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Identification of autophagy-related genes as potential biomarkers correlated with immune infiltration in bipolar disorder: a bioinformatics analysis

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Abstract

Background Bipolar disorder (BPD) is a kind of manic and depressive phase alternate episodes of serious mental illness, and it is correlated with well-documented cortical brain abnormalities. Emerging evidence supports that autophagy dysfunction in neuronal system contributes to pathophysiological changes in neurological disease. However, the role of autophagy in bipolar disorder has rarely been elucidated. This study aimed to identify the autophagy-related gene as a potential biomarker Correlated to immune infiltration in BPD.

Methods The microarray dataset GSE23848 and autophagy-related genes (ARGs) were downloaded. Differentially expressed genes (DEGs) between normal and BPD samples were screened using the R software. Machine learning algorithms were performed to screen the significant candidate biomarker from autophagy-related differentially expressed genes (ARDEGs). The correlation between the screened ARDEGs and infiltrating immune cells was explored through correlation analysis.

Results In this study, the autophagy pathway was abundantly enriched and activated in BPD, as indicated by Pathway enrichment analysis. We identified 16 ARDEGs in BPD compared to the normal group. A signature of 4 ARDEGs (ERN1, ATG3, CTSB, and EIF2AK3) was screened. ROC analysis showed that the above genes have good diagnostic performance. In addition, immune correlation analysis considered that the above four genes significantly correlated with immune cells in BPD.

Conclusions Autophagy - immune cell axis mediates pathophysiological changes in BPD. Four important ARDEGs are prospective to be potential biomarkers associated with immune infiltration in BPD and helpful for the prediction or diagnosis of BPD.

Keywords Autophagy, Bipolar disorder, Immune, Machine learning algorithms, Macrophage, Monocyte

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Introduction

Bipolar disorder (BPD) is a severe mental illness in which a person's mood swings back and forth between mania and depression. Existing research shows that BPD affects more than 1% of the global population and is one of the major causes of psychological change in young people [1]. The latest data from the World Mental Health Surveys involving 156,331 respondents across 29 countries from 2001 to 2022 reveal that the probability of the first episode peaks around the age of 15, with a median onset age of approximately 20 for bipolar disorder [2]. In addition, patients with bipolar disorder show variable courses and often leading to cognitive impairment and significantly reduced quality of life [3, 4], and tend to get worse as the disease progresses [5, 6], and in more severe cases, individuals with bipolar disorder may face life-threatening outcomes, with a heightened association with incidents involving harm, such as suicide, interpersonal violence, and road injuries, as well as cardiovascular diseases, notably ischemic heart disease [7].

The Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition, Text Revision outlines stringent criteria for the diagnosis of bipolar disorder, necessitating the presence of at least one episode of mania or hypomania [8]. This nuanced clinical framework underscores the intricacies associated with the characterization and identification of bipolar pathology. The challenges faced by clinicians encompass discerning subtle variations in mood episodes, disentangling bipolar manifestations from related mood disorders, and negotiating the inherent subjectivity in symptom reporting. Despite the meticulous delineation of these clinical parameters, the molecular diagnostic landscape for individuals with BPD remains characterized by uncertainty.

In some studies of recurrent affective bipolar disorder, progressive brain structure and function changes have been observed in patients [9]. Recurrent episodes of long-term illness have been linked to reduced thickness in parts of the cerebral cortex, such as the prefrontal cortex, which may play an essential role in stress regulation [10]. Epigenetic mechanisms [11], mitochondrial dysfunction, pathways that maintain neuroplasticity, increased inflammation, and oxidative stress are all thought to contribute to neural progression in bipolar disorder [9].

An increasing body of evidence suggests that BPD is correlated with the physiological processes mediated by autophagy [12–14]. Autophagy is primarily involved in protein degradation pathways and plays a vital role in helping the body remove protein aggregates and misfolded proteins from healthy cells [15]. According to the present researches, changes in autophagy-related factors in the prefrontal cortex are involved in regulating depression-like behaviors [16].The Akt-mTOR pathway, which plays a crucial role in the regulation of autophagy, is reduced in the prefrontal cortex in BPD patients, and decreased activity leads to cognitive impairment associated with changes in synaptic connectivity and function [12]. Moreover, imaging, biochemical and genetic research has suggested that mitochondrial dysfunction is the core of BPD features [17]. Previous studies have demonstrated a complex interplay between autophagy and mitochondria, particularly in stress responses such as oxidative stress [18] and endoplasmic reticulum stress [19, 20]. In the context of bipolar disorder, dysregulation of these stress responses is implicated in the pathophysiology of the condition [21]. The intricate signaling pathways activated by stressors contribute to the dysregulation of autophagy and mitochondrial dynamics, potentially playing a crucial role in the progression of bipolar disorder.

Numerous studies have noted that immune disorders accompany patients with BPD (e.g., increased production of pro-inflammatory cytokines, activation of monocytes and macrophages, reduced T cell numbers or activity) [22–26]. Moreover, autophagy interacts with immune system. Autophagy can control the immune response by regulating the activity of immune cells and the production of cytokines [27]. In turn, autophagy is also significantly affected by a wide variety of immune cells and cytokines [28]. Consequently, it becomes crucially critical to examine the relationship between immune cells and autophagy-related genes (ARGs) in BPD.

Nevertheless, understanding the pathophysiology and molecular mechanisms leading to BPD is still limited, and more unambiguous evidence for the correlation between BPD and autophagy should be provided further. Early diagnosis is quite difficult for professionals who did not previously comprehend the longitudinal evolution of the disease because there are no exact biomarkers available.

This research sought to link the ARG signature to likely diagnosis of BPD. Furthermore, the immune status of BPD patients was further explored to reveal the potential immune infiltration pattern of ARGs in BPD, which may be helpful for diagnosing and treating BPD.

Materials and methods

Gathering and processing of gene expression omnibus (GEO) datasets

The GEO database was used in this investigation to explore the gene expression profile of BPD [29]. Data from 20 BPD group and 15 control group samples' peripheral blood gene expression matrices were included in the GSE23848 dataset, and the GPL6106 platform was used to evaluate the aforementioned information. The composition of BPD samples includes both patients undergoing medication treatment and those without medication treatment. Considering the diversity within the actual patient population, we did not conduct

separate analyses, aiming to provide a more comprehensive reflection of the characteristics of bipolar disorder. All data employed in the study originated from GEO, such that ethical approval and informed consent were not required. The workflow chart is illustrated in Fig. 1.

Selection and functional enrichment of DEGs in BPD patients

The GEO2R online analytic tool was used to find the DEGs between the BPD and normal groups [29]. In the identification of DEGs associated with BPD, our approach focused on an adjusted P-value threshold of less than 0.05. Unlike conventional methodologies, we opted not to incorporate a specific Log2 fold change threshold in our criteria. This decision was grounded in the consideration that BPD, as a complex and multifaceted disorder, may involve subtle yet biologically significant changes in gene expression. By exclusively utilizing the adjusted P-value criterion, we aimed to capture a comprehensive view of gene expression alterations without imposing restrictions on the magnitude of fold changes. This approach aligns with the intricate nature of psychiatric disorders, where nuanced expression changes can hold biological relevance. Based on the "clusterProfiler" package in the R programming language, enrichment studies for the Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) were carried out.

