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Mult- Omics Approach-Based Autophagy Pathway Analysis in Alzheimer's Disease

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ABSTRACT

Autophagy, as an important cellular protective mechanism, is closely associated with the occurrence and progression of neurode- generative diseases such as Alzheimer's disease. However, the specific mechanisms underlying autophagy in neurodegenerative diseases remain unclear, and there are still many unknowns regarding its detailed regulation and involvement mechanisms. In this study, we aimed to use a multi-omics bioinformatics approach to integrate large-scale transcriptomic data and identify a series of autophagy key genes, namely CDKN2A, CXCR4, and IFNG, whose expression levels significantly differ between disease pa- tients and normal controls. Additionally, single-cell omics data will be supplemented to further investigate these findings. Our goal is to provide important clues for unraveling the pathogenesis of Alzheimer's disease and potentially identify novel targets for future therapeutics and diagnostics. Furthermore, by incorporating Mendelian randomization analysis, we will explore the causal relationships between vascular inflammation, depression , and mood fluctuations with Alzheimer's disease to provide insights into the associations among these related diseases and offer a theoretical basis and clinical application value for their treatment and prevention.

INTRODUCTION

Alzheimer's disease is a progressive neurodegenerative dis- order characterized by pathological changes in the brain, lead- ing to a gradual loss of cognitive and memory functions Ossenkoppele et al. (2015). It ultimately results in the loss of the ability to perform activities of daily living, posing significant psychological and physiolog-ical challenges for patients and their families, as well as a major challenge for medical research Poncet et al. (2011).

Currently, there is no effective treatment available for Alzheimer's disease, and interventions with medications of- ten only provide symptomatic relief without slowing down the progression of the disease Mahmoudi et al. (2014). Research has indicated that Alzheimer's disease is associated with the accumulation of beta-amyloid protein in the brain, leading to the formation of neurofibrillary tangles and amyloid plaques, which are linked to neuronal dysfunction and cell death, ultimately resulting in the decline of cognitive and memory functions Dohler et al. (2014), Yilmazer-Hanke et al. (1999).

Autophagy, a cellular mechanism that involves the break- down and recycling of cellular components, plays a crucial role in maintaining neuronal function and structure by clearing ab- normal proteins and organelles Li et al. (2015), Xu et al. (2017).

It holds potential signifi- cance in Alzheimer's disease by aiding in the clearance of beta- amyloid accumulation. Thus, autophagy has emerged as an im- portant area for research and the development of therapeutic approaches for Alzheimer's disease Funderburk et al. (2010). However, the specific mechanisms and regulatory pathways of autophagy in neurode- generative diseases remain incompletely understood, and there is still much unknown regarding the detailed regulation and in- volvement of autophagy Bostancikliogl u et al. (2019).

Therefore, in this study, we propose to employ a multi- omics approach to comprehensively analyze gene expression and reg- ulation within the autophagy pathway. By integrating transcrip- tomics with single-cell genomics, we aim to gain a deeper un- derstanding of the expression patterns, regulatory networks, and changes associated with neurodegenerative diseases. This will provide important research data for further investigation into the role of autophagy in the development and progression of neurodegenerative diseases.

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MATERIALS AND METHODS

Multi-omics data preparation andpreprocessing

We will call the" GEOquery" package in R software Davis et al. (2007) to obtain the information for two datasets, GSE5281 Liang et al. (2007), Liang et al.(2008), Readhead et al.(2018), Liang et al.(2008) and GSE138260 Nitsche et al. (2021), from the GEO database (http://www.ncbi.nlm.nih.gov/geo/) online. We will then use the annotation platform GPL570 - 55999 to perform the probe name-to-gene name conversion operation on the GSE5281 dataset, and the alternative annotation platform GPL27556 - 55246 to complete the corresponding annotation operation for the GSE138260 dataset. Subsequently, we will organize and merge the expression matrices with their respec- tive clinical information to obtain a merged expression ma- trix. We will then perform a union operation with the 232 autophagy genes provided by the autophagy information web- site (http://www.autophagy.lu/index.html) to complete the ini-tial data organization. Next, we will use the "factoextra" Soreq et al.(2023) and "FactoMineR" Alsema et al.(2020) packages in R software to perform PCA data dimensionality reduction, and calculate and display the first and second principal components. We will use the Com- Bat function in the " sva" package Mundt et al.(2020) in R software to correct for batch effects in the merged data. Finally, we will use the " limma" package Husson SeLeaJJaFcco et al.(2008) in R software to standardize the merged data. The aforementioned steps will complete the data prepro- cessing and organization for the two datasets, preparing for sub- sequent data mining and analysis.

