Bioinformatics-Based Analysis of Circadian Rhythm Regulation Mechanisms in Alzheimer's Disease

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Abstract.
Background: There is a close association between Alzheimer's disease (AD) and circadian rhythms, and neuroinflammatory-related pathways are associated with both interactions.
Objective: To reveal the relationship between circadian rhythm (CR) and AD at the level of genes, pathways, and molecular functions through bioinformatics.
Methods: We analyzed the differential genes between AD and control groups in GSE122063 and found the important gene modules; obtained CR-related genes from GeenCard database; used Venn 2.1 database to obtain the intersection of genes of AD important modules with CR-related genes; and used STRING database and Cytoscape 3.7.1 to construct the gene protein-protein interaction network. The MCODE plugin was used to screen pivotal genes and analyze their differential expression. We trranslated with www.DeepL.com/Translator (free version) to obtain transcriptional regulatory relationships from the TRRUST database and construct a hub gene-transcription factor relationship network.
Results: A total of 42 common genes were screened from AD and CR genes, mainly involving signaling pathways such as neuroactive ligand-receptor interactions. A total of 10 pivotal genes were screened from the common genes of CR and AD, which were statistically significant in the comparison of AD and control groups ($p < 0.001$), and ROC analysis showed that all these pivotal genes had good diagnostic significance. A total of 36 TFs of pivotal genes were obtained.
Conclusion: We identified AD- and CR-related signaling pathways and 10 hub genes and found strong associations between these related genes and biological processes such as inflammation.

Keywords: Alzheimer's disease, bioinformatics, circadian rhythm, hub genes

INTRODUCTION

Alzheimer's disease is a chronic and progressive decline in cognitive function, mainly manifested by cognitive decline, decline in daily living ability and mental behavior abnormalities, which seriously endangers the health of the elderly. Its pathogenesis is

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closely related to genetics, aging, lifestyle, environmental factors, and nervous system diseases [1–4], but so far, the etiology and pathogenesis of AD is not clear. Although researchers have proposed the hypothesis of amyloid-β (Aβ) cascade damage [5], tau hyperphosphorylation [5], cholinergic injury hypothesis [6], the ApoE hypothesis [7], neuroinflammatory hypothesis [8], and mitochondrial dysfunction hypothesis [9] for AD, they have yet to fully explain the complex mechanism of this disease. The prevalence of AD continues to rise, seriously affecting healthcare systems worldwide [10].

The complex connection between the body's intrinsic biological rhythms and AD has recently attracted a lot of attention. The "sunset phenomenon" is a symptom of a distinct temporal pattern in AD, manifested by symptoms such as hallucinations, hallucinations, emotional instability, anxiety, and cognitive impairment at dusk, lasting several hours in mild cases or affecting sleep in severe cases [11]. Circadian rhythm (CR) is a biological timer [12], a 24-hour physiological and behavioral cycle generated by endogenous biological clock oscillations, which can regulate the biological processes of the body at different levels, such as neural, molecular, and cellular [13]. Sunset syndrome in AD patients is both one of the symptoms of circadian rhythm disorder and aggravated by circadian rhythm disorder [14, 15]. There is an intricate relationship between AD and CR. On the one hand, $A\beta_{42}$ production and secretion increase when the body is awake, while $A\beta$ clearance increases and production decreases during sleep, and sleep in the elderly mostly shows an increase in daytime sleep fragmentation and a decrease in nighttime sleep [16, 17]. Sleep deprivation also increases tau protein in the interstitial fluid, and its hyperphosphorylation may destabilize the microtubule system, leading to neurogenic fiber tangles and neurodegeneration of neuronal synaptic terminals [18], causing the onset of AD. Interestingly, melatonin correction of the sleep/wake cycle has achieved positive results in improving sunset/agitation behavior in patients with dementia [19]. On the other hand, clinical studies have shown a significant shortening of rapid eye movement (REM) sleep and increased fragmentation of slow-wave sleep in AD patients, which may be caused by a decrease in cholinergic activity in AD [20–22].

The intrinsic mechanism link between AD and CR is not yet clear, and bioinformatics has a bright future in the analysis of disease differential genes and the screening of important pivotal genes and pathways.

We use bioinformatics to analyze biomarkers and pathways commonly associated with AD and CR, and to point out new directions for future research in the prevention and treatment of AD from correcting circadian rhythm disorders.

METHODS

Acquisition of genes

Differential genes in AD

Using "GSE122063" as the search term, we obtained the differential genes between the AD group and the control group in this study from the GEO database [23] (https://www.ncbi.nlm.nih.gov/geo/), removed the duplicate genes, and established the AD differential gene database. A volcano diagram was used to visualize the differential genes that were screened based on gene expression.

Construction of co-expression network and identification of key modules

WGCNA was used to explore gene modules highly related to the external traits of the samples. The weighted gene co-expression network of differentially expressed genes (DEGs) in our study was constructed through the R package WGCNA (V1.69). First, we calculated the MAD (Median Absolute Deviation) of each gene in the gene expression profile separately, eliminated the top 50% of genes with the smallest MAD, used the good Samples Genes method of the R package WGCNA to eliminate outlier genes and samples, and further used WGCNA to construct a scale-free co-expression network. To classify genes with similar expression profiles into gene modules, average linkage hierarchical clustering was performed based on the TOM-based dissimilarity measure with a minimum gene dendrogram size (genome) of 30, setting the sensitivity to 3. To further analyze the modules, we calculated the dissimilarity of the module feature genes, selected a cut line for the module dendrogram, and merged some modules. In addition, we merged modules with distance less than 0.25, and finally obtained 3 co-expression modules. It is worth noting that gray modules are considered as the set of genes that cannot be assigned to any module.

After identifying significant interest of modules, gene significance (GS) and module membership (MM) were calculated for each gene. In WGCNA, MM refers to the correlation between genes and gene expression profiles. Hub genes are a subset of highly

interconnected genes (nodes) within key modules of co-expression network and are significantly associated with biological functions.

Targets of CR

The CR-related genes were retrieved from the GeneCard database (https://www.genecards.org/) [24] using the search term "circadian rhythm," and a CR gene database was created.

Targets for CR intervention in AD

Using Venny2.1(http://bioinformatics.psb.ugent.b e/webtools/Venn/), the AD differential genes obtained earlier were intersected with the CR-related genes to create a gene database for CR interventions in AD, and the corresponding Venn diagrams were plotted.