Acquisition of autophagy-related differential genes (ARDEGs)

We were able to extract 221 genes associated with autophagy from the human autophagy database (HADb, http://www.autophagy.lu/). The "venn" package in R was used to find the genes that are differentially expressed in autophagy. Principal Component Analysis (PCA) was employed to provide a comprehensive exploration and analysis of the identified genes.

Screening of potential biomarkers for diagnosis of bipolar disorder based on machine learning algorithm and construction of PPI network

A support vector machine (SVM), random forest (RF), and generalized linear model (GLM) were established depending on GSE23848. The response variable was BPD

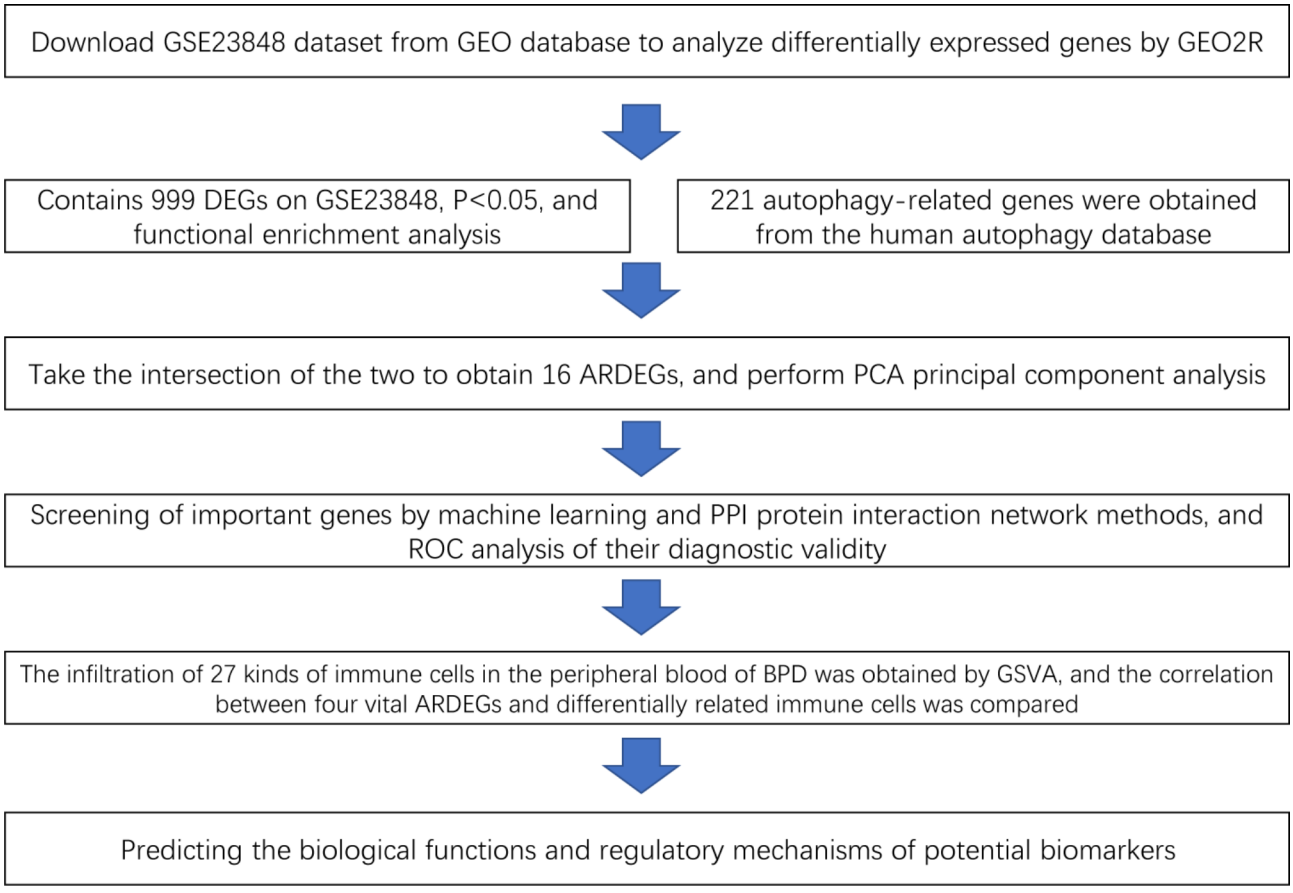

Fig. 1 Workflow of the research. Abbreviations are defined as follows, Gene Expression Omnibus database (GEO), differentially expressed genes (DEGs), Autophagy-related differentially expressed genes (ARDEGs), protein-protein interaction (PPI), Principal Component Analysis (PCA), Receiver Operating Characteristic (ROC)

diagnosis, while the explanatory variable was ARDEGs. The three models were examined using the interpretative capabilities of the "DALEX" package in R, and the residual distribution was improved using the test set. This research additionally examines the significance of these factors, screens the core genes employing the PPI network, and derives four significant explanatory variables. Finally, the "glmnet" package in R was used to build the Least Absolute Shrinkage and Selection Operator (LASSO) analysis with penalty settings for 10-fold cross-validation, which was utilized to validate the screened genes.

Evaluation of expression levels and diagnostic significance of crucial genes identified

The expression levels of the crucial genes were examined using the Wilcoxon rank-sum test. Additionally, a receiver operating characteristic curve (ROC) analysis was conducted to assess the diagnostic performance of these genes in differentiating between samples with BPD and healthy controls.

Immune status assessment

The ESTIMATE algorithm, employed in this study, was utilized to compute immune scores and stromal scores in the GSE23848 dataset for DEGs between the control and BPD groups. ESTIMATE (Estimation of Stromal and Immune cells in samples using Expression data) is a computational approach designed to infer the presence of stromal and immune cells within samples based on gene expression profiles.

Immune infiltration

A total of 27 immune-related cells and related genes were collected (for details, see Supplementary material_1) [30]. The R package "GSVA" was used to analyze the differential immune cell infiltration in the GSE23848 dataset; "ggpubr" was used for visualization; and "corrplot" was used to analyze the relationship between four crucially significant genes and immune cells. The Pearson test was performed to calculate the correlation coefficient, and the significance test.

Prediction of transcription factors

Using ChIP-X Enrichment Analysis (ChEA3), the transcription factors associated with ARDEGs were predicted [31]. A tool for TFs enrichment analysis and evaluating TFs of user-submitted gene sets, the ChEA3 database contains several gene banks from various sources.

Statistical analysis

Statistical analysis was conducted using the R programming language, specifically version 4.3.1. A significance threshold of $p<0.05$ was employed, indicating statistical significance.

Result

Differentially expressed genes and their functional enrichment in peripheral blood of BPD patients

In order to investigate if autophagy plays a role in the development of BPD, we analyzed the DEGs and their functional enrichment in peripheral blood in BPD patients compared to healthy individuals. 400 up-regulated genes and 599 down-regulated genes, totaling 999 DEGs, were found in GSE23848 (Fig. 2A). The expression profile of all DEGs was shown in the heatmap (Fig. 2B). Furthermore, the 999 DEGs were subjected to GO and KEGG functional enrichment analysis. The genes mentioned above, located inside of cytosol, which mostly display catalytic activity and nucleic acid binding, are positive regulators of the cellular protein metabolism process (Fig. 2C). According to the KEGG enrichment analysis (Fig. 2D), the highest-level classical pathways correlated with differentially expressed genes (DEGs) included autophagy, apoptosis, and Alzheimer's disease modulation. These findings suggest a close association between bipolar disorder (BPD) and autophagy, with a potential pivotal role of the immune system in BPD.

