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Bioinformatics Screening and Experimental Validation for Deciphering the Immune Signature of Late-Onset Depression

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Background: Late-onset depression (LOD) is often associated with more severe cognitive impairment and a higher risk of disability and suicide. Emerging evidence suggests that immune system problems may be involved. This study aims to systematically characterize the genetic signature of LOD based on the immune landscape.

Methods: The expression profile of GSE76826 was obtained from the Gene Expression Omnibus (GEO) database to gather gene expression data for 10 LOD patients and 12 healthy controls (HC). Various analyses, such as Single-Sample Gene Set Enrichment Analysis (ssGSEA) and Weighted Gene Co-expression Network Analysis (WGCNA), were used to mine key genes closely related to LOD. ImmuCellAI helped us understand differences in the immune environment between LOD patients and controls, and we used an LOD animal model to validate the relevant immune characteristics.

Results: We found enriched immune pathways linked to LOD and adaptive immune responses. Using advanced bioinformatics techniques, we identified two key genes: *apelin (APLN)* and *leptin (LEP)*, which have good diagnostic efficacy (AUC=0.925, 95% CI=1.00–0.83) for LOD. Neutrophil infiltration increased significantly in LOD, while CD8+ T lymphocytes (CD8_T) decreased. We finally constructed an animal model of LOD, validated two key genes and microglia marker genes in blood and hippocampus, and detected elevated pro-inflammatory factors such as interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α).

Conclusion: We identified and validated the presence of aberrant expression of *APLN* and *LEP* in LOD and described a possible immune mechanism involving increased release of IL-6 and TNF-α, leading to decreased CD8_T infiltration and increased neutrophil infiltration. Meanwhile, peripheral inflammation across the blood-brain barrier further promotes microglia activation, leading to neuronal damage.

Keywords: late-onset depression, ssGSEA, immune cell infiltration, *APLN*, *LEP*, microglia

Introduction

The aging population poses significant challenges for the elderly, emphasizing the need to address late-life depression (LLD). Late-onset depression (LOD),[1] a subtype emerging after age 60, is associated with cognitive impairments and comorbidities such as osteoporosis, menopause syndrome, cerebrovascular disease, and dementia,[2,3] which elevate risks of disability, mortality, and suicide.[4] Studies show that major depression affects about 13.3% of the elderly globally,[5] with severe depression reaching 7.2% among those 75 and older.[6] LOD's clinical presentation and progression are highly variable, and its complex pathogenesis—still not fully understood—has been linked to microvascular dysfunction, neuroinflammation, and other aging-related processes.[7]

The vascular hypothesis of depression is closely linked to immune dysregulation. Evidence increasingly associates depression with systemic immune activation, marked by cytokine imbalances, immune cell dysfunction, and dysregulated

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inflammatory pathways.[8] Major depressive disorder (MDD) patients often show elevated levels of proinflammatory cytokines such as interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), soluble IL-2 receptor,[9] and C-reactive protein (CRP) in peripheral blood. These cytokines activate the hypothalamic-pituitary-adrenal (HPA) axis,[10] disrupting serotonin metabolism and neurogenesis, which leads to depressive symptoms. Dysregulated cytokines further recruit immune cells, amplifying inflammation. In depression, increased CD4/CD8 ratios,[11] higher levels of white blood cells, neutrophils, monocytes, impaired natural killer (NK) cells and helper T cell maturation are commonly observed.[8,12] In the central nervous system, activated microglia inhibit neurogenesis and increase immune sensitivity.[13] Key immune-related pathways, including Toll-like receptor signaling,[14] NK cell activation,[15] and IFN-α/β signaling,[16] are also implicated in depression. While research suggests a link between immune dysregulation and LOD, exact mechanisms remain unclear. Further study of immune-mediated LOD pathogenesis and identification of new biomarkers are essential for improving prevention and treatment.

This study examines the complex relationship between LOD and immune dysfunction, focusing on Hub Immune-DEGs (HIDRs) and immune cell dynamics. We identified differentially expressed genes (DEGs) distinguishing LOD patients from healthy controls (HC) to clarify functional distinctions. Using Weighted Gene Co-expression Network Analysis (WGCNA), immune-related gene modules were identified, isolating HIDRs by integrating DEGs with immune-related genes (IRGs). Employing ImmuCellAI, we analyzed immune infiltration patterns differentiating LOD from HC, alongside correlations between HIDRs and immune cell changes. HIDRs were validated through KEGG pathway enrichment, receiver operating characteristic (ROC) curves-based diagnostic assessment, gene-drug interaction networks, and animal model studies. The technical framework is illustrated in Figure 1.

Materials and Methods

Preparation of Gene Sets and Identification of DEGs

We searched the GEO database (https://www.ncbi.nlm.nih.gov/geo/) for gene expression profiles associated with "late-onset depression" and focused on the GSE76826 gene chip dataset.[17] From the initial 32 samples in this dataset, we selected 22 blood samples: 10 from patients with LOD and 12 from matched HC (Table S1). Probes were then converted to gene symbols, and for genes with multiple probe data, we computed the median expression values.

The dataset underwent analysis utilizing the "Limma" software package, aimed at identifying DEGs meeting the criteria of $p< 0.05$ and |Fold Change| $\geqq 2$. Visualization using "heatmap" and "ggplot2" R packages.

Gene Set Enrichment Analysis (GSEA)

The Sangerbox tool (http://vip.sangerbox.com/) serves as a complimentary online platform offering data analysis and visualization services. Employing GSEA, we aimed to discern enriched biological pathways correlated with LOD. The GSEA software (version 3.0) was acquired from the platform, and a subset of c2.cp.kegg.v7.4.symbols.gmt was retrieved specifically for GSEA analysis. Normalized Enrichment Score |(NES)|> 1, $p< 0.05$, and the false discovery rate (FDR)< 0.25 were considered significant differences.

SsGSEA Analysis of Immunoreaction Gene Lists

The 17 lists of immunoreactive genes from the ImmPort database (https://www.immport.org/) cover a range of immune responses. Using these lists gives us a clearer understanding of how immunity affects disease development.[18] We used ssGSEA in R version 4.0.2 and the "GSVA" package on the Sangerbox platform to calculate enrichment scores for these gene lists in both groups. Differences in enrichment levels were shown using boxplots. Immunoreactive categories with differing enrichment scores between LOD and HC ($p < 0.05$) were considered significant. We also scaled the single sample enrichment scores using Z-scores and visualized them as heatmaps using the "heatmap" R package.