Target analysis and enrichment analysis

Protein-protein interaction (PPI) network construction of key genes

Common genes were imported into String database [25] (https://cn.string-db.org/) to obtain protein interactions; the obtained protein information was imported into Cytoscape 3.7.2 software to construct protein-protein interaction networks and calculate the Degree values of nodes, and adjust the nodes in the network according to the Degree values. The PPI network was generated by adjusting the properties of the nodes in the network according to the Degree value.

KEGG signaling pathway enrichment analysis

The genes of CR intervention AD were uploaded to the Metascape platform (https://metascape.org/gp/index.html#/main/step1) [26] for Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis ($p < 0.05$), and the top 20 signaling pathways were filtered according to p-value to draw bubble maps for visualization.

Gene ontology analysis

The genes of CR intervention AD were uploaded to the Metascape platform for Gene ontology (GO) analysis enrichment analysis ($p < 0.05$), and the enrichment results of molecular function, biological process, and cellular component were obtained.

Hub genetic analysis

Screening of HUB targets and construction of PPI networks

Using cytohHubba plug-in and MCODE plug-in in Cytoscape 3.7.2 [27], the top 10 differential genes were screened as hub genes based on EPC from the results obtained above, and the PPI network maps of the hub genes were drawn.

Differential expression of Hub genes in AD

The expression of the hub genes in GSE122063 was downloaded from the GEO database for each sample in the AD and Control groups, respectively. Cluster analysis of the hub genes was performed and box plots of the differences in expression of the different genes within the two groups were plotted separately. The diagnostic and discriminant values of DEGs in the AD group and control group were evaluated by receiver operating characteristic curve analysis. GSE122063 datasets were used as the external validation datasets.

ROC curve analysis of hub genes

ROC analysis was performed using the R package pROC (version 1.17.0.1) to obtain AUC, and the ci function of pROC was used to evaluate AUC and confidence intervals to obtain the final AUC results.

KEGG and GO analysis of HUB gene

KEGG signaling pathway and GO analysis of hub genes using Metascape, and visualization of results.

Analysis of hub gene-associated transcription factor

Each of the 10 HUB genes was imported into Transcriptional Regulatory Relationships Unraveled by Sentence-base Text mining database (TRRUST) [28] (https://www.grnpedia.org/trrust/) to obtain the transcription factors (TFs) of HUB genes. Then Cytoscape 3.7.2 was applied, and the hub gene-IF network was constructed.

Statistical analysis

In the above parts, it is considered that the research results meet $p < 0.05$ was statistically significant.

RESULTS

Acquisition of gene

In the GEO database GSE122063 study, 56 samples in the AD group and 44 samples in the Control group, and 14,200 differential genes were obtained between the AD and Control groups, volcano plot in Fig. 1A, and the sample clustering plot in Fig. 1B. A total of 2,320 CR-related genes were obtained.

WCGNA analysis and module significance calculation

The gene expression matrix of 14,200 pretreatment genes from AD patients in the GEO dataset was used for the WGCNA of the R package. Sample clustering is shown in Fig. 2A. To ensure high scale independence (0.85), we set $\beta = 10$ in this analysis (Fig. 2B). To ensure average connectivity (15.24), we set $\beta = 10$ in this analysis (Fig. 2C). Based on the average hierarchical clustering and dynamic tree clipping, we obtained a total of 9 co-expression modules, module feature vector clustering (Fig. 2E), module-phenotype correlation heat map (Fig. 2D), turquoise module's GS and MM correlation scatter plot (Fig. 2F).

Screening of common targets and construction of PPI networks

A total of 381 AD-related genes were obtained in the turquoise module and intersected with 2,320 CR-related genes to obtain 42 key genes acting on both AD and CR, Venn diagram (Fig. 3A) and PPI network diagram (Fig. 3B).

Enrichment of the KEGG signaling pathway

Analysis of DEGs by Metascape revealed a total of 42 signaling pathways enriched by CR and AD common targets $p < 0.05$, mainly including Neuroactive ligand-receptor interaction, Cytokine-cytokine receptor interaction, Viral protein interaction with cytokine and cytokine receptor, Chemokine signaling pathway, Human cytomegalovirus infection, etc. (Fig. 4A).

Enrichment of the GO

Analysis of DEGs by Metascape revealed a total of 289 items ($p < 0.05$). There were 19 molecular functions, including receptor ligand activity, signaling receptor activator activity, signaling receptor regulator activity, neuropeptide hormone activity; 23 cellular components, including postsynapse, asymmetric synapse, neuron to neuron synapse, glutamatergic synapse, postsynaptic specialization membrane; and 247 biological processes, including regulation of monoatomic ion transport, regulation of metal ion transport, regulation of transmembrane transporter activity, regulation of transmembrane transport, regulation of transporter activity; the top 10 p-values in each of the three groups were selected for visualization (Fig. 4B).

Hub genetic analysis

Hub target screening

A total of 10 hub genes were screened in this study, included CCL2, CCR5, GDF15, HSPA1A, IL1R1, MPO, NEDD4L, PPBP, SNCA, and YWHAH. The specific information calculated by Cytoscape 3.7.2 is shown in Table 1.

The PPI network of Hub genes

A total of 10 nodes and 28 edges were obtained from the analysis of the interrelationships of the 10 hub genes by the string database and Cytoscape 3.7.2 software (Fig. 3C).

Differential expression of HUB gene

We determined the differential expression levels by comparing Hub gene expression between the AD group and the control group in the GSE122063 disease gene pool (Fig. 5A). Box plots showed significant differences in the levels of all 10 genes between the AD and control groups (Fig. 5B).

ROC analysis of HUB gene

The diagnostic ability of the 10 DEGs to distinguish AD from control group samples in the GSE122063 dataset showed good diagnostic value: HSPA1A (AUG = 0.8705); NEDD4L (AUG = 0.7918); CCL2 (AUG = 0.8409); PPBP (AUG = 0.7666); CCR5 (AUG = 0.8393); MPO (AUG = 0.7416); GDF15 (AUG = 0.8425); YWHAH (AUG = 0.7526); IL1R1 (AUG = 0.7390); SNCA (AUG = 0.7403) (Fig. 6).