Acquisition of autophagy-related differential genes (ARDEGs)

The intersection of 999 DEGs and 221 Autophagy relative genes were further taken to screen out autophagy relative genes from DEGs. On that basis, 16 differential ARGs were obtained (Fig. 3A). Subsequently, ARDEGs were classified through PCA analysis. The results of the principal component analysis revealed that 16 genes exhibit a clear distinction between BPD and control group samples. This observation suggests the potential significance of these genes in the diagnosis of BPD; however, it should be noted that experimental confirmation is required to validate their diagnostic relevance (Fig. 3B). Figure 3C and D present the expression profiles of these 16 ARDEGs in different samples, displayed in the form of a heatmap and violin plot, respectively.

Selection of signature genes via support vector machine model algorithms, LASSO analysis and PPI network

The 16 ARDEGs above were employed as crucial genes to build three models to further narrow the scope of ARDEGs. As illustrated in Fig. 4(A-B), the SVM model is considered the optimal match for minimizing sample residues. Five significant explanatory variables (i.e., KLHL24, ERN1, ATG3, CTSB, and EIF2AK3) were selected from the SVM model (Fig. 4C). Next, the functional interaction of 16 ARDEGs was studied using STRING 11.5 (http://string-db.org). The KLHL24 gene

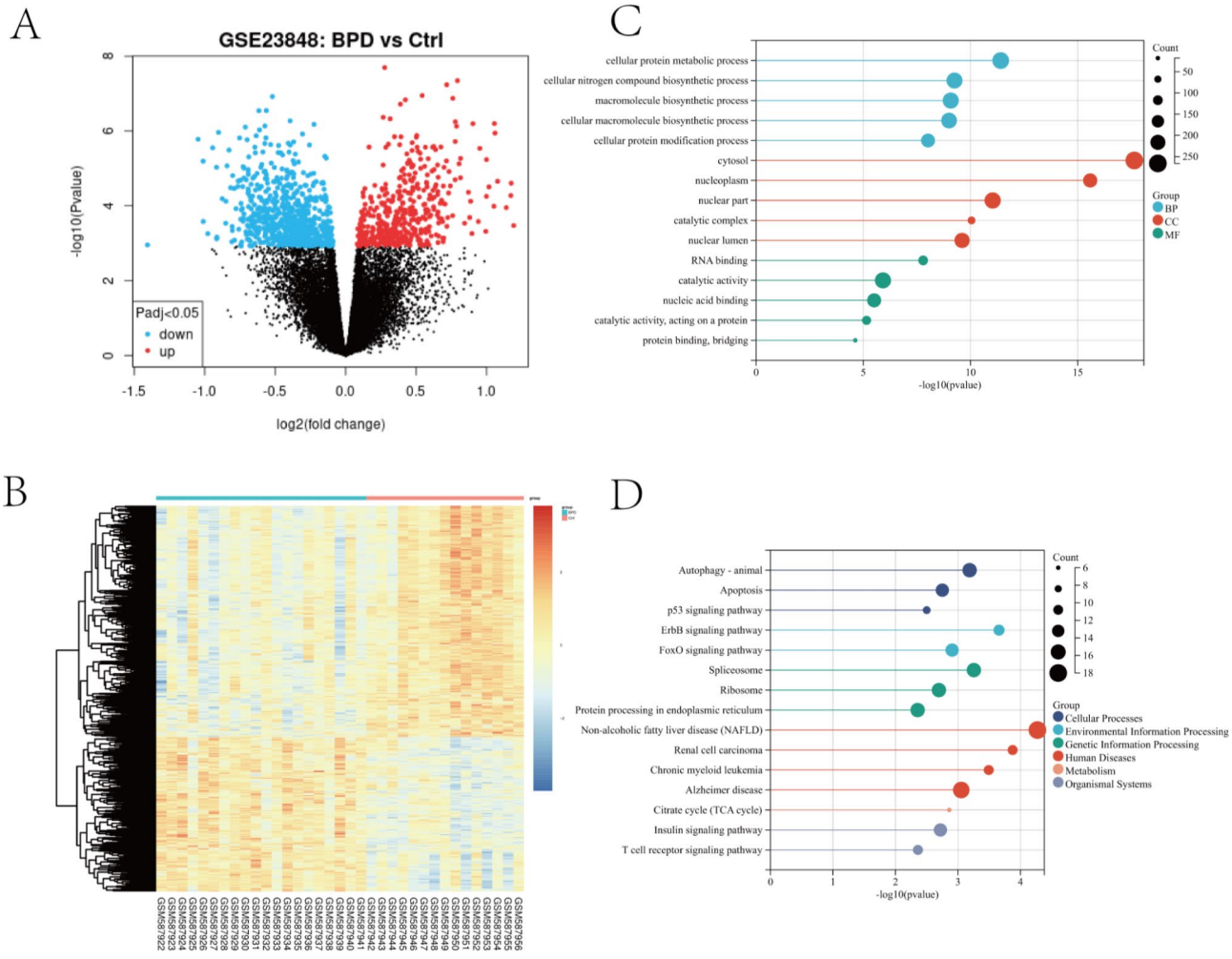

Fig. 2 Differentially expression analysis and Gene Ontology and KEGG pathway enrichment analysis. (**A**) Volcano plot of significant DEGs between BPD and Ctrl samples (BPD: Bipolar disorder; Ctrl: Control). (**B**) A heatmap of the DEGs. (**C**) The results of GO are presented in cnetplot. (**D**) The results of KEGG are presented in Lollipop illustration

without protein interaction was deleted, and four essential genes were obtained (Fig. 4D). Finally, on the basis of 16 ARDEGs expression matrices, the regression analysis model of LASSO was established, and the LASSO model was optimized and selected with the optimal λ value by using the minimum standard of 10-fold cross-validation (Fig. 4E). It was decided to employ a value of =0.0115532 with log (λ)= -4.460793. The log (λ) sequence was plotted against a coefficient profile (Fig. 4F). ERN1, ATG3, CTSB, and EIF2AK3 coefficients were 0.37669785, 0.33988395, 0.12711732, and −0.22049001, respectively.

Evaluation of the selected genes' diagnostic efficacy and expression levels in BPD

Subsequently, we performed further analysis on the four significant variables that the SVM model had filtered. The chromosomal locations of ERN1, ATG3, CTSB, and EIF2AK3 were shown in Fig. 5B. Patients with BPD reported higher peripheral blood levels of ERN1, ATG3, and CTSB than patients without the condition. However, compared to control group patients, EIF2AK3 expression in peripheral blood was lower in BPD patients (Fig. 5A and C). The following four markers can effectively discriminate between BPD and control group patients, as demonstrated in the PCA analyses in Fig. 5D, demonstrating that they are essential for diagnosing BPD. In this regard, ROC analysis verified the diagnostic validity of the above four genes for BPD. AUC greater than 0.800 could diagnose BPD with excellent specificity and sensitivity. The AUC values of ERN1, ATG3, CTSB, and EIF2AK3 were 0.827 (95%CI 0.933-0.600), 0.867 (95%CI 0.933−0.750), 0.862 (95%CI 0.867−0.850) and 0.882 (95%CI 0.733–0.950), respectively, As shown in Fig. 5E-H. Furthermore, a multi-gene combined diagnosis model was built, and the result indicated that the AUC of those models are all greater than 0.9 and the 4-gene model was 1 through logistic regression analysis (Fig. 5I).