Transcriptomic gene differential expression studies

We performed differential gene analysis on the transcrip- tomic data using the "limma " package in R software. A log fold change (logFC) threshold of +0 .4 and -0 .4 was set, and genes with a P-value less than 0.05 were selected for further analysis Wang et al.(2022). The resulting differentially expressed genes for each sample were obtained, and volcano plots and heatmaps were generated to visualize the differential gene expression.

To construct the volcano plot, differentially expressed genes were plotted based on their logFC values on the x-axis and their corresponding statistical significance (e .g. , -log10(P-value)) on the y-axis. Genes with a larger absolute logFC and a smaller P-value were represented with larger dots, indicating more sig-nificant differential expression.

The heatmap was generated to visualize the expression patterns of differentially expressed genes across samples. Clus-tering algorithms were applied to group genes and samples based on their expression profiles, and the resulting heatmap displayed the expression levels of genes, with different colors representing different expression levels.

These visualizations provide a comprehensive overview of the differential gene expression patterns and aid in identifying potentially important genes and pathways associated with the condition under investigation Wang et al.(2022) .

Multi-omics combined immunoinfiltration analysis

We utilized the " IOBR" package Xiong et al.(2023) in R software to in- vestigate the degree and composition of immune cell infil- tration between different samples using the Cell-type Identi-fication By Estimating Relative Subsets Of RNA Transcripts (CIBERSORT) method. CIBERSORT employs machine learn-ing algorithms to estimate the relative abundance of various cell types within a complex mixture of cells based on the gene expression profiles. By comparing the expression profiles to pre-constructed reference labels of different cell types, CIBER- SORT predicts the relative abundance of each cell type in the mixed cell sample. We then constructed multiple boxplots to observe any significant differences in immune cell populations.

Furthermore, using single-cell transcriptomics data, we fur-ther analyzed the differences in cell proportions between Alzheimer's disease patients and healthy individuals. We cal-culated module scores for gene sets using a custom dataset and employed t-SNE dimensionality reduction method to visualize the expression profiles of cells at different levels of immune scores.

These analyses allow us to gain insights into the differences in immune cell composition and function between various sam-ples, providing valuable information about the potential role of immune cells in disease pathology.

GO,KEGG, GSEA enrichment analysis

We used the " clusterProfiler" package Guangchuang et al.(2012) in R software to perform Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG) , and gene set enrichment analysis (GSEA) on the differentially expressed genes obtained earlier. We set the threshold for both P- value and q-value at 0 . 05 and utilized the Benjamini-Hochberg procedure, denoted as "BH," for multiple test adjustment.

This enrichment analysis allowed us to identify the signif-icantly enriched GO terms, KEGG pathways, and gene sets associated with our differentially expressed genes. The BH method was employed to control for false discovery rate and ensure the reliability of the enrichment results.

By examining the enriched pathways and functional annota-tions, we gained insights into the biological processes, molecu-lar functions, cellular components, and signaling pathways that are potentially influenced by the differential expression of genes in our study.

PPI network construction and Hub gene screening

We further analyzed the differentially expressed genes from the transcriptomic analysis by importing them into the String database (https://cn.string-db.org). This database enabled us to construct a protein-protein interaction network and obtain rele-vant data information.

By utilizing the Cytoscape software, we employed the MNC algorithm (Multiple-Napsack Clustering Algorithm) to select the top 10 hub genes with the highest values. The MNC al-gorithm allowed us to identify key genes that potentially play important roles in the protein interaction network.

The protein-protein interaction network and the selected hub genes provided insights into the potential functional modules and biological processes that are influenced by these key genes. This analysis further enhanced our understanding of the regu-latory mechanisms and molecular interactions underlying the observed differential gene expression in our study.

Construction of Lasso regression model

To perform single-variable logistic regression on the differen-tially expressed genes from the transcriptomic data, we utilized the " glmnet" package in R software Tay et al.(2023) . We selected genes with a p-value ¡ 0 . 05 as candidate genes for logistic regression. To ensure reproducibility of the results, we set a random seed and specified a maximum of 1000 iterations.

To reflect the best performance of the model on the validation set, we determined the optimal regularization parameter value using cross-validation and plotted the number of best genes se-lected during model training. Using the coefficients obtained from the model, we integrated the data to calculate the risk scores for each sample.

To evaluate the predictive performance of the model, we em-ployed the "pROC" package in R software Robin et al.(2011) to compute and plot the ROC curve. This quantitative evaluation provides a measure of the model's discriminatory ability.

By implementing these procedures, we performed logistic re-gression on the differentially expressed genes, identified the best genes using cross-validation, calculated risk scores for each sample, and evaluated the model 's performance using ROC analysis.