KEGG signaling pathway and Gene Ontology

Analysis of DEGs by Metascape revealed a total of 8 signaling pathways enriched by 10 hub genes
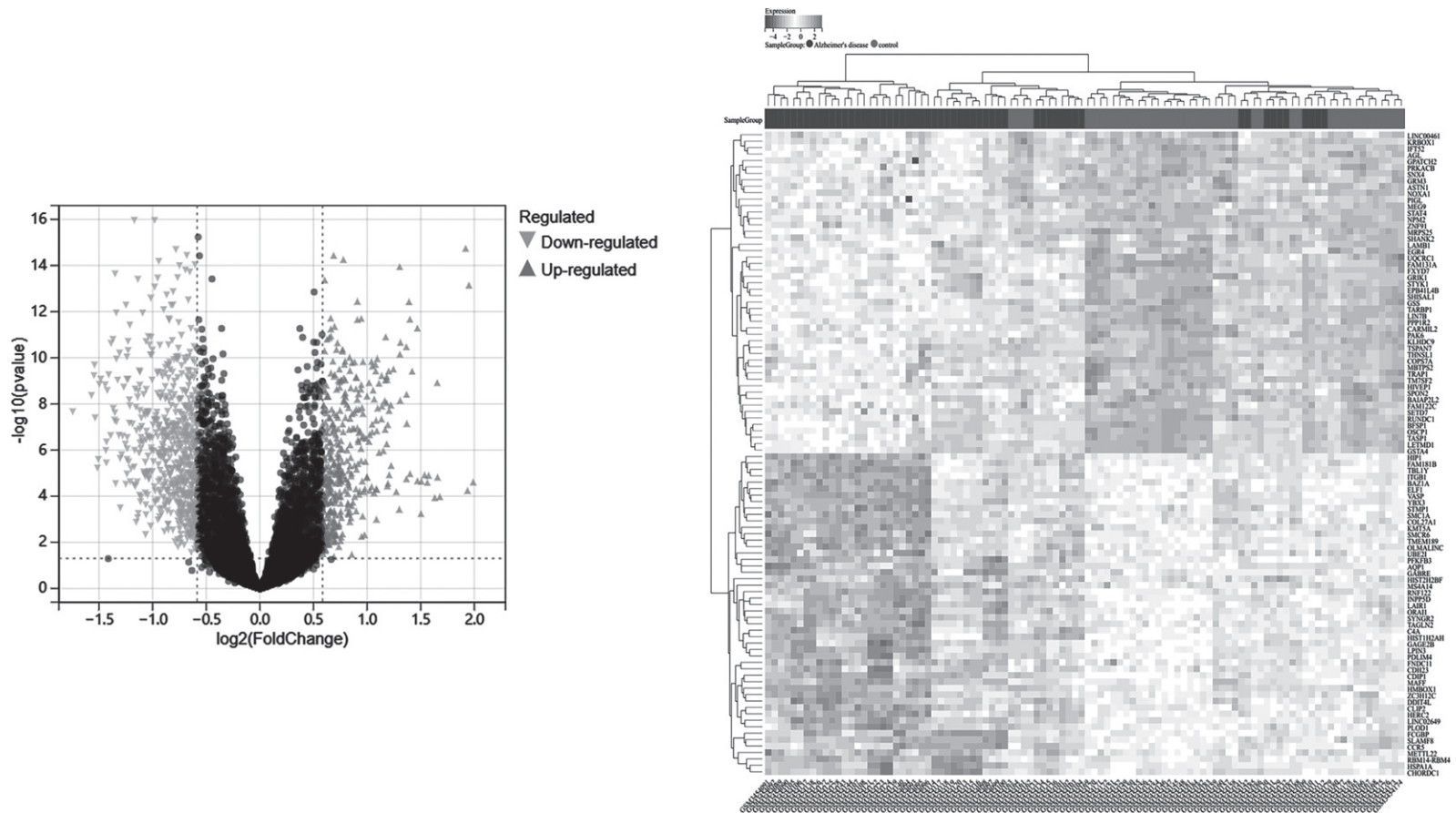
Fig. 1. A) Differentially expressed genes between the blank and Alzheimer's disease groups in the GSE122063 study in the GEO database; B) sample information.
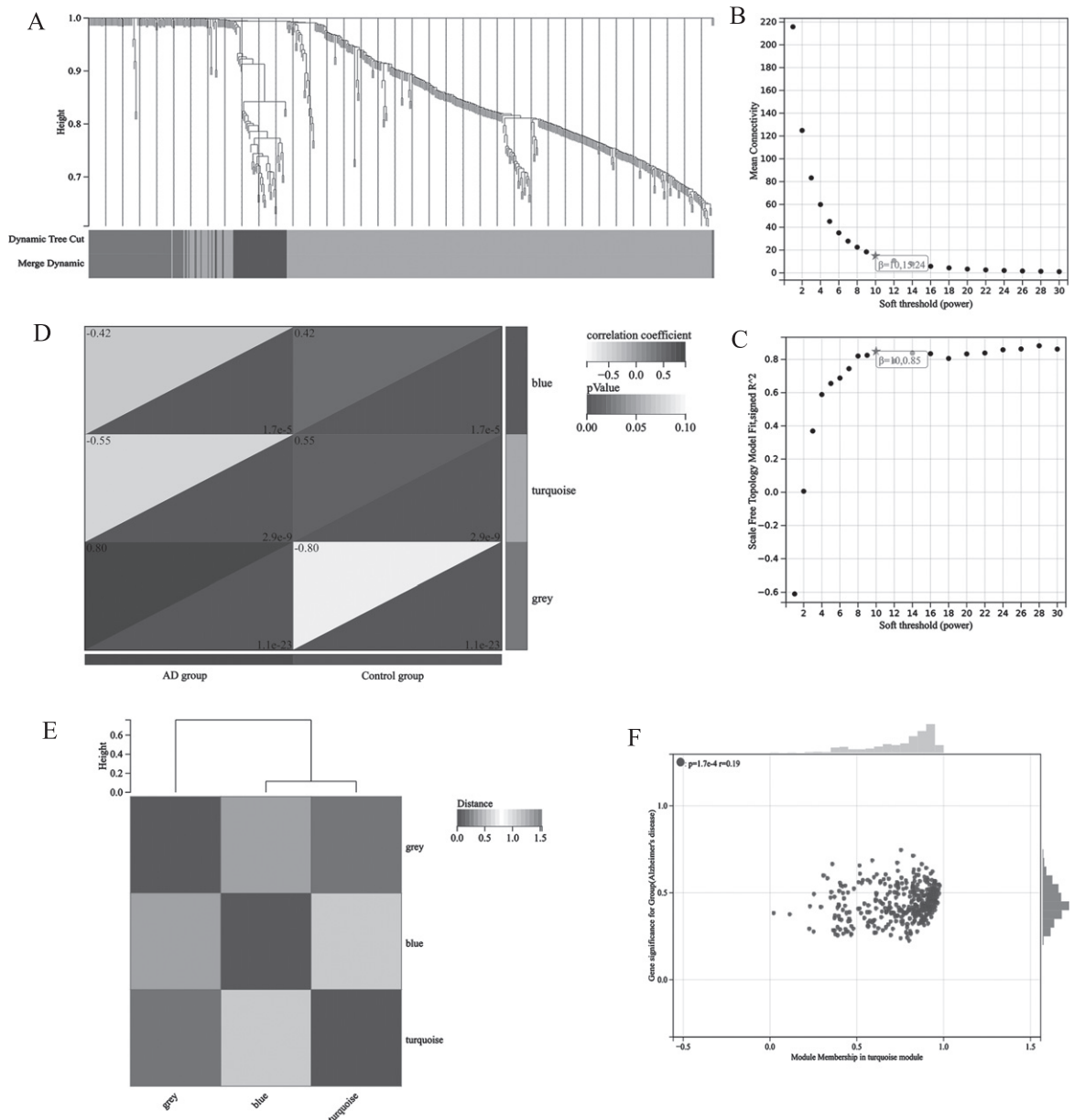
Fig. 2. A) sample clustering; B) Scalar independence; C) average connectivity; D) module-phenotype correlation heat map; E) module feature vector clustering; F) turquoise module's GS and MM correlation scatter plot.