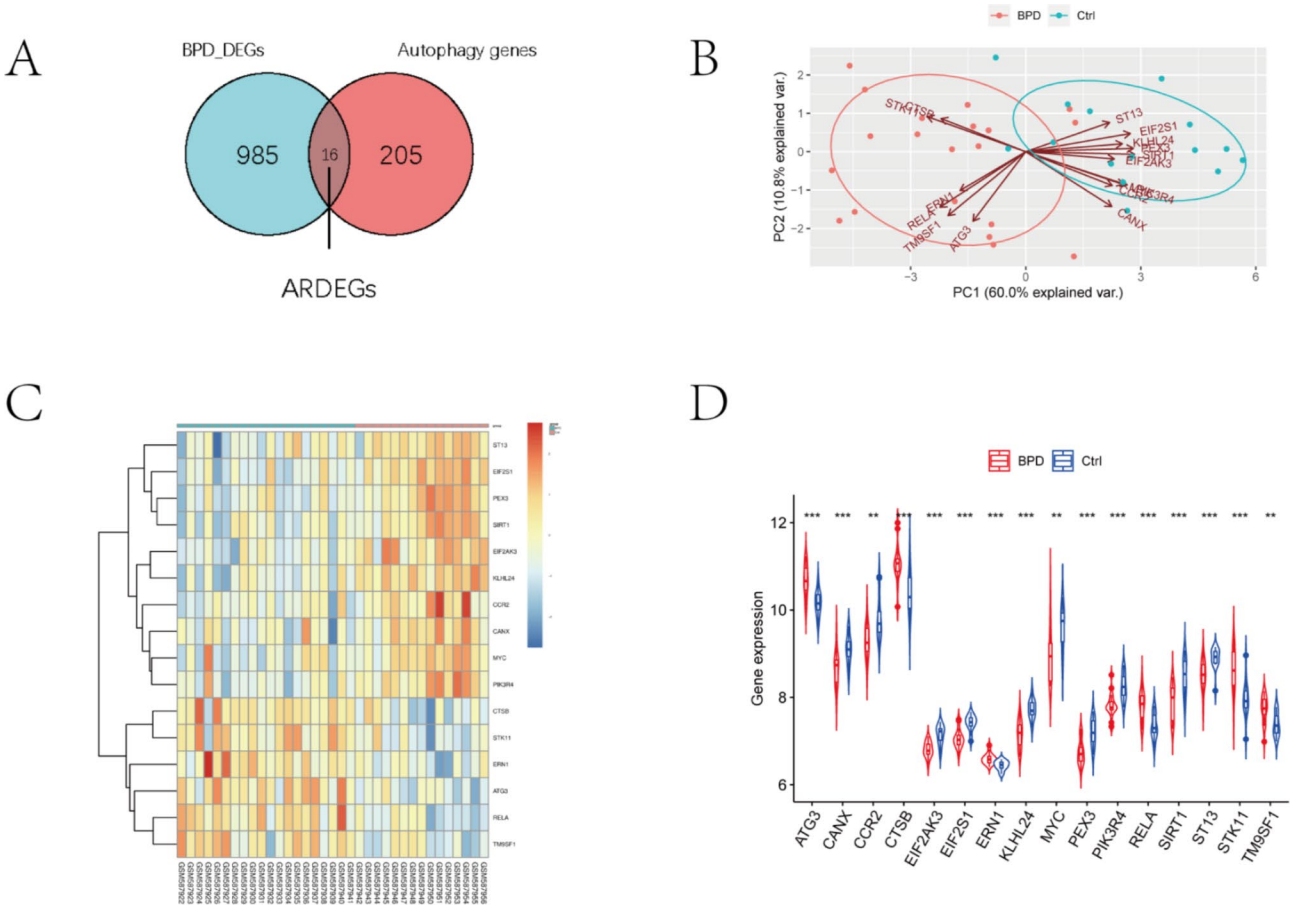

Fig. 3 Identification of ARDEGs. (**A**) Venn diagram was generated to obtain the intersection of DEGs in GSE23848 dataset and ARGs originating from the Human Autophagy Database covered 16 ARDEGs. (**B**) PCA analysis was performed to classify infiltrating ARDEGs between BPD and normal peripheral blood samples. The expression levels of ARDEGs are presented in the heatmap (**C**) and vioplot (**D**). *$p<0.05$, **$p<0.01$, ***$p<0.001$

Distribution of Immune Infiltration Pattern in BPD patients

Existing research has suggested that autophagy is significantly correlated with the immune system [27, 32–35]. To gain insights into the interaction between immunity and autophagy in BPD, we evaluated the immune status of DEGs in patients with BPD compared with healthy people based on the ESTIMATE algorithm. As shown in Fig. 6, BPD patients had a lower immune score (Fig. 6A. Wilcoxon test, $p=9.8E-07$) and higher stromal scores (Fig. 6B. Wilcoxon test, $p=3.1E-07$), which indicated immune dysfunction might participate in the occurrence and development of BDP patients.

Subsequently, we utilized the R package 'GSVA' to assess the relative proportions of the 27 immune cell biomarkers in each sample, aiming to gain a more comprehensive understanding of the immune microenvironment's functionality in BPD. The analysis revealed statistically significant associations for 21 distinct types of immune cells. Using a heatmap and a boxplot, we could compare 21 immune cell infiltrations across samples BPD and normal. We found a variety of immune cell infiltration abundances that were statistically significant between BPD and control group samples and a correlation between the above differentially associated immune cells. In this regard, we found that the four essential ARDEGs above were correlated with differentially related immune cells, such as ERN1 showed a positive correlation with Macrophage ($R=0.38$, $P=7.83E-07$) and Monocyte ($R=0.39$, $P=3.68E-07$), ATG3 showed a positive correlation with Macrophage ($R=0.51$, $P=1.43E-07$) and Monocyte ($R=0.56$, $P=1.16E-09$), and CTSB showed a positive correlation with Macrophage ($R=0.70$, $P=5.82E-13$) and Monocyte ($R=0.69$, $P=4.62E-12$). In addition, EIF2AK3 showed a negative correlation with Macrophage ($R=-0.70$, $P=3.36E-15$) and Monocyte ($R=-0.65$, $P=1.02E-13$). (Fig. 6F)

Biological function analysis and transcription factor regulation mechanism of the above four genes

We conducted GO and KEGG functional enrichment analyses for these four putative biomarkers in order to learn more about the biological processes and regulatory mechanisms of ERN1, ATG3, CTSB, and EIF2AK3. The result indicated that the above markers were not only