WGCNA Analysis

We used WGCNA,[19] developed by Langfelder and Horvath in 2008, to identify key gene modules in LOD. Initially, we filtered out genes with low variation, then removed outlier genes and samples using the "goodSamplesGenes" function in the

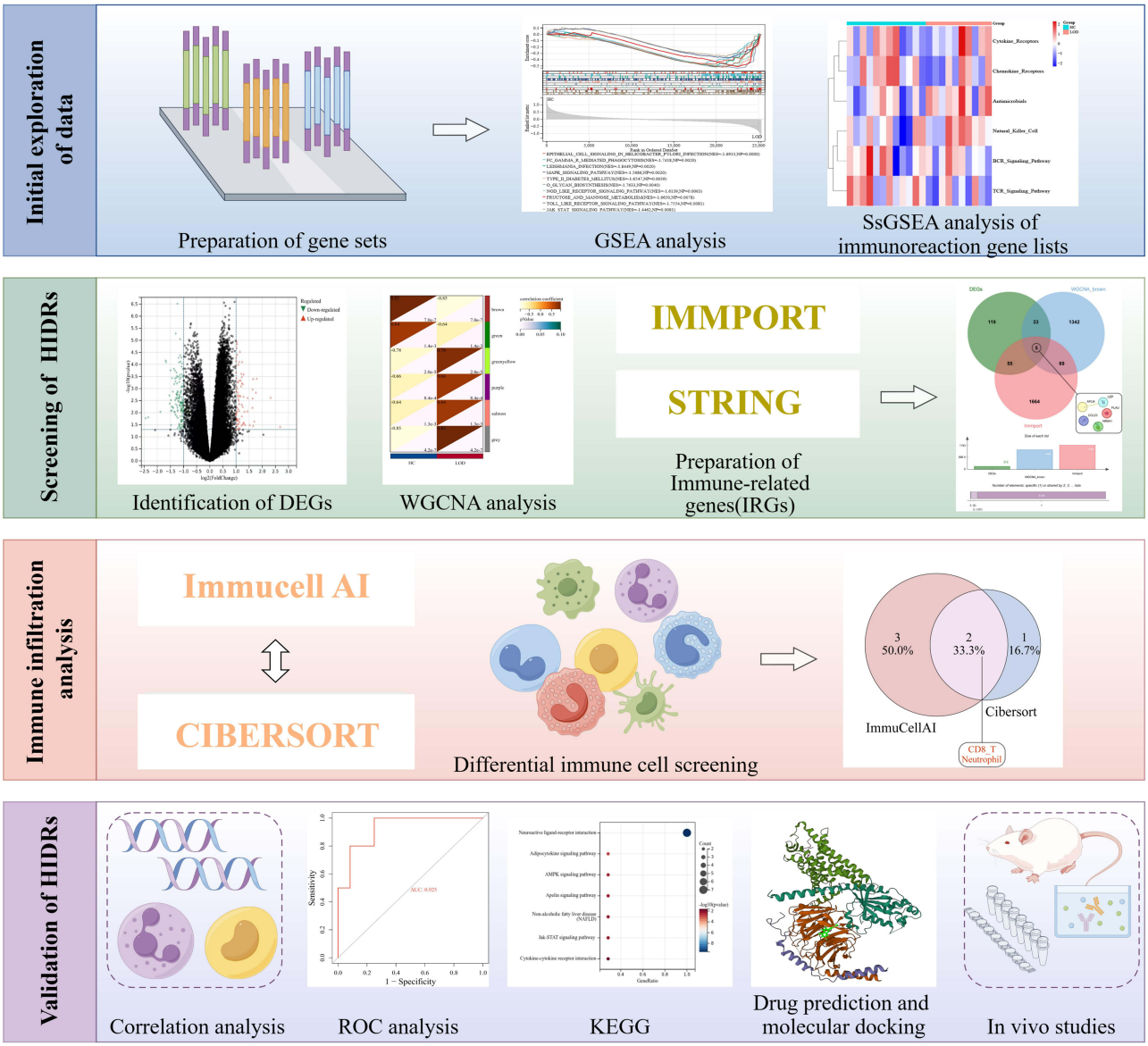

Figure 1 Flow chart of the whole research design (by Figdraw).

R package "WGCNA". This helped us construct a weighted gene co-expression network, followed by hierarchical clustering to group genes with similar expression patterns into modules. We set a minimum of 30 genes per module with a sensitivity of 3.

Screening and Characterization of HIDRs

IRGs were sourced from the ImmPort database (https://www.immport.org/). Using the "venn" package in R, we identified HIDRs by finding the overlap between IRGs, DEGs, and genes in the brown module dataset. Subsequently, the HIDR interactions were visualized using the STRING database (http://string-db.org/).

We assessed the diagnostic power of HIDRs by plotting ROC curves and calculating the area under the curve (AUC) using the "Proc" tool for visualization.

Immune Infiltration Analysis

We used the ImmuCellAI online tool (http://bioinfo.life.hust.edu.cn/web/ImmuCellAI/) to predict 24 immune cell abundance in LOD samples and compared differences between groups using the Wilcoxon rank sum test.[20]

Visualization was done with R packages "stats" and "vioplot". Additionally, we applied the CIBERSORT algorithm to calculate immune cell types in the dataset and displayed their composition between groups using a box plot.[21] Significance was set at $p < 0.05$.

Subsequently, we investigated the relationship between different immune cells and the correlation between immune cells and hub genes, using Spearman correlation analysis with screening criteria of |Correlation Coefficient|>0.5 and $p < 0.05$.

Prediction of Potential Therapeutic Agents and Docking Analysis of Drug-Gene Interaction

To explore potential therapeutics for LOD, we employed the Connectivity Map (https://clue.io/query) to match identified key genes against a database of gene expression profiles from various cell lines treated with over 2000 compounds, many of which have received approval from the US Food and Drug Administration (FDA).[22] Furthermore, molecular docking analyses were conducted using Autodock Vina v.1.2.2 to determine binding poses and interactions of candidate small molecule compounds with proteins.[23] This process generated binding energies for each interaction, aiding in the assessment of drug efficacy.

Animal Experimental Validation

To validate our screening results, we used Chronic Unpredictable Mild Stress (CUMS) to establish an LOD animal model. Detailed experimental methods and statistical analyses are provided in the Supplementary Information. Twelve male Sprague-Dawley rats (20 months old) were obtained from Beijing Vital River Laboratory Animal Technology Co., Ltd., and housed under controlled conditions with food and water ad libitum. After a 7-day acclimatization, rats were divided into two groups and subjected to 28 days of CUMS modeling.