$p < 0.05$, mainly including Cytokine-cytokine receptor interaction, Viral protein interaction with cytokine and cytokine receptor, Chemokine signaling pathway, Human cytomegalovirus infection, Endocytosis, Toxoplasmosis, Fluid shear stress and atherosclerosis, and Viral carcinogenesis (Fig. 7A).

Analysis of DEGs by Metascape revealed a total of 567 items ($p < 0.05$): there were 60 molecu-

lar functions, including signaling receptor binding, sodium channel regulator activity, cytokine activity, chemokine activity, g protein coupled receptor binding; 4 cellular components, including inclusion body, secretory granule, platelet alpha granule, secretory vesicle; and 503 biological processes, including response to molecule of bacterial origin, response to lipid, regulation of transporter activity, cellular
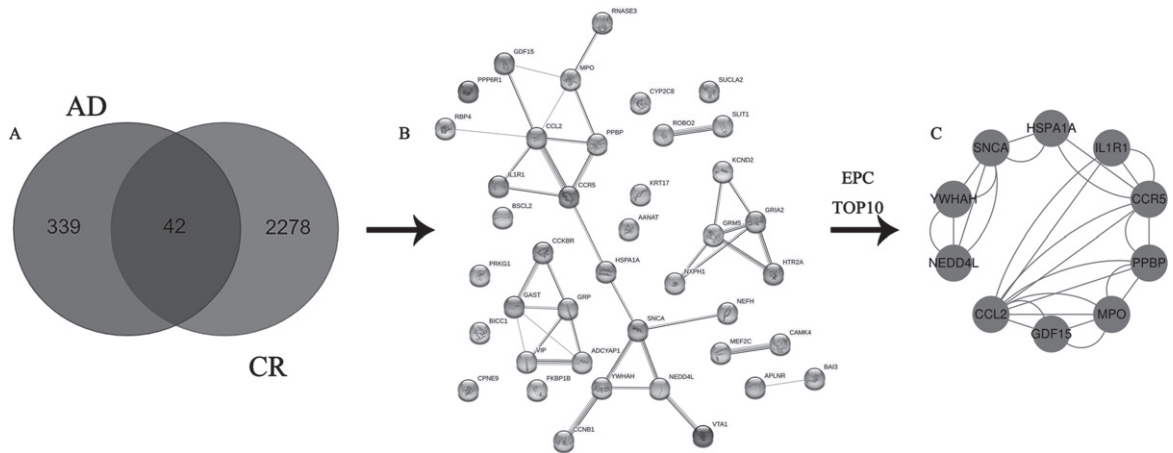
Fig. 3. A) Venn diagram of the intersection of differential genes and circadian rhythm-associated genes in Alzheimer's disease; B) PPI network diagram of intersecting genes; C) PPI network diagram of 10 hub genes.

response to oxygen containing compound, response to cytokine; the top 10 *p*-values in each of the three groups were selected for visualization (Fig. 7B).

The regulatory relationship between Hub gene and TFs

A total of 36 TFs of 10 Hub genes were obtained through the TRRUST database, and the regulatory relationship between TFs and target genes are shown in Fig. 8.

DISCUSSION

With the advent of the aging of the world population and the increase in life expectancy, AD has attracted widespread attention because of its insidious onset, irreversible neurodegenerative changes, and severe reduction in patients' ability to perform daily living and social functions [29]. AD is a multifactorial disease that is determined by the interaction of genetic susceptibility and environmental factors throughout the life course [30]. Circadian rhythms are the periodic rhythms inherent in many lives and the most common form of rhythm in human life activities, and changes in people's lifestyles often disrupt this rhythmic cycle. Researchers have thoroughly investigated the complex interrelationships that exist between lifestyle changes and AD-related mechanisms: 24.5%–40% of AD patients are reported to suffer from chronic sleep disorders [31]. In addition, sleep disorders causing circadian rhythm disturbances may be early signs of age-related neurodegenerative diseases and memory loss in the elderly. Melatonin, whose main function is