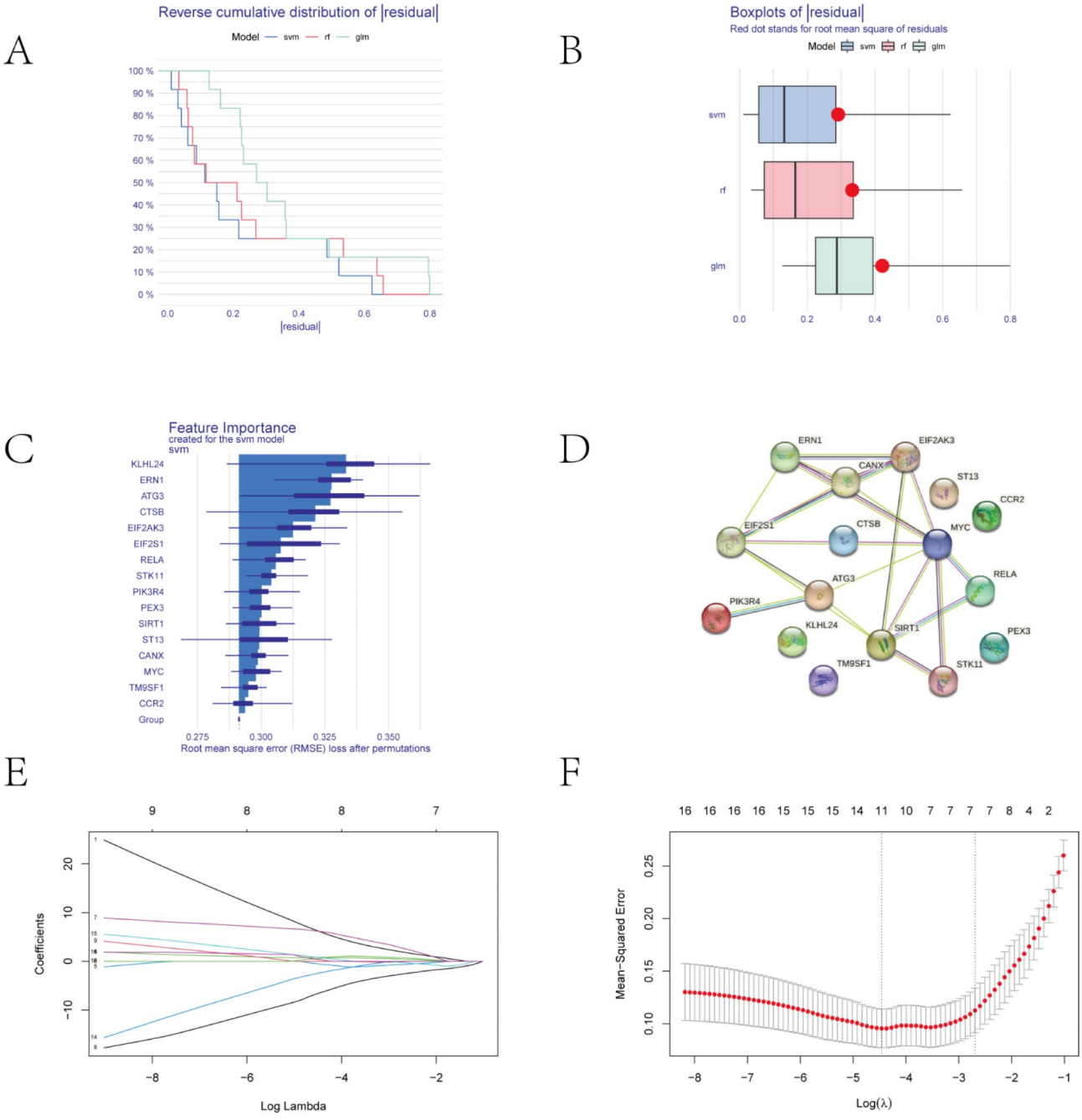

Fig. 4 Construction and assessment of RF, GLM and SVM model. (**A**) Cumulative residual distribution map of the sample. (**B**) Boxplots of the residuals of the sample. Red dot stands for root mean square of residuals. (**C**) The significance of the variables in the SVM model. (**D**) Functional protein association network of ARDEGs constructed using STRING dataset. (**E**) LASSO coefficient profiles. (**F**) Ten time cross-validation for tuning parameter selection in the LASSO model

correlated with autophagy, but they were likely to regulate pathophysiological processes (e.g., apoptosis (adjust. $P<0.001$) and Alzheimer's disease (adjust. $P<0.01$)). (Fig. 7A-D)

Furthermore, the transcription factors regulating the above four genes were predicted through the CHA3E website to study the regulatory mechanisms of ERN1, ATG3, CTSB, and EIF2AK3, and 1632 transcription factors were obtained in total. A total of 377 transcription factors were obtained by removing blank values (Fig. 7E, Please refer to Supplementary material_2 for detailed content). The above four genes were compared with the TF target gene set ChEA3 library assembled from multiple orthogonal omics datasets. The Fisher precision