Key gene screening and establishment of disease risk as-sessment model

To generate a Venn diagram summarizing the intersection of genes from different categories, we utilized the "VennDia- gram" package in R software Chen et al.(2022) . We defined five categories: a common gene set (Common gene), an autophagy gene set (Au-tophagy), a significantly differentially expressed gene set (Dif-ference) , a Lasso modeling key gene set (Lasso) , and a PPI hub gene set (Hub). By visualizing the intersections of these sets, we obtained candidate key genes for each category.

To identify the final set of strong candidate key genes, we defined them as genes that are expressed in at least four of the module parts. With the final selection of key autophagy genes, we constructed a Nomogram risk plot to develop a predictive model for the occurrence of Alzheimer's disease. To evaluate and validate the model, we employed ROC curves and calibra-tion plots.

By implementing these procedures, we generated a Venn di-agram to display the intersections of genes from different cat- egories (Common gene, Autophagy, Difference, Lasso, and Hub). We obtained candidate key genes for each category and selected the final set of strong candidate key genes that are ex- pressed in at least four parts. Using these final key autophagy genes, we constructed a Nomogram risk plot to develop a prob-abilistic model for the onset of Alzheimer's disease. Finally, we evaluated and validated the model using ROC curves and calibration plots.

Key gene localization and quasi- temporal analysis

To investigate the expression differences and temporal se- quencing relationships of key autophagy genes at the single- cell level, we utilized the " Seurat" package Hao et al.(2024) and the "mon-ocle" package Trapnell et al.(2014) in R. This allowed us to perform single- cell transcriptomic cell localization and pseudo-time analysis, en-abling a deeper understanding of the dynamic changes in gene expression within and between individual cells. By mining the dynamic changes in gene expression both intra- and extracel-lularly, we obtained more comprehensive and in-depth insights into the complexity of Alzheimer's disease. This knowledge will contribute to the development of novel therapeutic strate-gies and a better understanding of neuroregulatory mechanisms at the cellular level, ultimately facilitating the deciphering of the intricacies of the disease.

Cell communication analysis

By utilizing the " Cell Chat" package Jin et al.(2024) to analyze single- cell transcriptomic data, we can gain deeper insights into the mechanisms underlying the interactions between cells.

This approach allows us to explore the occurrence and progression of Alzheimer's disease from multiple perspectives, potentially providing new clues for the development of innovative treat- ment strategies.

By deciphering the complex communication networks between cells, we can identify key signaling pathways and gain a better understanding of the changes in cell types, functions, and interactions.

This, in turn, provides substantial support for the research and treatment of Alzheimer's disease.

Mendelian randomization analysis

By utilizing the "TwoSampleMR" package Hemani et al.(2017) in R, we per- formed Mendelian randomization (MR) analysis to examine the causal relationships between pairwise variables.

We obtained data from the IEU OpenGWAS project (http://mrcieu.ac.uk) GWAS database. In the MR analysis, we designated a set of traits— depression (GWAS ID: ieu-a- 1187) , vasculitis (GWAS ID: finn-b-L12 VASCULITISNAS) , and moodswings (ukb-b - 14180)— as exposure variables for Alzheimer 's disease (GWAS ID: ieu-b-5067), which served as the outcome variable. We con- ducted two-sample MR analysis after preprocessing the data to ensure compatibility of effect alleles and effect sizes.

We generated odds ratio (OR) values for this binary outcome and performed tests for heterogeneity and multiple outcomes.

We visualized the results using scatter plots and conducted sensi- tivity analysis with leave-one-out plots. Finally, we displayed the results using visual representations.

RESULTS

Data collation and transcriptomic difference analysis re- sults

After visualizing the distribution of the merged expression matrix derived from transcriptomic data, it became evident that there was a substantial batch effect between the two original datasets used. Batch effects can lead to cumulative errors and ultimately affect subsequent gene expression analysis and in- terpretation.

To address this issue, we performed batch correc- tion and normalization procedures (Figure 1a) to ensure that the gene expression data are more reliable and comparable.

Next, we took the obtained gene data and performed a union operation with 232 autophagy-related genes provided by the Autophagy Database (http://www.autophagy.lu/index.html) .

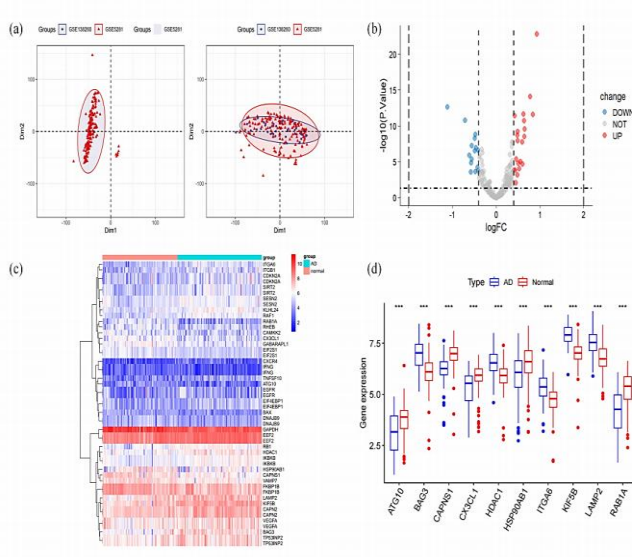
We conducted differential analysis and identified 36 differen- tially expressed autophagy-related genes, including 21 upregu- lated genes and 15 downregulated genes (with a log-fold change threshold of 0 .4 and a p-value cutoff of 0 . 05) . We visual- ized the differentially expressed autophagy-related genes using a volcano plot (Figure 1b).