to improve sleep disorders, has achieved significant efficacy in the treatment of AD in clinical studies, further confirming the existence of some linkage between the two [32]. Existing studies confirm that untimely light exposure or sleep deprivation inhibits the expression of Bmal1 (brain and muscle arnt-like) in neurons and glial cells of mice, a central factor in the transcriptional-translational feedback loops of the biological clock, and that Bmal1 deficiency leads to cortical and hippocampal astrocyte proliferation and memory impairment, resulting in an AD-like pathological development [33–35]. In addition, Wagner et al. autopsied brain samples from 32 volunteers and performed single nucleus RNA sequencing (snRNA-seq) on them, and the analysis revealed that *APOE ε*4, the most critical gene leading to the development of AD, causes abnormal accumulation of cholesterol in oligodendrocytes in AD patients, which makes the outer myelin sheath of neuronal axons become less and thinner, affecting neuronal myelin involved in neuronal metabolism and insulation function [36]. At the same time, Hita-Yañez et al. found a significant shortening of rapid eye movement (REM) sleep time in patients with cognitive impairment carrying the *APOE ε*4 gene. AD also has a significant disruptive effect on sleep rhythm [20]. The relationship between circadian rhythm disorders and AD is reciprocal, and a vicious circle is formed between the two, with the occurrence of either one leading to a high risk state for the other [37]. Therefore, an in-depth study of specific biomarkers sensitive to AD and CR is imminent. In this study, we deeply analyzed the differential genes between AD and control in the GEO database, and successfully constructed PPI
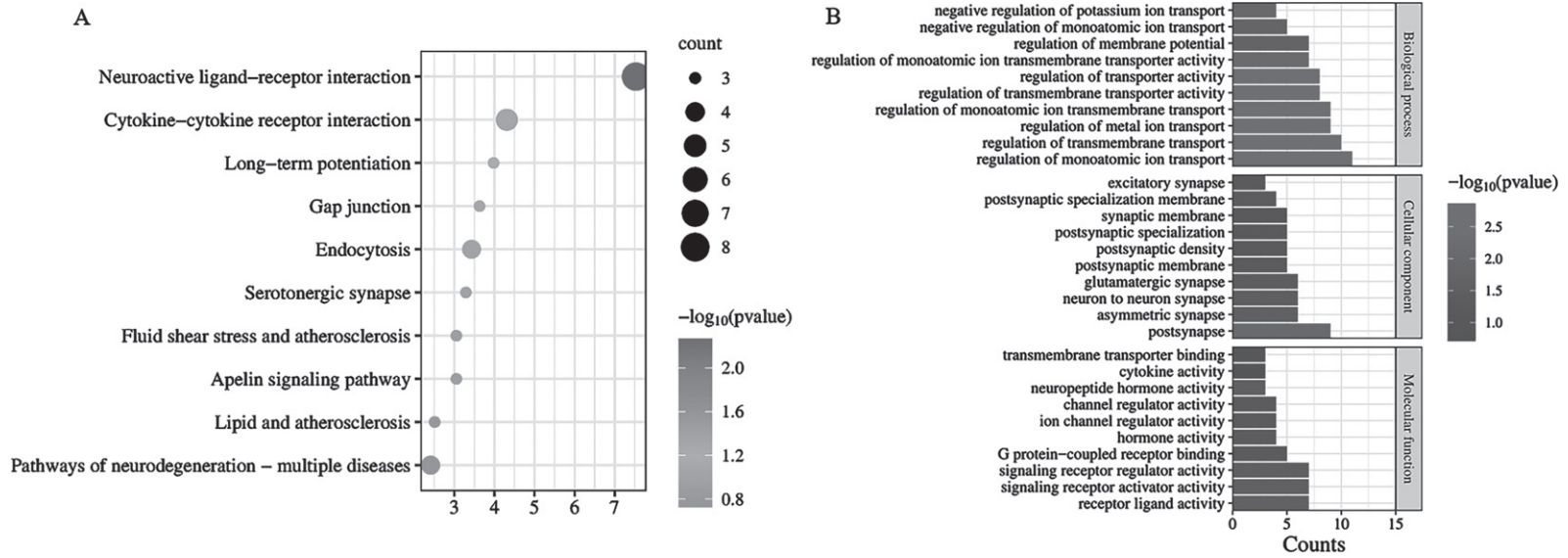
Fig. 4. A) Bubble diagram of KEGG signaling pathway; B) bar graph of Gene Ontology analysis diagram.

Table 1

| Name	Degree	Average Shortest PathLength	Betweenness Centrality	Closeness Centrality	Clustering Coefficient	Neighborhood Connectivity	Number Of Directed Edges	Radiality
CCL2	6	2.5	0.362637	0.4	0.266667	2.666667	6	0.785714
MPO	4	3.142857	0.148352	0.318182	0.333333	3	4	0.693878
CCR5	4	2.285714	0.543956	0.4375	0.333333	3.25	4	0.816327
SNCA	4	2.571429	0.538462	0.388889	0.166667	2.25	4	0.77551
PPBP	3	2.714286	0.087912	0.368421	0.666667	4.666667	3	0.755102
YWHAH	3	3.214286	0.142857	0.311111	0.333333	2.666667	3	0.683673
NEDD4L	3	3.214286	0.142857	0.311111	0.333333	2.666667	3	0.683673
GDF15	2	3.285714	0	0.304348	1	5	2	0.673469
IL1R1	2	2.857143	0	0.35	1	5	2	0.734694
HSPA1A	2	2.357143	0.527473	0.424242	0	4	2	0.806122
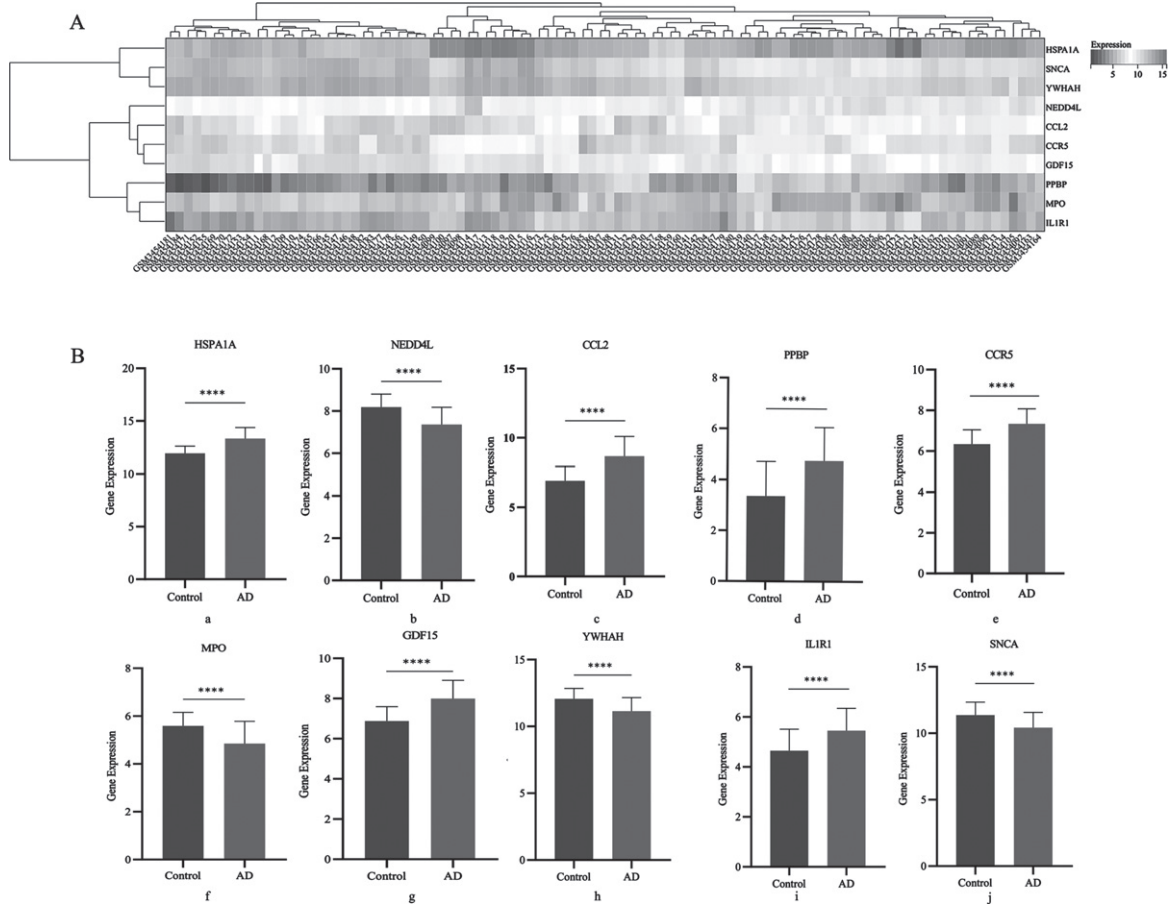
Fig. 5. A) Heat map of DGEs expression; B) Differential expression of 10 central genes in AD group and Control groups (a: HSPA1A; b: NEDD4L; c: CCL2; d: PPBP; e: CCR5; f: MPO; g: GDF15; h: YWHAH; i: IL1R1; j: SNCA).