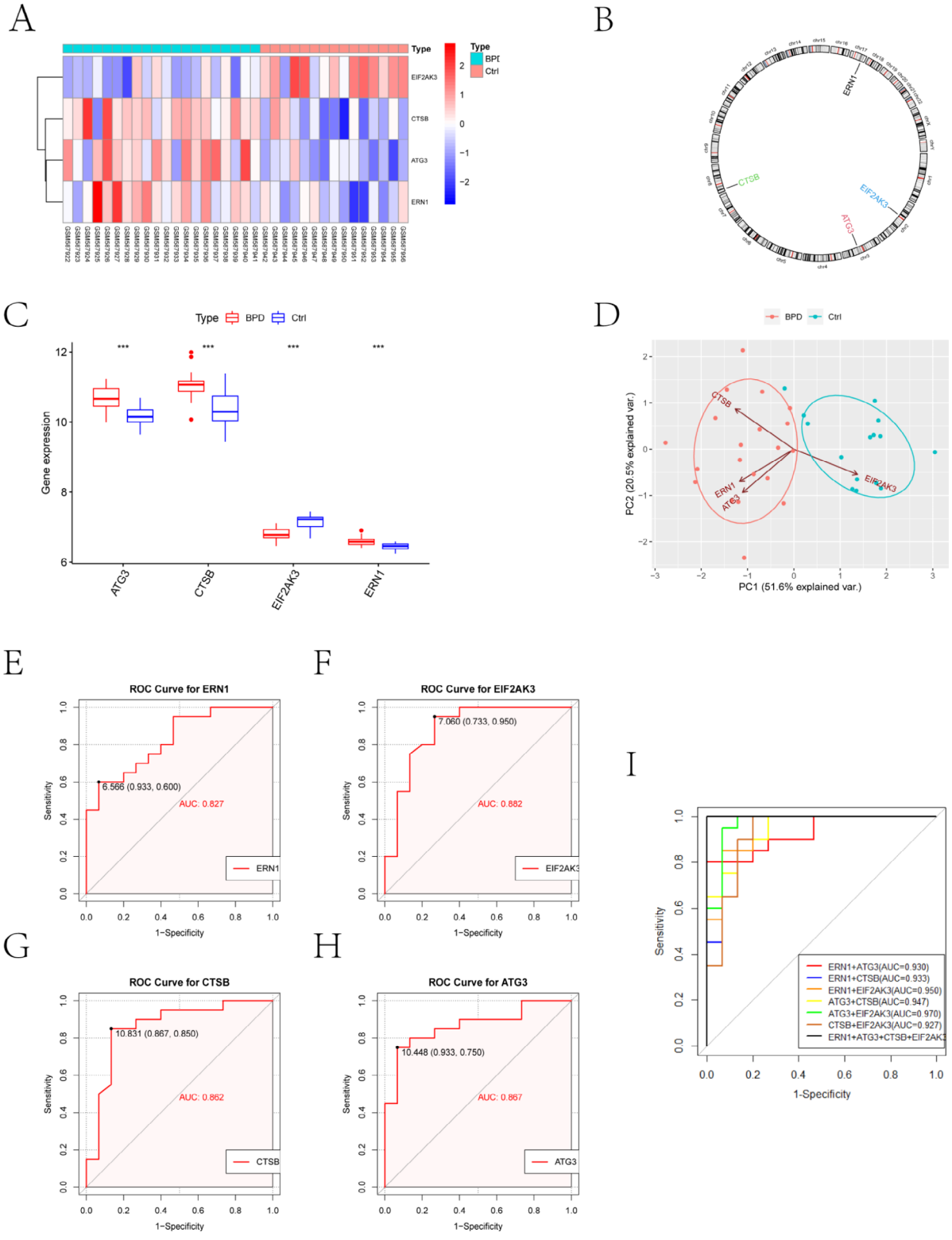

Fig. 5 Relative expression level of the 4 ARDEGs. (**A**) Heat map of the expression pattern of the 4 ARDEGs. (**B**) The chromosomal locations of the 4 ARDEGs (**C**) The relative expression level of the 4 ARDEGs between BPD and control group from GSE23848 dataset. (**D**) Principal component analysis shows that the four genes aforementioned can clearly distinguished BPD and control group. (**E**-**H**) The GSE23848 dataset was used to validate the diagnostic effectiveness of the four important ARDEGs by ROC analysis. (**I**) The diagnostic performance of multi-gene combined model was calculated based on the 4 genes' expression. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

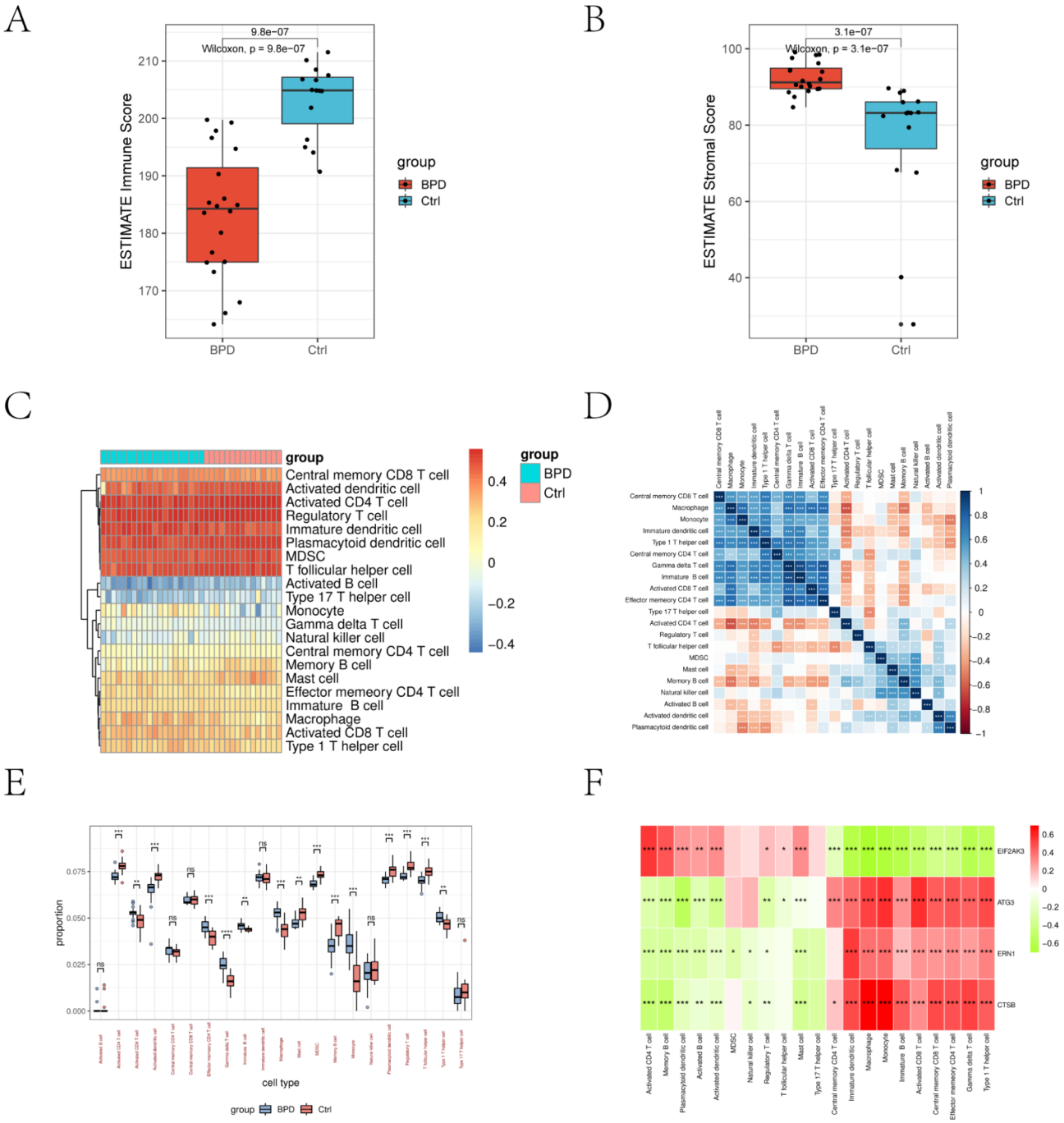

Fig. 6 Assessment of immune status and immune cell infiltration patterns in BPD samples and control group samples. The immune score (**A**) and the stromal score (**B**) of DEGs in patients with bipolar disorder compared to healthy people based on the ESTIMATE algorithm. (**C**) Heatmap of 21 immune cell subpopulations in each BPD and control group sample. (**D**) Correlation heatmap of 21 immune cells. (**E**) Boxplot showing the differentially infiltrated immune cells between the two groups. (F) Correlation among four important ARDEGs and 21 differential immune cells. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

test (background size 20000) was used to compare the 4 screened genes with the TF target gene set to determine which TF might be most closely related to the 4 genes and to screen out the top 10 core transcription factors most related to each gene. (Fig. 7F-I. Table 1)

Discussion

In the clinical setting, the precise and timely diagnosis of BPD continues to pose a formidable challenge, regardless of the overt manifestation of symptoms. Therefore, the diagnosis and treatment of BPD still need to be vigorously promoted. This work identifies useful diagnostic