To validate the LOD animal model, we conducted behavioral tests including the Open field,[24] Sucrose preference,[25] Y-maze,[26] and Novel Object Recognition tests.[27] We further validated HIDRs using real-time quantitative reverse transcription polymerase chain reaction (RT-qPCR, Beijing Quanshijin Biotechnology Co., Ltd, PerfectStart™ Green qPCR SuperMix: AQ601-02) and Enzyme-Linked Immunosorbent Assay (ELISA, Jiangsu Jingmei Biotechnology Co., Ltd, Rat TNF-α ELISA detection kit: J3056-A, Rat IL-6 ELISA detection kit: J3066-A).

Statistical Analysis

We used SPSS (v25.0) to analyze the data. Continuous variables were compared using the Student's *t*-test for normally distributed variables and the Wilcoxon rank-sum test for non-normally distributed variables. Binary logistic regression was employed to construct joint ROC diagnostic predictive values for the two key genes. Spearman correlation analysis was conducted to assess correlations between immune cells and between key genes and immune cells. Statistical significance was set at $p < 0.05$.

Results

Screening of DEGs

In our investigation, we pinpointed 212 DEGs in patients with LOD, comprising 78 up-regulated and 134 down-regulated genes. Visualization through volcano plots and clustered heat maps vividly illustrated the distinct distribution of gene expression levels between the LOD and control groups (Figure 2A and B).

GSEA and ssGSEA Analyses

To analyze gene function, we performed GSEA on the dataset, revealing enrichment of 39 KEGG pathways in GSE76826 (Table S2). Notably, these pathways predominantly relate to immune response processes in LOD. From these pathways, we selected 10 based on their core functions, FDR, and NES, ranking by p-value (see Figure 2C). Among these, five classical immune-related pathways stood out: the Fc gamma R-mediated phagocytosis (NES=−1.7418, p=0.002), MAPK signaling pathway (NES=−1.5686, p=0.002), NOD-like receptor signaling pathway (NES=−1.6139, p=0.0063), Toll-like receptor signaling pathway (NES=−1.7554, p=0.0081), and JAK-STAT signaling pathway (NES=−1.4462, p=0.0081).

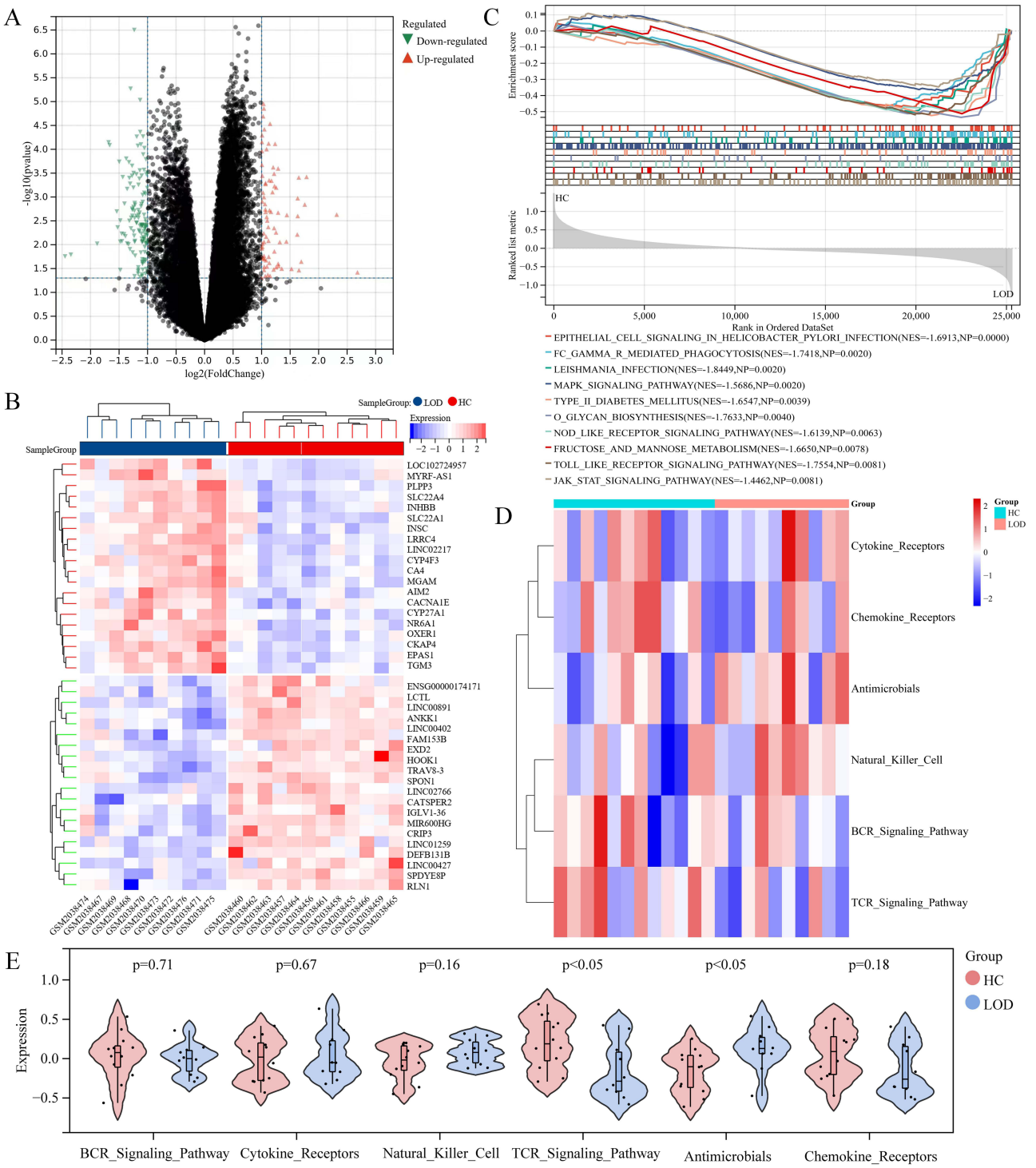

Figure 2 LOD pathways are closely linked to immune response processes. (**A**) Volcano plot for DEGs between HC and LOD. |FC|≧2 and $p<0.05$ were considered statistically significant. (**B**) Heatmap of the top 20 upregulated and downregulated genes. (**C**) Ten pathways were obtained by GSEA analysis in GSE76826. |(NES)|> 1, $p<0.05$ and FDR<0.25 were considered statistically significant. (**D**) Heatmap of immunoreaction gene lists in GSE76826. (**E**) SsGSEA scores of immunoreaction gene lists in each subgroup in GSE76826. $p<0.05$ was considered statistically significant.