and displayed the changes in up- and downregulated autophagy-related genes using a heatmap (Figure 1c) . Among the 36 differentially expressed genes, the top five upregulated autophagy-related genes were ITGA6, HDAC1, LAMP2, BAG3, and KIF5B , while the top five down- regulated ones were RAB1A , CAPNS1 , CX3CL1 , HSP90AB1 , and ATG10 (Figure 1d) .

Therefore, it can be inferred that Alzheimer 's disease alters the expression levels of relevant autophagy-related genes.

For the single-cell datasets GSE5281 and GSE138260 (in- cluding samples from healthy individuals and Alzheimer's disease patients), we selected four single-cell data samples (GSM5348375, GSM5348374, GSM5348377, GSM4403286) labeled as HC1, AD1, HC2, and AD2, respectively.

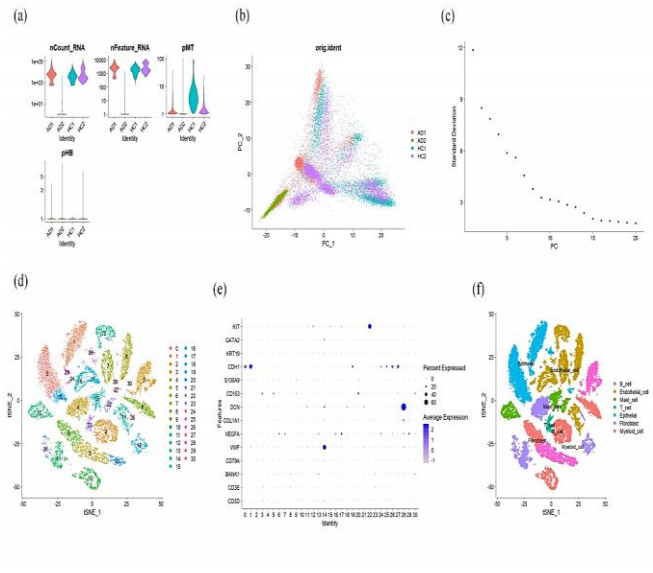
Figure 1:



(a) Distribution of transcriptome data from GSE5281 and GSE138260 before and after batch effect re- moval. (b) Volcano plot of differentially expressed autophagy genes (p ¡ 0 .05 , logFC thresholds for upregulated and downreg- ulated genes set at 0 .4 and -0.4 , respectively) . (c) Heatmap of differentially expressed autophagy genes. (d) Boxplots show- ing the top 5 upregulated and top 5 downregulated autophagy genes.

We compared the total RNA count (nCount RNA), the number of RNA features (nFeature RNA) , the percentage of mitochon- drial genes (pMT), and the percentage of hemoglobin genes (pHB) among these groups using violin plots (Figure 2a) . Sim- ilar to the previous analysis, we observed a noticeable batchef- fect. To address this, we applied the "LogNormalize" method to perform a logarithmic transformation of the raw data, achieving standardization and comparability between different samples. We then visualized the processed single-cell data using PCA to effectively correct the data (Figure 2b). The elbow plot indi- cated that the optimal number of principal components (PCs) was 15 (Figure 2c) . Based on the relevant parameters, we vi- sualized the reduced-dimensional single-cell dataset in t-SNE space and identified 31 clusters (Figure 2d) . We further dis- played the expression patterns of feature genes in different cell clusters using a bubble plot (Figure 2e) and performed enrich- ment analysis for different cell clusters (Figure 2f) . The annota- tion resulted in six major cell types: T cells, B cells, endothelial cells, fibroblasts, myeloid cells, and epithelial cells.

Figure 2:



(a) Violin plots comparing the data distribution of 4 single-cell samples in the dimensions of nCount RNA, nFeature RNA, pMT, and pHB. (b) Overall data distribution of the 4 single-cell samples after PCA dimensionality reduction. (c) Elbow curve showing the selection of the optimal number of principal components (PCs) . (d) Visualization of the single- cell dataset in t-SNE space after dimensionality reduction. (e) Bubble plot showing the expression of feature genes in different clusters of the single-cell dataset. (f) Visualization of different clusters in the t-SNE space of the single-cell dataset after man- ualannotation .