network of DEGs using the STRING database and network construction tool, and enriched the differential genes; based on this, 10 important HUB genes were also screened and analyzed, including CCL2, CCR5, GDF15, HSPA1A, IL1R1, MPO, NEDD4L, PPBP, SNCA, and YWHAH.

Global gene expression studies (WGCNA) can help us better understand the specific pathobiology between AD and CR. The DGEs of the most important sections were further screened using WGCNA based on obtaining all DGEs of the AD and control groups in GSE122063. To gain more insight into
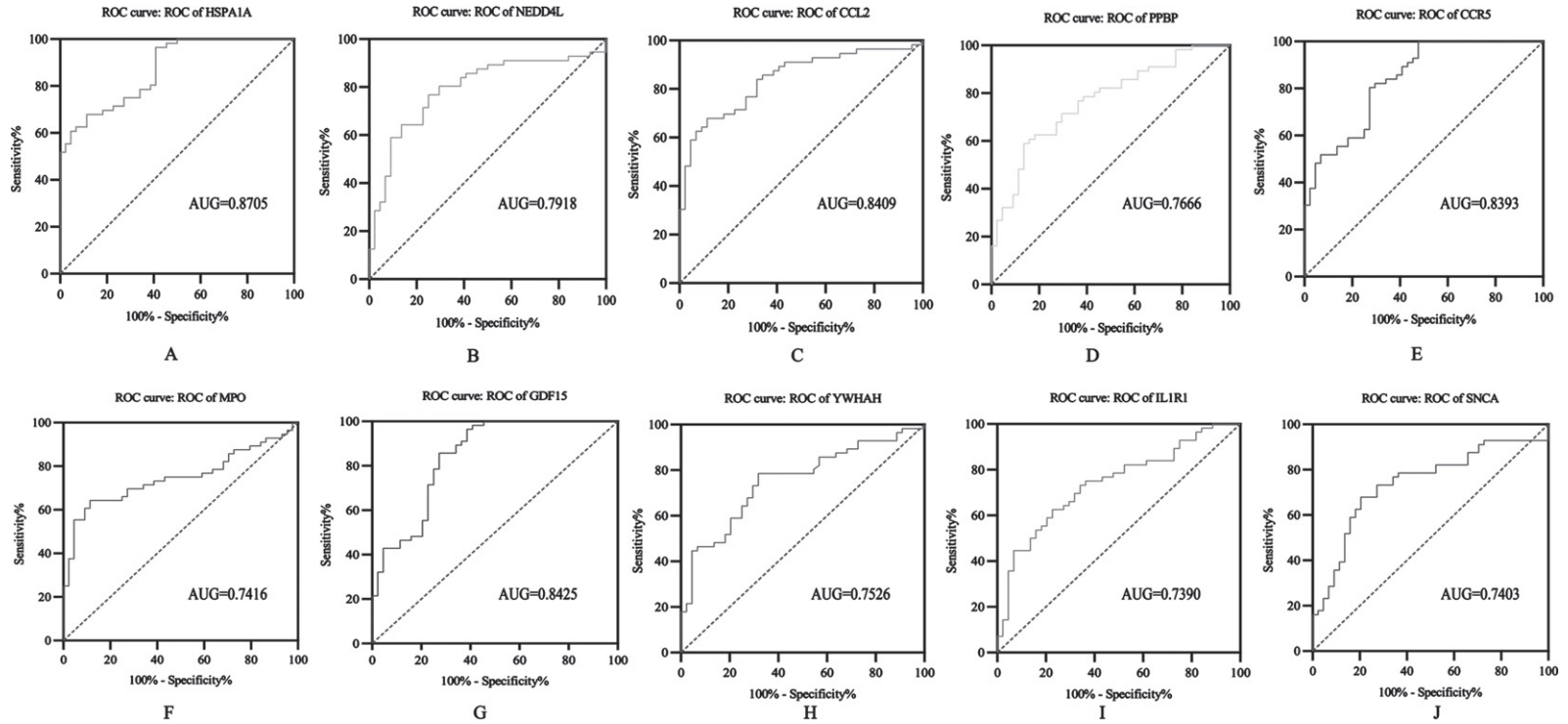
Fig. 6. ROC analysis of HUB gene: A) HSPA1A; B) NEDD4L; C) CCL2; D) PPBP; E) CCR5; F) MPO; G) GDF15; H) YWHAH; I) IL1R1; J) SNCA.
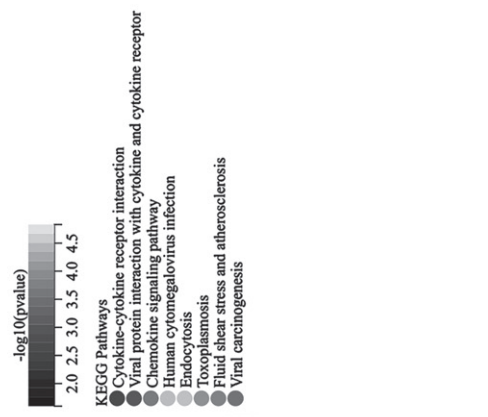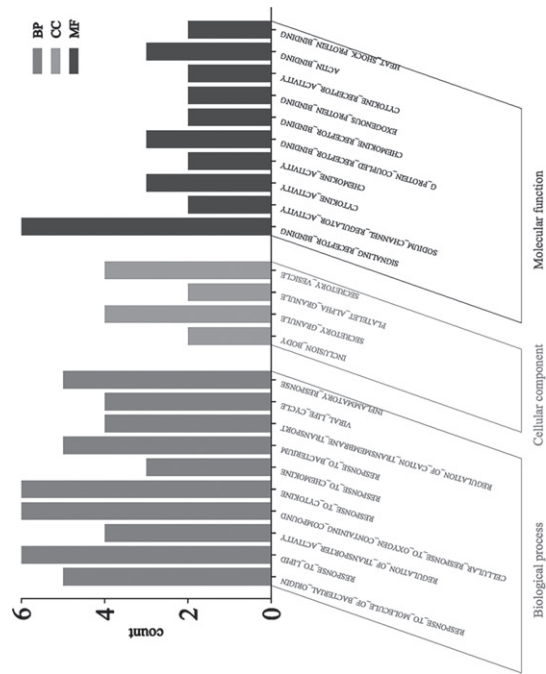
Fig. 7. A) String Chart of KEGG signaling pathway; B) bar graph of Gene Ontology analysis diagram.
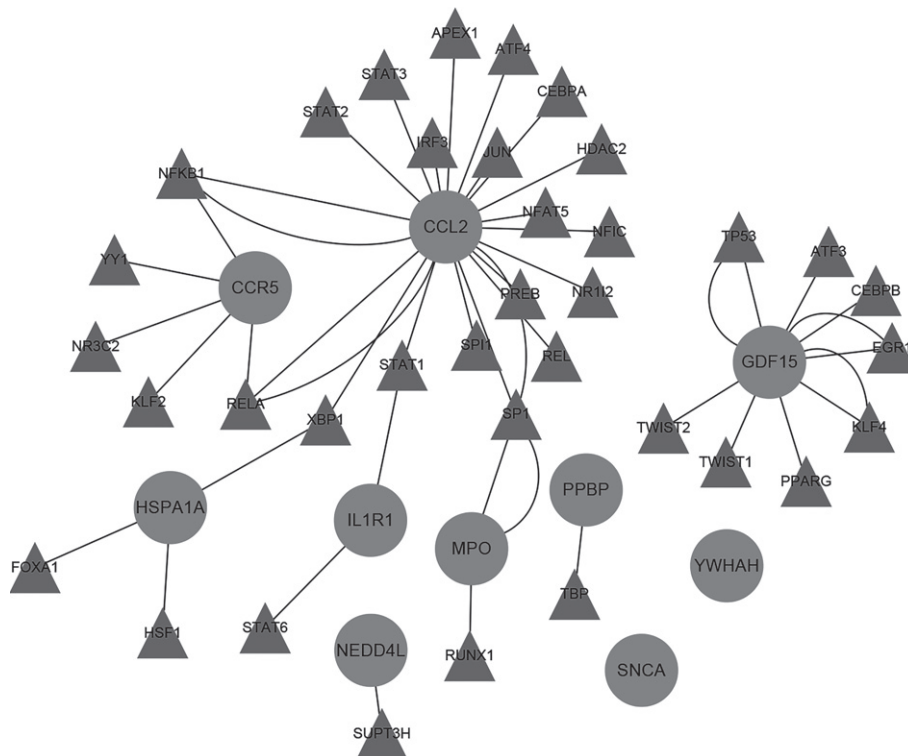
Fig. 8. Interaction network diagram of hub gene-TFs (Pink circular nodes are hub genes; purple triangular nodes are transcription factors).

these DEGs, we analyzed selected genes used for GO and KEGG enrichment analysis and found Neuroactive ligand-receptor interaction, Cytokine-cytokine receptor interaction, Long-term potentiation, Gap junction, Endocytosis, Serotonergic synapse, Fluid shear stress and atherosclerosis, Apelin signaling pathway, Lipid and atherosclerosis, Pathways of neurodegeneration - multiple diseases and other signaling pathways play important roles in AD. The pathways play an important role in AD. By studying circadian rhythm disorders in zebrafish larvae, Xiao et al. discovered significant differential expression of clarm1a, bmal1b, per1b, and per2 on neuroactive ligand-receptor interaction [38]. Neuroactive ligand-receptor interaction is also essential in neuroinflammatory diseases. Mesenchymal stem cells (MSCs) were found to activate neuroactive ligand-receptor interactions by secreting trophic factors, anti-apoptotic factors, and intervening in the immune response process, thereby activating the downstream PI3K-Akt signaling pathway, reducing neuroinflammatory response, enhancing neurogenesis, and improving efficacy in the treatment of neurological injury [39]. Thus, the interaction of neuroactive ligands with receptors and the PI3K-Akt

signaling pathway may play an important role in neuroinflammation and neurodegenerative diseases caused by CR disorders.