The ssGSEA of immune response gene lists in LOD highlights specific immune responses involved in its pathogenesis. Out of six enriched gene lists (Figure 2D), two—the TCR Signaling Pathway and Antimicrobials—showed significant differences in enrichment scores between LOD and control groups (Figure 2E). This suggests a strong link between LOD pathogenesis and adaptive immune response.

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WGCNA Analysis

We used WGCNA to identify key modules related to LOD. After evaluating module gene similarity and connectivity, we selected a soft threshold β = 12 (scale free R2 = 0.86) (Figure 3A and B). By merging modules with a distance of less

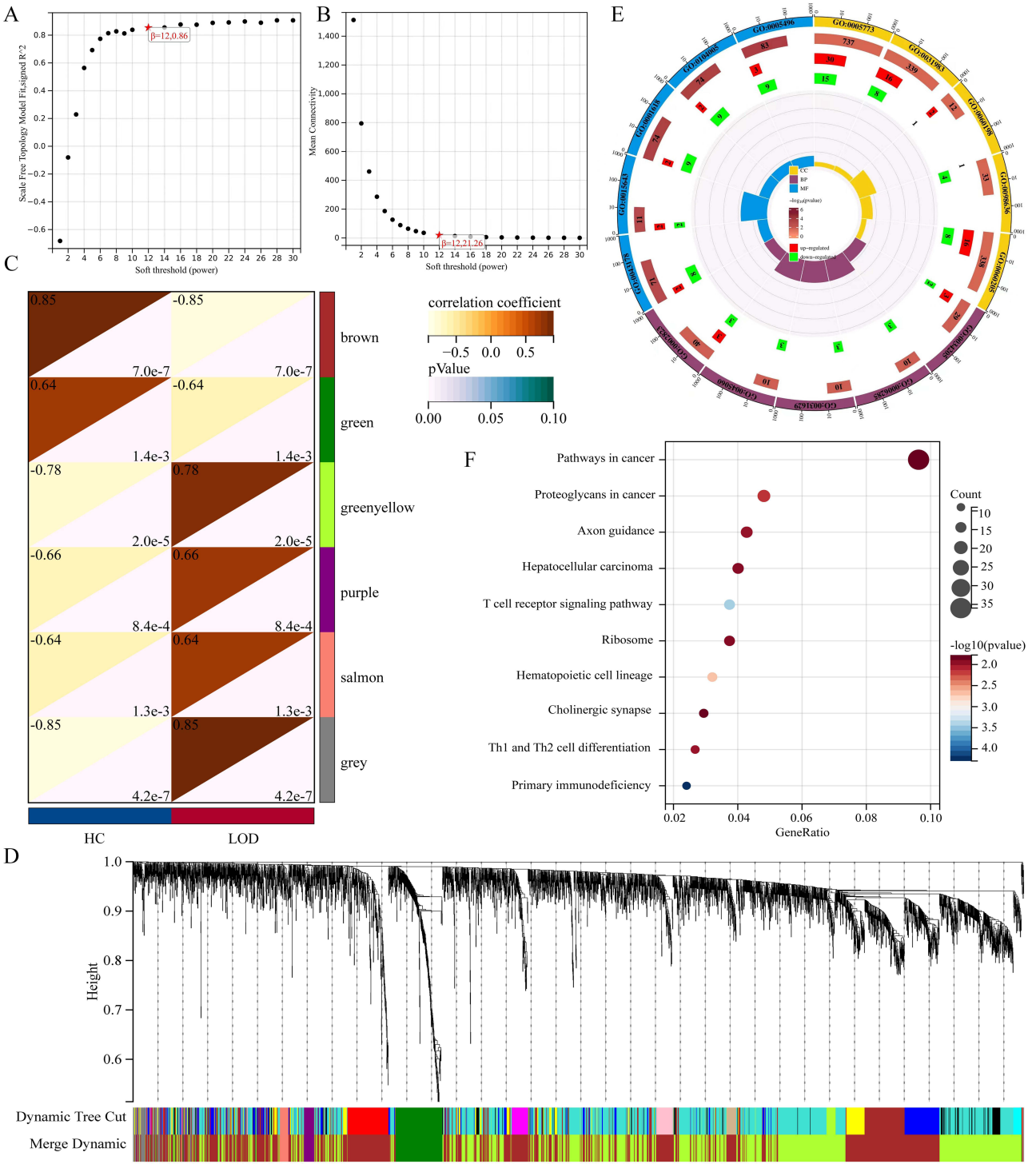

Figure 3 WGCNA analysis. (**A**) Analysis of network topology for soft-thresholding powers, the x-axis reflects the soft-thresholding power, the y-axis reflects the scale-free topology model fit index.(**B**) Mean connectivity graph, the x-axis reflects the soft-thresholding power, the y-axis reflects the mean connectivity. (**C**) Heatmap of the association between module genes and clinical traits. Each row corresponds to a module, and each column corresponds to a trait. Each cell is divided into two sections, with the upper left section showing the corresponding correlation and the lower right section showing the P value. (**D**) Clustering dendrogram of genes, each branch represents a gene and on the bottom each color represents a co-expression module. (**E**) GO enrichment analysis of brown module genes. (**F**) KEGG pathway enrichment analysis of brown module genes.

than 0.25, we obtained six co-expression modules (Figure 3C and D), with grey modules representing unassigned genes. Correlating each module with LOD, we found that one brown module ($r = -0.85$, $p < 0.001$) stood out as the most relevant to LOD (Figure 3C).

We analyzed the brown module genes through GO and KEGG pathway enrichment analysis (Figure 3E and F) to explore their biological processes and signaling pathways. Results highlighted significant enrichment in pathways like axon guidance, T cell receptor signaling, Cholinergic synapse, Th1 and Th2 cell differentiation, Primary immunodeficiency, among others. These genes are closely tied to immune cell activation, differentiation, and recruitment, suggesting their association with immune inflammation.

Screening of HIDRs

The study suggests that LOD might involve significant immune-inflammatory mechanisms. Thus, the identification of key immune genes associated with the disease will aid in its early detection and diagnosis. We compared 1793 IRGs from the ImmPort database with 212 DEGs and genes from the brown module, resulting in the identification of five HIDRs: *APLN, CCL23, NR6A1, LEP*, and *PLAU*. Among them, only *APLN* and *LEP* exhibited interactions in the protein interaction network (Figure 4A), making them the key genes of interest for further investigation.