Results of multi-omics analysis of infiltration degree of im- mune cells

The analysis of differentially expressed genesis an important data basis for immune infiltration analysis. We further con- ducted immune infiltration analysis based on this to identify changes in immune cell types under the regulation of differ- entially expressed autophagy genes (Figure 3a). This helps us gain a deeper understanding of their potential roles in disease development and immune response. We labeled the experimen- tal group as "high" (high-risk group for Alzheimer's disease) and the control group as "low" (low- risk group for Alzheimer 's disease) (Figure 3b) .

Through immune infiltration analysis, we identified path- ways with significant differences in infiltration levels: Ossenkoppele et al.(2015) CD4+ T cells that have not undergone antigen stimulation, dif- ferentiation, or functional maturation: the decrease in CD4+ T cells that have not undergone antigen stimulation may reflect an immune system imbalance Taams et al.(2002). This could be due to cer- tain issues in the immune system's response to antigen stimu- lation or abnormalities in maintaining immune balance, which affects the body's ability to respond to potential pathogens or abnormal proteins. Poncet et al. (2011) Activated CD4+ memory T cells: the activation of CD4+ T cells suggests that the immune system is trying to respond to potential inflammation or infection Swain et al.(2000). This partly reflects the immune system's reaction to problems in the nervous system. Mahmoudi et al. (20- 14) T cell co -regulatory proteins: the presence of these proteins indicates that the immune system is attempting to regulate its own activity or play a role in mitigat- ing inflammatory responses Jones et al.(2020) . Dohler et al. (2014) Both activated and unac- tivated NK cells: an increase in the number of NK cells in both states may indicate that the immune system is responding to pathological reactions related to Alzheimer's disease, such as inflammatory reactions or the clearance of abnormal proteins Chang et al.(2003) . Yilmazer-Hanke et al.(1999) Resting dendritic

cells: the activity state of dendritic cells directly affects the information transmission and synaptic connections of neurons.

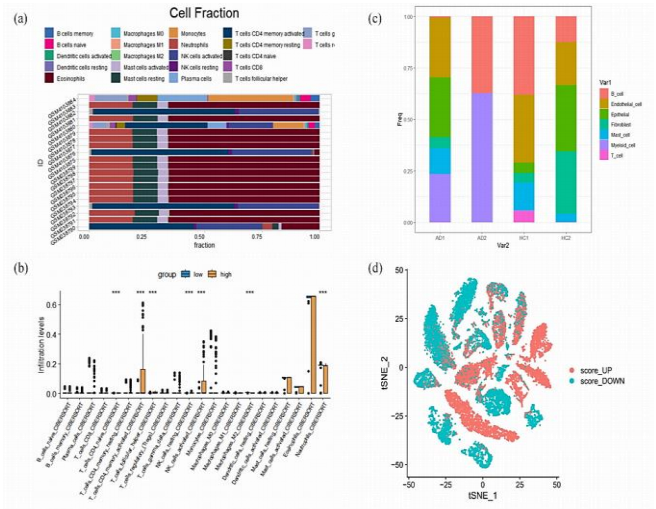
Their decreased infiltration level may affect neural signal transmission, exacerbating cognitive dys - function in Alzheimer 's disease patients Fernandez et al.(1999) . Li et al. (2015) Neutrophils: as the main granulocytes in the immune system, the significant differences in their presence may indicate the presence of patho- logical processes such as inflammation reactions or infections in Alzheimer 's disease patients Teillet-Thiebaud et al.(1985).

Comprehensive analysis of immune infiltration helps reveal the key role of the immune system in biological processes and is of significant importance for understanding disease mech- anisms and discovering therapeutic targets. Subsequently, to further explore immune pathway information in Alzheimer's disease patients, we visualized the proportions of different cell types using the previously organized single-cell transcriptomics data (Figure 3c).

We observed an increase in myeloid cell pro- portions in Alzheimer's disease samples compared to healthy samples and visualized this through a heat map (Figure 3d).

This allows us to understand the specific distribution of cells with high or low immune levels and to discover that cells with high immune level scores are mostly myeloid cells.

Figure 3:



(a) Visualization of the proportion of immune cells in each sample. (b) Boxplots showing multiple groups of high and low-risk categories, where a higher number indi- cates greater significance.

(c) Bar plots displaying the differ- ential proportions of different cell types between healthy con- trol (HC) and Alzheimer's disease (AD) samples in single-cell omics data. (d) Visualization of the distribution of cells with high and low immune levels based on scoring in single-cell omics data.