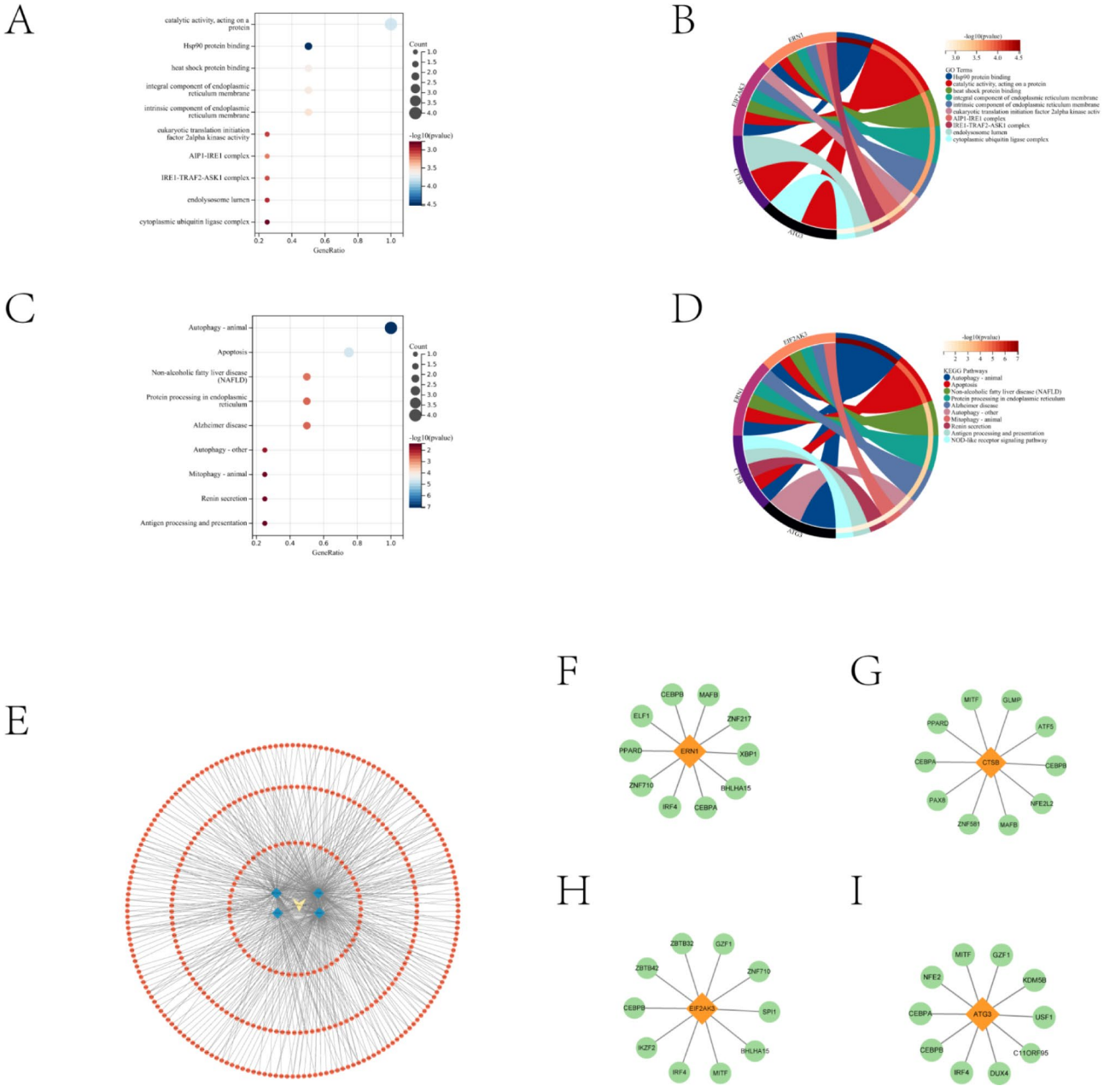

Fig. 7 Functional correlation analysis, and Prediction of transcription factors for ARDEGs. (**A**, **B**) The results of KEGG were presented by bubble and circle charts. (**C**, **D**) The results of GO were shown by bubble and circle graphs. (**E**) The TF enrichment analysis of four important ARDEGs. Blue diamond indicate ARDEGs, and red circles indicate predicted TFs (Supplementary material_2). (**F**-**I**) Top10 TFs network of four ARDEGs

biomarkers for BPD and sheds light on the landscape of autophagy associated with the disorder. Additionally, this study examines the relationship between immune cells and ARDEGs, which are involved in the onset and progression of BPD. In a nutshell, this study used bioinformatics analysis to first pinpoint four ARDEGs as possible biomarkers associated with immune infiltration in BPD patients.

We integrated the differentially expressed genes of patients with bipolar disorder and the gene set of the Human Autophagy Database. Based on machine learning methods, we screened four important ARDEGs (ERN1, ATG3, CTSB, and EIF2AK3). An essential sensor of unfolded proteins in the ER is the signaling enzyme endoplasmic reticulum nuclear signal 1 (ERN1) [36]. According to a recent study, exosomal microRNA-124-3p from bone marrow mesenchymal stem cells may reduce ERN1 levels and thereby lessen nerve damage in spinal cord ischemia reperfusion injury [37]. Unfolded protein response (UPR) results in neuronal apoptosis by

Table 1 The top 10 transcription factors of prediction results for the 4 ARDEGs

ERN1/Rank	TFs	Mean Rank	CTSB/Rank	TFs	Mean Rank
1	BHLHA15	19	1	NFE2L2	71.4
2	IRF4	62	2	ZNF581	71.67
3	MAFB	113.3	3	GLMP	105
4	CEBPA	129.4	4	MAFB	113.3
5	CEBPB	141	5	MITF	114.8
6	PPARD	144.3	6	CEBPA	129.4
7	ZNF710	159	7	PAX8	132.3
8	ZNF217	167.8	8	ATF5	137
9	XBP1	175.3	9	CEBPB	141
10	ELF1	178.2	10	PPARD	144.3
ATG3/Rank			EIF2AK3/Rank		
1	C11ORF95	31	1	BHLHA15	19
2	IRF4	62	2	IRF4	62
3	GZF1	68.33	3	GZF1	68.33
4	DUX4	109.5	4	MITF	114.8
5	MITF	114.8	5	ZBTB32	140.3
6	CEBPA	129.4	6	CEBPB	141
7	CEBPB	141	7	IKZF2	147
8	KDM5B	192.4	8	ZNF710	159
9	USF1	202	9	SPI1	165
10	NFE2	213.4	10	ZBTB42	165.5

This provides a ranking of transcription factors whose putative transcriptional targets are most closely similar to the query set. Integrated results, which take into account results from all libraries, are sorted in ascending order by score. Lower scores indicate more relevancy to the transcription factor

regulating ERN1/IRE1 or EIF2AK3/PERK pathways [38]. In mental diseases such schizophrenia, depression and post-traumatic stress disorder, aberrant neuronal death is mediated by endoplasmic reticulum stress [39]. The endoplasmic reticulum stress pathway involves ERN1, which has a strong correlation with autophagy [40–42]. Our results indicated that ERN1 was up-regulated in patients with BPD compared with normal, suggesting a complex interplay between endoplasmic reticulum function, autophagy, and BPD. Autophagy-related gene 3 (ATG3) refers to an enzyme primarily known for its role in the lipidation of LC3B, and it is of critical significance to autophagy. More recently, dysfunctional mitochondria in early BPD have been identified by co-localizing mitochondria (Hsp60) with autophagosomes (LC3B) in BPD patient cells [43]. This finding suggested that ATG3 was implicated in the development of BPD, and this research verified that the expression level of ATG3 in blood samples of BPD patients was greater than that of the control group. A member of the cysteine cathepsin family, CTSB (cathepsin B), contributes to neuropathological alterations in Alzheimer's disease and traumatic brain injury in addition to being strongly linked to autophagy [44–46]. The hippocampus and cortical areas of healthy individuals' brains were discovered to have significant levels of CTSB expression [47]. As revealed by the transcriptome analysis of different brain regions in the mouse limbic system, CTSB is closely correlated with mood [48]. Our investigation discovered that BPD patients had much greater levels of CTSB expression in their peripheral blood than the control group, indicating that CTSB may have a special function in BPD. EIF2AK3(Eukaryotic translation initiation factor 2α kinase 3) may be involved in the pathogenesis of BPD by regulating ER stress. The down-regulation of PERK/EIF2AK3 leads to increase of endoplasmic reticulum stress and impaired apoptosis induction, antioxidant response and autophagy impaired flux [49]. Our study found that the expression of EIF2AK3 in the blood samples of BPD patients was lower than that of the control group. In brief, it is speculated that the four ARDEGs may become the peripheral blood monitoring markers for BPD. In order to confirm our suspicions, ROC curves were created to assess the four aforementioned genes' diagnostic effectiveness. In this study, the AUCs of the above ARDEGs were all greater than 0.8, and the combination of multiple genes achieved a higher diagnostic efficiency.