Immune Infiltration Analysis

Considering the crucial role of the immune response in LOD, we used the ImmuCellAI tool to assess immune cell abundance and differences in infiltrating immune cells between the groups. ImmuCellAI stands out for its precise quantification of 18 distinct T-cell subgroups,[20] surpassing existing immune infiltration prediction tools. Widely applied, it is pivotal for immune infiltration analysis across non-oncological conditions.[28,29] Analyzing 24 immune cell subtypes for each sample (Figure 4D), we conducted Partial Least Squares Discriminant Analysis (PLS-DA) on their infiltration matrix. This analysis revealed significant inter-group differences in immune cell infiltration (Figure 4C), with a successful model construction indicated by the blue regression line intersecting the left vertical coordinate on the negative half-axis (Figure 4B).

We used violin plots to visualize immune cell differences in blood samples. Significant differences were noted at $p < 0.05$. Results showed lower levels of CD8_T ($p=0.01$), Gamma_delta ($p=0.02$), Exhausted ($p=0.03$), and MAIT ($p<0.01$), and higher levels of Neutrophil ($p=0.01$) in LOD group blood samples (Figure 4E). In addition, we demonstrated the correlations among 24 immune cell types (Figure 4F), with the results suggesting potential synergistic or antagonistic interactions between them.

Simultaneously, to enhance result reliability, we bolstered and corroborated findings through the classical method of immune infiltration analysis, CIBERSORT. This involved scrutinizing 22 immune cell types across both LOD and HC, visually represented through box plots (Figure S1). Combining the findings with another algorithm, we identified CD8_T and neutrophil as immune cells with significant differences between the two groups (see Figure 4G). Spearman correlation analysis revealed *APLN*'s strong negative correlation with CD8_T ($r = -0.51$, $p = 0.016$, Figure 5A) and strong positive correlation with Neutrophil ($r = 0.54$, $p = 0.0088$, Figure 5B). *LEP* showed trends of negative and positive correlation with CD8_T (Figure 5C) and Neutrophil (Figure 5D), respectively, but the differences were not statistically significant.

Validation of APLN and LEP

ROC analysis indicated *APLN* and *LEP* had AUC values of 0.867 and 0.800 (Figure 5E). Logistic regression showed a combined AUC of 0.925 (Figure 5F). These findings suggest promising utility and prospects for *APLN* and *LEP* in diagnosing clinical LOD.

The KEGG enrichment analysis of *APLN* and *LEP* (Figure 5G) revealed predominant enrichments in several pathways, notably: Neuroactive ligand-receptor interaction, Adipocytokine signaling pathway, and Apelin signaling pathway, alongside the Jak-STAT signaling pathway.

We employed the L1000 platform to identify potential therapeutic compounds for treating LOD, utilizing its extensive gene expression profiles associated with therapeutic agents. By inputting the interacting genes for *APLN* and *LEP* (Table S3), we identified five compounds with strong activity against LOD (Table S4), among which quercetin, a flavonol

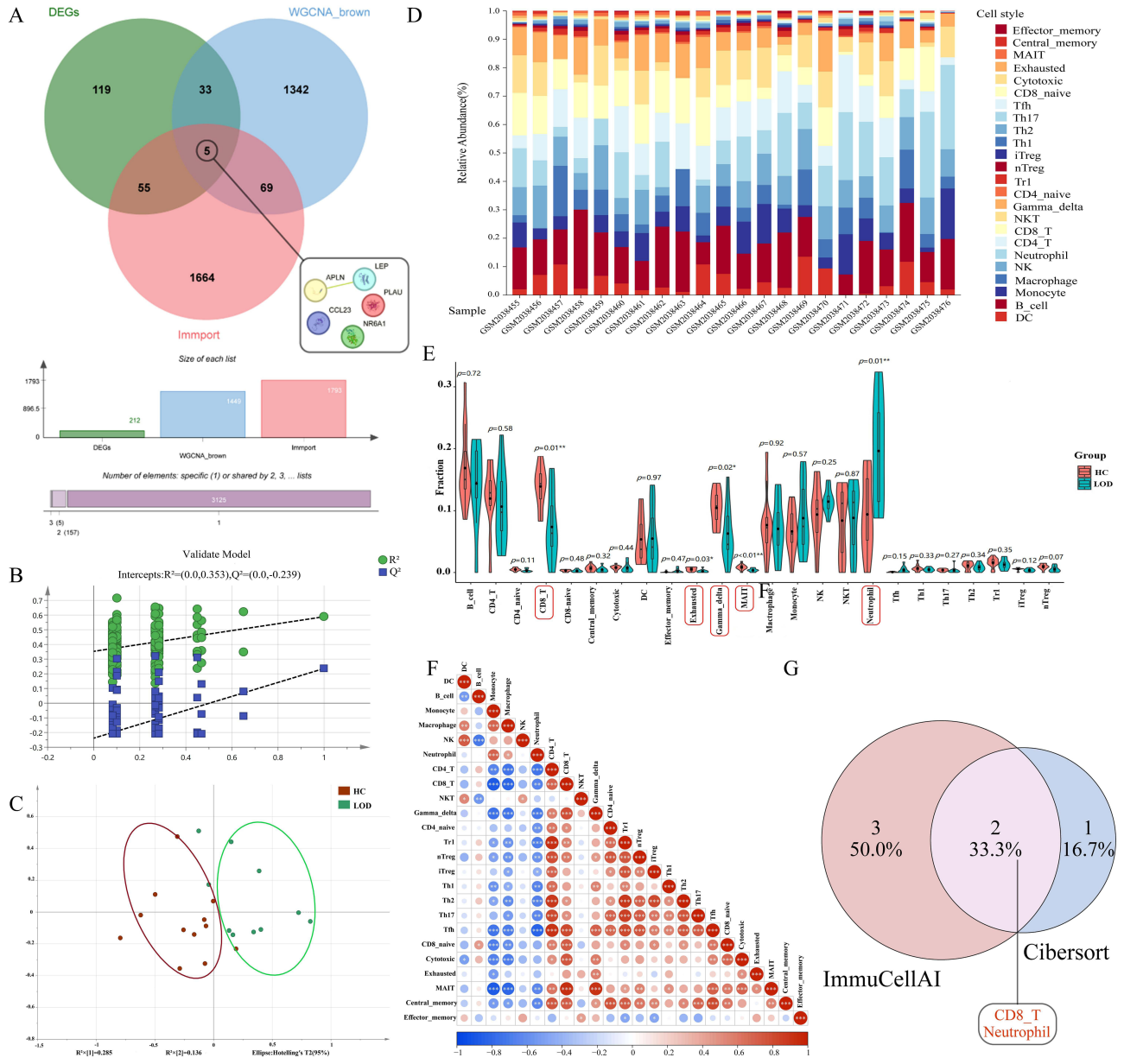

Figure 4 HIDRs screening and immune infiltration analysis. (**A**) Venn diagram of intersecting genes between IRGs, DEGs, and brown module genes. (**B** and **C**) PLS-DA score plot (R^2 X=0.285, R^2 Y=0.136) and permutation plot (200 times, R^2=0.353, Q^2=−0.239). (**D**) Stacked bar plots showing the proportion of each 24 subtypes of immune cells. (**E**) Violin plots of the abundance of 24 immune-cell subtypes between HC and LOD. (**F**) The correlation matrix diagram of 24 immune-cell subtypes. The larger the circle, the higher the correlation coefficient. The correlation coefficients were computed using the Spearman correlation coefficient. (**G**) Combining two algorithms to screen for differentially expressed immune cells.

compound known for its antidepressant effects, stood out. Molecular docking analyses indicated stable binding energies with APLN and LEP (−8.702 kcal/mol and −6.795 kcal/mol, Figure 5H and I), suggesting these proteins as potential molecular targets for quercetin in LOD treatment.