Enrichment results by multiple methods and pathways

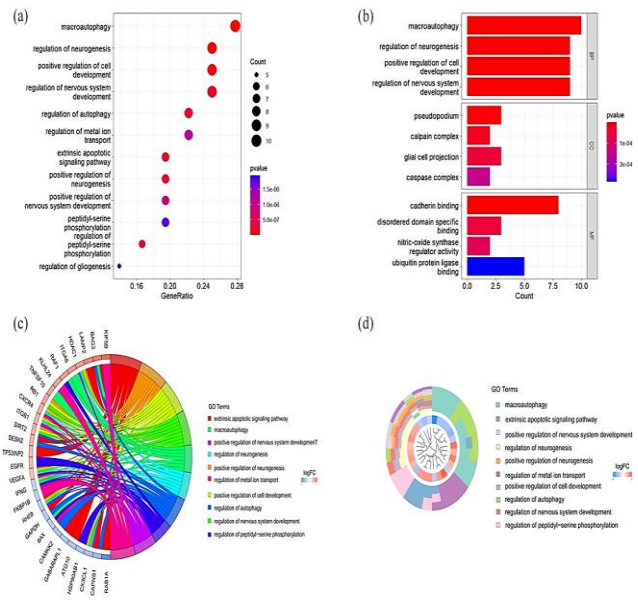
To gain a more comprehensive understanding of the role and regulatory mechanisms of these genes in cellular processes and biological functions, we performed gene enrichment pathway analysis based on the differentially expressed genes obtained from the previous differential analysis.

This analysis aimed to reveal the metabolic pathways, signaling pathways, and other related biological processes involved in the differentially ex- pressed autophagy genes.

This enables a deeper understanding of the important roles these genes play in disease development and physiological regulation.

After conducting comprehensive gene ontology (GO) enrichment analysis incorporating cellu - lar component (CC), biological process (BP), and molecular function (MF) (Figure 4a , b , c, d) , the most prominent path- ways identified included macroautophagy, regulation of neural growth, positive regulation of cell, regulation of the nervous system, and regulation of autophagy.

Figure 4:



(a) Bubble plot showing GO enrichment. (b) Bar plot showing significantly enriched GO terms (p¡0.05). (c) Cluster plot depicting the clustering of enriched GO terms. (d) Circle plot illustrating the enriched GO terms.

Macroautophagy is involved in clearing abnormal protein ag - gregates and damaged organelles within nerve cells and helps maintain the stability of the intracellular environment Wong et al.(2011).

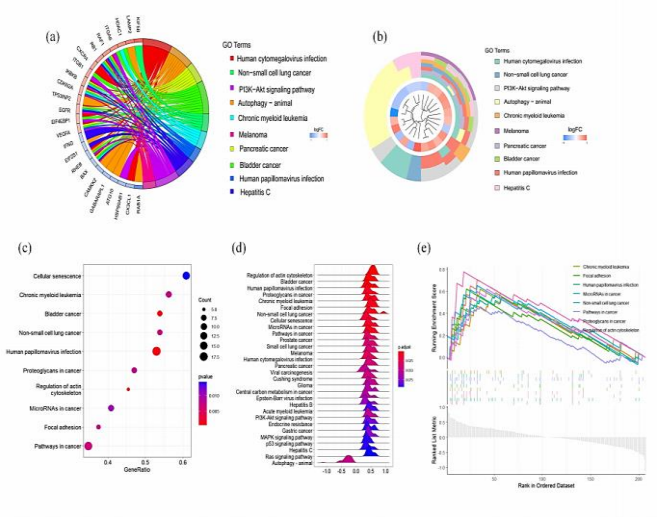
Re- search has shown that the expression and activity of the au- tophagy pathway in the brain are abnormal in Alzheimer's dis- ease patients, leading to protein aggregation and disruption of organelle function, thereby exacerbating the progression of Alzheimer's disease Ma et al.(2010).

On the other hand, the regulation of neural growth and positive regulation of cell are also associated with Alzheimer 's disease Fernandez -Verdecia et al.(2009) .

In the nervous system, neuroge- nesis and synapse formation require precise regulation and co- ordination of both intracellular and extracellular environments, with positive regulation of cells playing a critical role in this process.

When the positive regulation of cells is imbalanced, neurogenesis and synapse formation may be affected, thereby exacerbating the abnormal state of the nervous system.

Figure 5:



(a) Cluster plot depicting KEGG enrichment (p¡0 . 05) . (b) Circle plot illustrating enriched KEGG pathways. (c) Bubble plot showing GSEA enrichment (p¡0 .05) . (d) Ridge plot illustrating GSEA enrichment. (e) Top 8 enriched path- ways displayed in GSEA.

Furthermore, after performing KEGG enrichment and GSEA enrichment analyses, the results indicate that the autophagy pathway is also enriched in various cancer phenotypes (Figure 5a , b , c, d, e) .