The dopaminergic synapse is an important signal transduction pathway in AD that regulates synaptic function and modulates neurotransmitters, thereby enhancing memory [40]. Dopaminergic synapse is also an important neuromodulatory pathway for animals to maintain circadian rhythm periodicity within their physiological range and has been identified in Drosophila as an important pathway that innervates arousal-promoting neurons. Dopaminergic neurons innervate the large ventral lateral neurons (l-LNvs) of Drosophila and increase cAMP levels in the l-LNvs via signaling to induce the generation of action potentials that inhibit sleep and lead to arousal [41]. Fernandez-Chiappe et al. [42] confirmed this in his study. In his study, he found that Dopamine receptor 1 (Dop1R1) and Dopamine receptor 2 (Dop1R2) regulate daytime and nighttime sleep duration, respectively. In addition to higher receptor levels, correspondingly longer sleep duration, he also found that inhibition of the dopamine pathway following inhibition of the Dop1R2 receptor similarly reduced nighttime sleep duration in

Drosophila, and that there is a complex mechanism for maintaining circadian periodicity [42]. Thus, we propose that circadian rhythm disorders may contribute to the development of AD via dopaminergic synapse signaling, neurodegeneration pathways, and multiple diseases.

The human master biological clock is located in the supraoptic nucleus (or core) (SCN). Circadian rhythm-related genes (CRY1, CRY2, DBP, NPAS2, PER1, PER3, PER2, CLOCK, etc.) regulate transcriptional and translational loops in cells. Circadian locomoter output cycles protein kaput (Clock), Basic helix-loop-helix ARNT-like protein 1 (Bmal1), Period circadian protein homolog 1 (PER), and Cryptochrome (Cry), whose expression levels fluctuate in a 24-hour cycle, together form a "transcription-translation-suppression of transcription" feedback loop that regulates the human biological rhythm. Neuronal PAS domain-containing protein 2 (NPAS2), CRY, and PER genes heterodimerize in the nucleus to drive positive feedback regulation of circadian rhythms and encode cyclin (PER and CRY); PER proteins accumulate mainly at night, and a certain amount of accumulation enters the nucleus to repress PER gene retrotranscription to close the feedback loop [43]. The operation of the feedback loop causes a circadian rhythm in the release of neural excitations, transmitters, and hormones, and this affects the cyclical fluctuations in brain function. In physiological states, amyloid β-protein (Aβ) secretion in brain tissue is reduced during sleep, whereas disrupted circadian rhythms cause abnormal Aβ release and accumulation, forming Aβ plaques and leading to the development of AD [44].

Then, we further analyzed the expression of 10 hub genes. The results showed that CCL2, CCR5, GDF15, HSPA1A, IL1R1, and PPBP were highly expressed in the AD group compared with the blank group ($p < 0.05$), while NEDD4L, MPO, YWHAH, and SNCA showed low expression ($p < 0.05$). C-C motif chemokine 2 (CCL2), also known as monocyte chemotactic protein 1 (MCP-1), is a small molecular weight cytokine of the CC chemokine family and is partially responsible for the chemotaxis of mononuclear phagocytes (microglia, peripheral monocytes, and macrophages). Increased CCL2 in serum and cerebrospinal fluid has been reported to be associated with neurodegeneration and negatively correlated with cognitive scores in AD patients [45, 46]. Previous studies have demonstrated that transgene overexpression of CCL2 enhances microglia proliferation and induces diffuse amyloid plaque

deposition in Tg2576 mice [47]; further studies have shown that CCL2 transgene overexpression in mice as young as 2-3 months is able to accelerate spatial and working memory and deficits in hippocampal synaptic transmission in amyloid precursor protein (APP) mice, mainly because CCL2 is a potent enhancer of Aβ oligomerization, microglia accumulation and cognitive dysfunction in animal models of Aβ degeneration, and can directly promote Aβ uptake, intracellular Aβ oligomerization, and protein secretion [48]. Another study found that the upregulation of circadian gene albumin D site binding protein (DBP) is involved in the activation process of CCL2, while the dynamic changes of CCL2 expression level showed oscillatory changes similar to DBP [49]. C-C chemokine receptor type 5 (CCR5) is a suppressor of neuroplasticity and memory, that is highly enriched in the CA1 region of the hippocampus with the ligand CCL5, regulates learning-related cell signaling and neuronal plasticity, and is involved in many forms of regulation of learning and memory [50]. CCR5 has a role in regulating the expression and secretion of cytokines, promoting the expression of pro-inflammatory mediators such as interleukin (IL)-1β, tumor necrosis factor (TNF)-α, IL-6, and IL-17a, causing neuroinflammation and leading to the development of AD [51]. It was confirmed that elevated levels of CCR5 expression also reduced the overlap of activated neurons present in the memory fragments of mice, thereby inhibiting memory capacity and leading to further aggravation of AD [50]. This suggests that CCR5 could be an effective target for the treatment of AD. There is also a strong link between circadian rhythms and neuroinflammation. A study in depressed mice claimed that the Per2 gene induces neuroinflammatory responses through chemokines (especially CCR5 antagonists) [52]. Myeloperoxidase (MPO) is produced by neutrophils and is involved in the development of AD by participating in the molecular pathway leading to Aβ deposition [53, 54]. Also, MPO binds tightly to the vascular endothelium by interacting with negatively charged glycocalyx in other chronic inflammatory diseases, including heart disease, leading to cerebrovascular endothelial dysfunction and reducing blood-brain barrier function [55]. Other studies have shown a significant daily rhythmic cyclic variation in MPO activity [56]. There is a complex relationship between circadian rhythm and AD in the aspects of inflammation and metabolism, among which the role of CCR and MPO cannot be ignored.

In addition, we performed a ROC analysis of the diagnostic value of the HUB gene in AD. The results showed that HSPA1A showed a high diagnostic value (AUC >0.85). Therefore, HSPA1A is considered an important biomarker to study the relationship between AD and circadian rhythm.

Conclusion

We have simultaneously explored the common related genes, pathways and biological processes of AD and CR, and clarified that there are complex molecular and cellular mechanisms between AD and CR. Most importantly, we have identified signaling pathways such as Neuroactive ligand-receptor interaction, Cytokine-cytokine receptor interaction, Viral protein interaction with cytokine and cytokine receptor in our study, further analyzed 10 important potential biomarkers such as CCL2, CCR5, and MPO, and found strong correlations between these associated genes and biological processes such as metabolism and inflammation. We elucidated that CR-related factors play a key role in the development of AD. We hope that our ideas will provide new ideas for further research in the prevention and treatment of AD.

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CONFLICT OF INTEREST

The authors have no conflict of interest to report.

DATA AVAILABILITY

Data supporting the results of this study are available from the corresponding author upon reasonable request.

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