohort (ACC and serum) confirmed the stability of the above results.

## 5. Limitations

There are also some limitations in this study. Firstly, MDD was a disease involving in multiple brain regions, and only ACC was selected in this study, so the other brain regions also need to be further explored. Secondly, the raw datasets did not provide patients' antidepressant medication information, which may affect the gene expression and cause bias. Thirdly, the robust DEGs obtained using the RRA algorithm were also enriched to a similar pathway as the DEGs, while it would lose some important DEGs information. Fourthly, the hub DEGs was mainly derived from the PPI, and the role of left robust DEGs also should not be neglected. Fifthly, we validated the hub DEGs level in the MDD patients' serum, while the molecule mechanisms between the hub DEGs and MDD need to be further explored. Last but not the least, the association between the central and peripheral of MDD also deserves further investigation.

## 6. Conclusions

Through an integrated analysis, we found the biomarker panel composed of these hub DEGs showed a promising diagnostic efficacy for MDD patients, and humoral immune response may be the key link between the brain and blood in MDD.

### Data availability statement

The data in this study was the public available datasets, which can be found in Gene Expression Omnibus (GEO, <https://www.ncbi.nlm.nih.gov/geo/>) database.

### Ethics statement

This study was approved by the ethical committee of Chongqing Medical University (No:2017013), and all participants provided the informed consent.

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## CRedit authorship contribution statement

**Xiaogang Zhong:** Writing – review & editing, Writing – original draft, Visualization, Formal analysis, Data curation, Conceptualization. **Xiangyu Chen:** Conceptualization. **Yiyun Liu:** Funding acquisition, Data curation. **Siwen Gui:** Methodology, Funding acquisition, Data curation. **Juncaï Pu:** Writing – review & editing, Funding acquisition, Data curation. **Dongfang Wang:** Funding acquisition, Formal analysis, Data curation. **Wei Tao:** Visualization, Data curation. **Yue Chen:** Validation. **Xiang Chen:** Validation. **Weiyi Chen:** Visualization, Data curation. **Xiaopeng Chen:** Visualization, Validation. **Renjie Qiao:** Formal analysis. **Xiangkun Tao:** Formal analysis. **Zhuocan Li:** Formal analysis. **Peng Xie:** Project administration, Funding acquisition, Conceptualization.

## Declaration of competing interest

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

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.heliyon.2024.e28960>.

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