Animal Experimental Validation

We created a LOD animal model using CUMS and performed behavioral experiments on the model. In the open field test, compared to the HC group, the LOD group exhibited significant decreases in total distance traveled (Figure 6A), sucrose preference index (Figure 6B), free alternation rate in the Y maze (Figure 6C), and ability to recognize new objects (Figure 6D). These findings collectively confirm the successful establishment of the model.

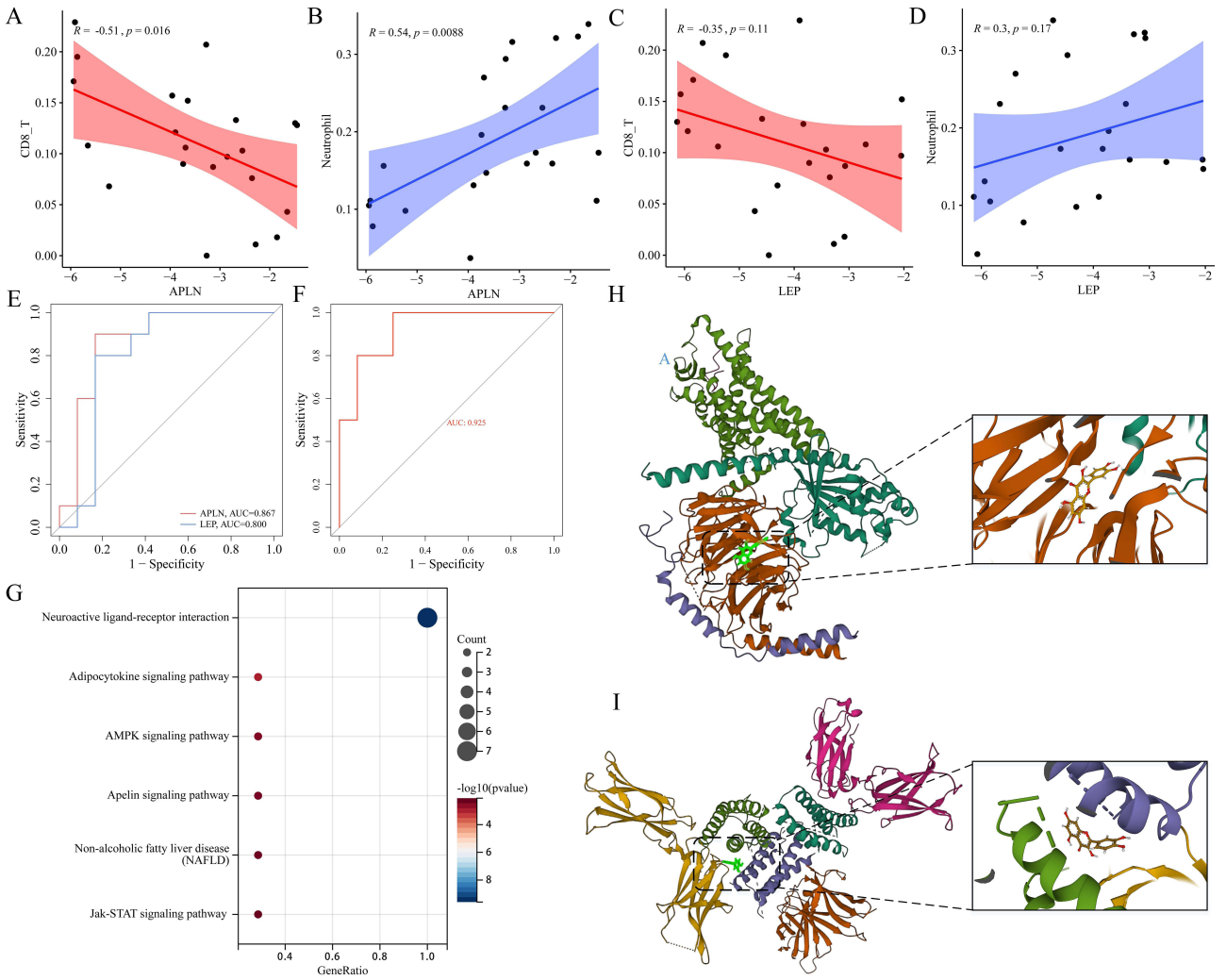

Figure 5 Validation of APLN and LEP. (**A** and **B**) The correlation plots between APLN and differential immune cells. (**A**) CD8_T. (**B**) Neutrophil. Analysis was performed by Spearman correlation. (**C** and **D**) The correlation plots between LEP and differential immune cells. (**C**) CD8_T. (**D**) Neutrophil. Analysis was performed by Spearman correlation. (**E**) ROC curves analysis of APLN and LEP. (**F**) ROC curve of the two-gene signature. (**G**) Bubble plots of functional signaling pathways for two-gene signature. (**H**) Combination pattern diagram of APLN and quercetin. (**I**) Combination pattern diagram of LEP and quercetin.

Next, we assessed the expression levels of *APLN* and *LEP* in plasma and hippocampal tissues. RT-qPCR analysis revealed significantly higher *APLN* mRNA levels in LOD (Figure 6E), whereas LEP mRNA levels were significantly lower (Figure 6G). Conversely, in hippocampal tissues, both *APLN* mRNA (Figure 6F) and *LEP* mRNA (Figure 6H) expressions were significantly elevated. Furthermore, ELISA results demonstrated markedly higher levels of immune factors, including IL-6 (Figure 6I) and TNF-α (Figure 6K), in the plasma of LOD compared to HC. In hippocampal tissues, IL-6 levels (Figure 6J) were significantly elevated in LOD, while TNF-α levels (Figure 6L) showed no significant difference, indicating a widespread peripheral and central nervous system inflammatory response in LOD. Given the close association between CNS inflammation and microglial activation, we validated the expression of three microglia activation marker genes: *MHC II, TLR2*, and *TLR4*. RT-qPCR analysis revealed significant upregulation of *MHC II, TLR2*, and *TLR4* mRNA levels in hippocampal tissues (Figure 6M-O).