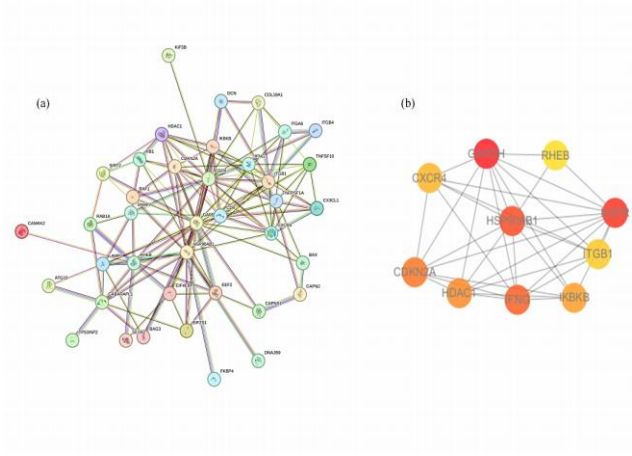
This further highlights the important role of autophagy in neurodegenerative diseases and cancer, providing insights into the complex regulation of autophagy in disease development and expanding the scope of autophagy research.

This shared regulatory mechanism may provide new research directions at the intersection of these two diseases and empha- sizes the universal importance of autophagy in cellular survival and metabolic regulation.

Protein interaction network and gene screening of network center node

Based on the gene pathway enrichment analysis, we pro - ceeded to construct a protein-protein interaction (PPI)

Figure 6:



(a) PPI network. (b) Top 10 hub genes based on MNC algorithm.

network and performed hub gene screening. By analyzing the protein- protein interaction network (Figure 6a),

we can explore the in- teraction relationships between genes more deeply and identify "hub" genes that play a key role in the entire biological net- work (Figure 6b) .

These "hub" genes often interact with mul- tiple other genes and have a significant impact on the stability and function of the entire network.

Therefore, identifying these critical protein-protein interaction networks and hub genes will help us better understand the patterns of interaction

Table 1: Top 10 hub genes and their changes based on the MNC algorithm.

Gene Symbol	Change Type
CXCR4	UP
GAPDH	DOWN
RHEB	DOWN
EGFR	UP
ITGB1	UP
IKBKB	UP
IFNG	DOWN
HDAC1	UP
CDKN2A	UP
HSP90AB1	DOWN

Note: The Change type corresponding to each gene is derived from the previous differential analysis results.

between genes and their biological functions, providing further insights and guidance for further bioinformatics analysis.

In this study, we used the multiple neighborhood-based clus- tering (MNC) algorithm to screen the top 10 hub genes in the PPI network (Table 1) . These hub genes are CXCR4 , GAPDH, RHEB, EGFR, ITGB1 , IKBKB, IFNG, HDAC1 , CDKN2A , and HSP90AB1 .

LASSO regression modelfeature geneselection results

After constructing the PPI network and screening for hub genes, a crucial step we took was to perform LASSO regression model to select key genes. By using machine learning methods, we were able to further narrow down the scope of analysis and identify the most critical genes, deepening our understanding of the molecular changes caused by interventions in Alzheimer's disease.

The application of LASSO regression model will help us identify genes that have a significant impact on disease status or treatment interventions, laying a solid foundation for further bioinformatics analysis and clinical translational research.

During the training process of the model, the complexity of the model decreases gradually on the training set as the regular- ization parameter increases, and the performance metric values become worse (Figure 7a). On the validation set, the perfor- mance metric reaches its optimum around a certain regulariza- tion parameter value and then gradually deteriorates as the reg- ularization parameter increases. This optimal regularization pa- rameter value reflects the best performance of the model on the validation set. By observing the cross-validation plot, it can be seen that the optimal number of genes in the model is 20 (Fig- ure 7b) , and the model 's coefficients for 19 relevant genes along with an intercept term are obtained (Table 2). Furthermore, a ROC curve is plotted to evaluate the performance of the model (Figure 7c) . The calculated AUC value is 0.970 , indicating that the built model can effectively distinguish between the healthy and Alzheimer's disease categories in this binary classification task, with a high accuracy rate and recall.