There is growing proof that immune dysfunction and inflammation play a significant role in the development of bipolar disorder [9, 50]. Actually, a number of autoimmune processes can have an impact on the brain, and this may explain various psychiatric problems. As evidenced by pathogenic microglial over-activation and elevated levels of pro-inflammatory cytokines, manic and depressive episodes are associated with the activation of neuroinflammation mechanisms [51–53]. 27 immune-associated cells from the BPD and healthy groups were examined for immune cell infiltration in this investigation. The expression of activated CD8 T cell, effector memory CD4 T cell, gamma delta T cell, immature B cell, macrophage, monocyte, macrophage, and Type 1 T helper cells in BPD was significantly up-regulated. However, the expression of myeloid-derived suppressor cell (MDSC), activated CD4 T cell (activated dendritic cell), memory B cell, mast cell, plasmacytoid dendritic cell, regulatory T cell, T follicular helper cell was significantly down-regulated. In existing research, significant changes in the circulating frequency of T cells and their subsets, playing a key role in cellular immune response, have been identified in patients with BPD [22–24]. The result indicated that patients with bipolar disorder showed a significant reduction in regulatory T cells and a significant bias towards high levels of Type 1 T helper cells rather than Type 2 T helper cells [25]. Drexhage, R.C. et al. verified that BPD patients had considerably higher levels of proinflammatory monocyte expression [54]. High levels of heterogeneity and plasticity allow macrophages to develop into M1 and M2 macrophages under various circumstances. Macrophages mostly differentiate into the pro-inflammatory M1 type in the peripheral blood

microenvironment of BPD [26]. Additionally, dendritic cells have been linked to the genesis of BPD. They exhibit abnormalities in BPD and totally recover or even activate after receiving lithium in vivo [55, 56]. In light of the fact that our study's findings agree with other studies, bioinformatics analysis is used to highlight the importance of the aforementioned immune cells in the pathogenesis of BPD.

Based on the significance of autophagy and immunological infiltration in BPD, the link between four efficient autophagy-related biomarkers (i.e., ERN1, ATG3, CTSB, and EIF2AK3) and various immune cells in BPD was further investigated. The results for Macrophage and Monocyte, which have a strong correlation with the four ARDEGs, are the most interesting. Macrophage and monocyte demonstrated positive correlations with ERN1, ATG3, and CTSB. Conversely, Macrophage and Monocyte had a negative connection with EIF2AK3. Existing research has suggested that ERN1 and EIF2AK3 mediate oxidative stress and induce macrophage autophagy [57], and they have a specific function in the control of monocyte alterations brought on by inflammation [58]. CTSB is mainly expressed in macrophage and promotes collagen synthesis in the infiltrated region of macrophage [59]. Thus, the notion that the autophagy-related gene-immune cell axis plays a crucial role in the early pathogenesis of BPD was put out. This theory still requires experimental confirmation.

Discussion section: limitations

In the pursuit of scientific inquiry, it is imperative to acknowledge and critically evaluate the limitations inherent in our study. By transparently addressing these limitations, we aim to provide a comprehensive understanding of the scope and potential impact of our findings.

One notable limitation of our study lies in the reliance on a single database, namely the Gene Expression Omnibus (GEO). While GEO offers valuable and relevant gene expression data related to bipolar disorder, the exclusivity of this database introduces a potential constraint. The lack of multiple database inclusion may limit the generalizability of our findings and could impact the robustness of the conclusions.

This study conducted a thorough exploration of the GEO database and considered datasets such as GSE46449, GSE62191, and GSE124326 for multifaceted validation of our experiments. However, discrepancies in sample composition and experimental design were identified, rendering these datasets unsuitable for meeting the specific objectives of our research. To elaborate, GSE46449 employed leukocyte samples, whereas our training dataset GSE23848 utilized peripheral blood tissue samples. This divergence may introduce distinct gene expression patterns, making GSE46449 inadequate for robustly validating our hypotheses. Similarly, GSE62191 utilized cells from the cerebral cortex, representing a significantly different tissue source compared to our study population, and was consequently excluded. Furthermore, the datasets from GSE124326 involved subjects under intense lithium treatment, introducing a potential confounding factor related to lithium therapy. To ensure the precision of our study in reflecting the distinct characteristics of bipolar disorder, we chose to exclude datasets with these specific limitations.

Furthermore, in this study, we included a total of 20 BPD patient samples and 15 control group patient samples. However, it is crucial to explicitly acknowledge that this relatively small sample size may introduce potential bias, impacting the generalizability of our research findings. Due to the limitation in sample size, the statistical power of our study may be constrained, preventing a comprehensive representation of the entire patient population. It is noteworthy that, particularly for diagnostic purposes, the small sample size could affect the accuracy of our understanding of the patient group. Future research endeavors should focus on enlarging the sample size, employing larger-scale study designs to ensure the reliability and generalizability of the results.

Conclusion

The autophagy-immune cell axis takes on a critical significance in the early pathogenesis of BPD. Four important ARDEGs (i.e., ERN1, ATG3, CTSB, and EIF2AK3) serve as potential biomarkers correlated with immune infiltration in BPD and be helpful for the prediction or diagnosis of BPD.

List of Abbreviations

BPD	Bipolar disorder
GEO	Gene Expression Omnibus
DEGs	Differentially expressed genes
ARDEGs	Autophagy-related differentially expressed genes
PPI	protein-protein interaction
ROC	receiver operating characteristic curve
GO	Gene Ontology
KEGG	Kyoto Encyclopedia of Genes and Genomes
LASSO	Least absolute shrinkage and selection operator
ERN1	Endoplasmic reticulum nuclear signal 1
ATG3	Autophagy-related gene 3
CTSB	cathepsin B
EIF2AK3	eukaryotic translation initiation factor 2 alpha kinase 3
PCA	Principal Component Analysis
RF	random forest
SVM	support vector machine
GLM	generalized linear model
TF	Transcription fact
ChEA3	ChIP-X Enrichment Analysis 3
KLHL24	Kelch like family member 24
AUC	Area Under Curve
UPR	Unfolded protein response
ER	Endoplasmic reticulum
LC3B	Microtubule associated protein 1 light chain 3 beta
Hsp60	Heat shock protein family D (Hsp60) member 1

Supplementary Information

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Supplementary Material 1

Supplementary Material 2

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Author contributions
YJL and JZ contributed to the study concepts and study design and helped in revising the manuscript. YJL provided funding acquisition. DC and YFL supervised the research, analyzed the data, and wrote the manuscript. JHM, SLY and CZ performed data management, and bioinformatics analysis. All authors read and approved the final manuscript.

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Data availability
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 in the article.

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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