Discussion

LOD, a distinct subtype of depression prevalent in older adults, presents unique challenges due to its association with severe cognitive decline,[30] heightened progression of dementia syndromes,[31] and potential resistance to treatment compared to early-onset depression (EOD).[32] The prevalence of depressive symptoms among individuals aged 75 and

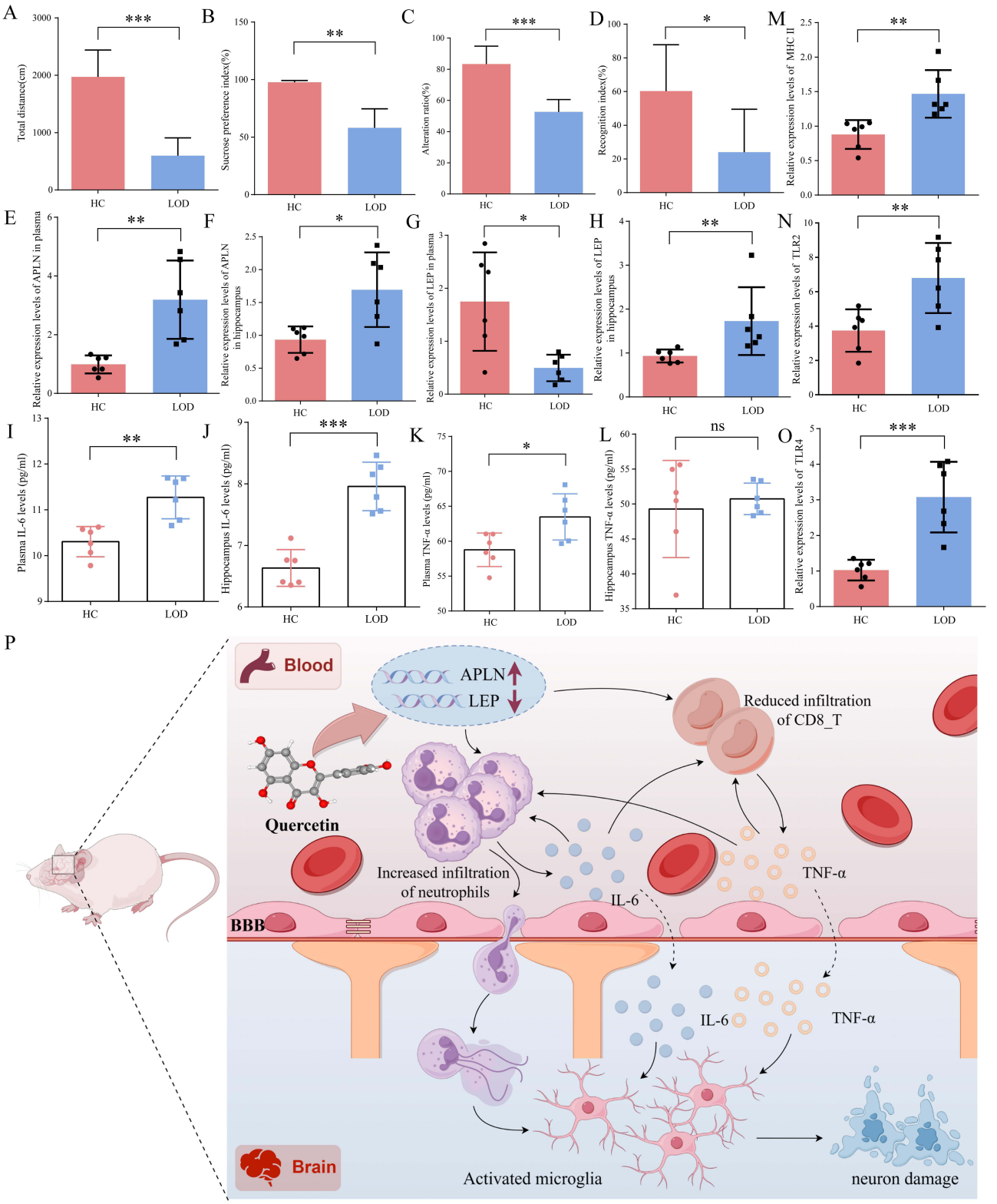

Figure 6 The dysregulation of APLN and LEP in LOD animal models. (**A-D**) Behavioral experiments. (**A**) Open field test, ***p<0.001. (**B**) Sucrose preference test, **p<0.01. (**C**) Y-maze test, ***p<0.001. (**D**) Novel object recognition test, *p<0.05. (**E** and **F**) The mRNA expression levels of APLN by RT-qPCR. (**E**) Plasma, **p<0.01. (**F**) Hippocampus, *p<0.05. (**G** and **H**) The mRNA expression levels of LEP by RT-qPCR. (**G**) Plasma, *p<0.05. (**H**) Hippocampus, **p<0.01. (**I** and **J**) Levels of IL-6 by Elisa. (**I**) Plasma, **p<0.01. (**J**) Hippocampus, ***p<0.001. (**K** and **L**) Levels of TNF-α by Elisa. (**K**) Plasma, *p<0.05. (**L**) Hippocampus, ns p>0.05. (**M-O**) Expression levels of microglia activation marker genes by RT-qPCR. (**M**) MHC II, **p<0.01. (**N**) TLR2, **p<0.01. (**O**) TLR4, ***p<0.001. (**P**) Hypothetical diagram. By Figdraw.

above is substantial, reaching up to 37%, with a notably elevated risk of suicide, especially among male patients.[33] Compounding the issue, LOD often masquerades as pseudodementia or vascular dementia, leading to frequent misdiagnosis in clinical practice.[34] The intricate and atypical symptomatology of LOD poses significant obstacles in its diagnosis and treatment. Despite ongoing research, the precise pathogenesis of LOD remains elusive, although emerging evidence suggests a potential link with inflammation and cognitive decline, thereby distinguishing it from recurrent depression.[35] The objective of this study was to elucidate the immune profile associated with late-onset depression.

Our investigation has unveiled a fascinating enrichment of immune response pathways in LOD through GSEA, which robustly supports the immune-inflammatory hypothesis of depression. Particularly noteworthy is our in-depth analysis of immune response-related pathways using ssGSEA, which revealed a striking enrichment of the T Cell Receptor (TCR) Signaling Pathway and Antimicrobial Gene Response in LOD.T cells, the backbone of adaptive immunity, undergo a sophisticated series of processes involving selection and activation via TCR signaling.[36] Our findings offer intriguing insights into the intricate dynamics of the TCR signaling pathway in LOD, potentially unraveling the nuanced mechanisms underlying the observed reduction in CD8_T infiltration in this complex condition.

In summary, LOD appears closely linked to immune response. Establishing a diagnostic system based on IRGs may be more effective than targeting individual genes. We identified 212 DEGs and focused on 1793 IRGs from the ImmPort database. Through analysis, we identified two key genes, *APLN* and *LEP*, shedding light on their potential roles in LOD.

APLN (apelin),[37] a polypeptide produced by the *APLN* gene on human chromosome Xq25-26.1, acts as a natural ligand for the G-protein-coupled receptor, APJ. Found in various tissues, APJ helps reduce inflammation by decreasing pro-inflammatory factors.[38,39] Research indicates that apelin/APJ signaling is prevalent in brain regions like the hippocampus, influencing stress response and depression treatment. Clinical studies show higher *APLN* levels in depressed peritoneal dialysis patients and genetic predispositions to depression and anxiety in certain allele carriers with coronary artery disease.[38,40] Preclinical studies support the antidepressant and anxiolytic effects of Apelin-13.[41] Our animal studies confirm elevated *APLN* mRNA expression in plasma and hippocampal tissues of LOD rats, implying the diagnostic promise of *APLN* for identifying LOD.

In our study, we identified *LEP (leptin)* as another key gene related to immune function. *LEP*,[42] a hormone produced by fat cells, plays a crucial role in enhancing immune function by binding to its receptors. It regulates various brain pathways involved in psychiatric disorders, such as the dopamine pathway and serotonin synthesis. Animal studies suggest that leptin may have antidepressant effects,[43] and clinical studies support this by showing reduced plasma leptin levels in major depression.[44] Furthermore, leptin acts as a pro-inflammatory factor, contributing to chronic inflammation.[45] Our results revealed significantly reduced *LEP* mRNA expression in the peripheral blood of LOD rats, suggesting *LEP* as a potential biomarker for immune-related mechanisms in LOD. However, inconsistent findings in hippocampal tissue warrant further investigation in larger samples.

After identifying HIDRs, we examined changes in immune cell populations. Using ImmuCellAI and CIBERSORT algorithms, we analyzed immune cell infiltration in LOD, bolstering result reliability. Our findings showed reduced CD8_T levels and increased neutrophil levels in LOD patient blood, consistent with prior studies.[8,46,47] Additionally, we observed elevated levels of pro-inflammatory cytokines IL-6 and TNF-α in both blood and hippocampal tissues of LOD rats, indicating significant peripheral and central inflammation. IL-6,[48] a major immune regulator, can both increase inflammation by recruiting immune cells like neutrophils and macrophages and trigger neuroinflammation by activating microglia in the brain.[49] Our research confirms microglia activation in the hippocampal tissues of LOD rats, marked by increased expression of microglia activation genes like *MHCII, TLR2*, and *TLR4*.[50] Leptin, part of the IL-6 family, may disrupt immune balance by boosting IL-6 release and increasing neutrophil infiltration. TNF-α, another critical cytokine, initiates inflammation and recruits more immune cells. Its presence in the periphery can induce neuronal damage by crossing the blood-brain barrier,[51] leading to apoptosis and further neuronal injury mediated by activated microglia.[52] In summary, our study identified two immune-related genes with diagnostic potential for LOD and elucidated their immune mechanisms.

Finally, we found that quercetin may be a potential therapeutic agent targeting these two key genes, and molecular docking showed that it binds highly and stably to both *APLN* and *LEP*. It is a flavonol compound widely found in nature in the form of a glucoside and has been shown to have anti-inflammatory and antidepressant effects in animal models or

clinical trials.[53,54] It has been shown that the expression of the pro-inflammatory factor *LEP* is suppressed in quercetin-treated obese rats.[55] There are no studies on quercetin-targeted *APLN* treatment, and our study may be a novel finding. In conclusion, the results of the study provide a new perspective for in-depth analysis of the mechanistic study of LOD.

However, there are still some limitations in this study: firstly the sample size of the gene chip datasets selected for this study is too small, and since there is only one set of sequencing datasets about LOD, further expansion of the sample size is still needed for subsequent validation and analyses of the value of these two genes as highly sensitive and specific diagnostic markers for LOD. Secondly, this study has only initially explored the immune mechanism in an animal model, which needs to be further validated in clinical samples. Moreover, the potential of quercetin as a therapeutic agent targeting *APLN* and *LEP* remains unvalidated, highlighting the need for further studies using animal models. Finally, single-cell sequencing to explore the interactions of different cell types may be a more valuable strategy. The results of the study provide a new perspective for in-depth analysis of the mechanistic study of LOD.

Conclusion

We identified two key genes that are strongly associated with LOD and described that their immune mechanism may involve increased release of pro-inflammatory cytokines IL-6 and TNF-α leading to immune cell dysregulation, including decreased CD8_T infiltration and increased neutrophil infiltration. Meanwhile, peripheral inflammation across the blood-brain barrier further promotes microglia activation, leading to neuronal damage.

Abbreviations

LOD, late-onset depression; GEO, Gene Expression Omnibus; LLD, late-life depression; MDD, major depressive disorder; CRP, C-reactive protein; HPA, hypothalamic-pituitary-adrenal; NK, natural killer; HIDRs, Hub Immune-DEGs; DEGs, differentially expressed genes; HC, healthy controls; WGCNA, Weighted Gene Co-expression Network Analysis; IRGs, Immune-Related Genes; ROC, receiver operating characteristic; GSEA, Gene set enrichment analysis; AUC, area under the curve; FDA, Food and Drug Administration; CUMS, Chronic Unpredictable Mild Stress; RT-qPCR, real-time quantitative reverse transcription polymerase chain reaction; ELISA, Enzyme-Linked Immunosorbent Assay; PLS-DA, Partial Least Squares Discriminant Analysis; EOD, early-onset depression; TCR, T Cell Receptor; APLN, Apelin; LEP, Leptin.

Data Sharing Statement

The data of this study are available from the corresponding author on reasonable request.

Ethics Approval and Consent to Participate

All studies covered in this thesis were approved by the Ethical Review Committee of the First Hospital of Shanxi Medical University. The experimental procedures were conducted following the National Institutes of Health Guide for the Care and Use of Laboratory Animals. (Opinion number: NO.KYLL-2024-066).

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors report no conflicts of interest in this work.

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