Venn and Nomogram mapping of key genes

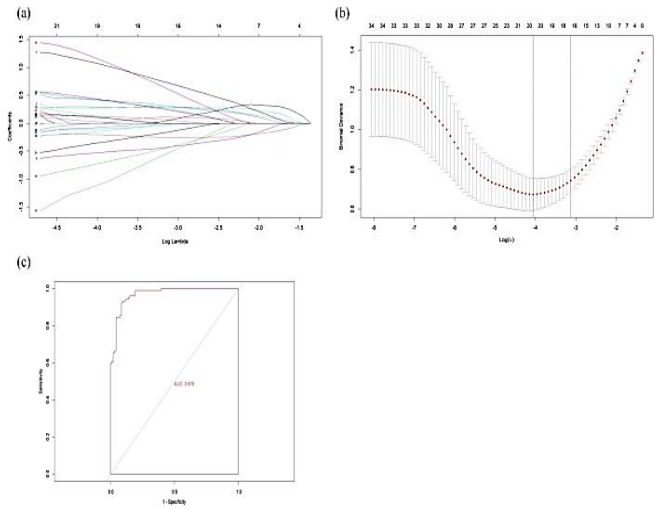
After a series of gene screening operations, we ultimately take the intersection of the screening results from the various parts mentioned above to obtain the final set of selected genes. The purpose of this step is to integrate the results from different screening methods, retain genes that are consistent and impor- tant, and further highlight the key roles of these genes in bi- ological processes. In this study, we set the original common gene set (Common gene)

Table 2 : Coefficients and intercept for the 19 key genes in the Lasso constructed model

Gene	Coef
(Intercept)	-8.482628529
RAB1A	-0.203382697
KLHL24	0.27250216
BAG3	0.430830843
EEF2	0.291031202
CAPNS1	-0.536966269
RAF1	1.089670214
VAMP7	-1.144675163
CAPN2	0.419745604
SESN2	0.130015567
RB1	1.186959801
BAX	-0.745967665
CX3CL1	-0.083016706
CAMKK2	-0.106564441
CXCR4	0.443820169
ATG10	-0.368013442
TNFSF10	0.128330603
EIF4EBP1	3.08E-02
IFNG	-0.157499347
CDKN2A	0.108990092

Note: (Intercept) represents the intercept term.

Figure 7:



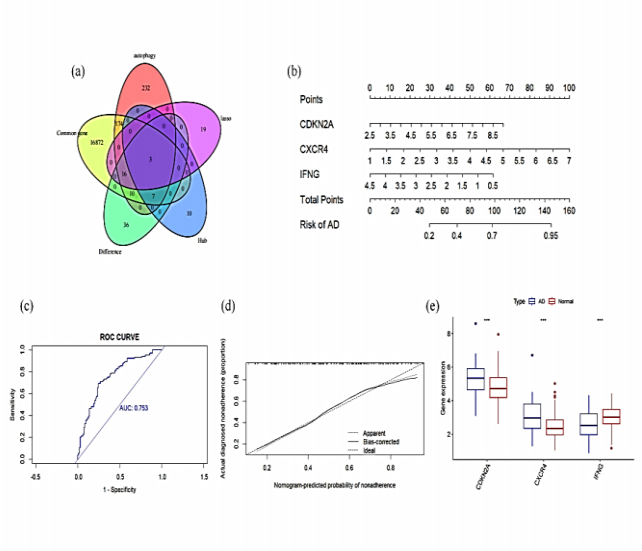
(a) Regularized pathway diagram. (b) glmnet cross-validation plot. (c) ROC curve.

autophagy gene set (autophagy) , sig- nificantly differentially expressed gene set (Difference), Lasso modeling key gene set (Lasso), and PPI key hub gene set (Hub) as the 5 parts for intersection (Figure 8a) . We identified CDKN2A, CXCR4, and IFNG as strong candidate key genes that were expressed in all 5 modules (Figure 8e) . These genes were used as variables for logistic regression analysis, and a Nomogram column chart was constructed to build a model for assessing the degree of autophagy deterioration in Alzheimer's disease (Figure 8b). The ROC curve of the model was plotted (Figure 8c), and the AUC value of the model was found to be 0 . 753 , indicating a good model performance. Furthermore, the calibration curve was used to

further evaluate and observe the performance of the model, and it was found that the calibrated curve had a good correlation with the ideal straight line (Figure 8d), once again demonstrating the good performance of the established multi-factor logistic regression model in assessing the degree of autophagy deterioration in Alzheimer's disease.

CDKN2A, CXCR4, and IFNG genes can serve as reliable in- dicators for evaluating the degree of autophagy deterioration in Alzheimer's disease.

Figure 8:



(a) Gene Venn diagram under multiple screening methods. (b) Nomogram plot of Alzheimer's disease risk col- umn. (c) Model calibration curve test plot. (d) ROC curve model test. (e) Box plot of three strong candidate key genes CDKN2A, CXCR4, and IFNG.

Location of key autophagy genes and results of pseudo - time series analysis

After completing the screening of key autophagy genes in Alzheimer ' s disease using transcriptomic data, we used single- cell genomics data to localize the expression of the strong can- didate key autophagy genes CDKN2A, CXCR4, and IFNG in different types of cells (Figure 9a, b). After performing dis- persion analysis and selection on the single -cell genomics gene data and reordering them (Figure 9c),

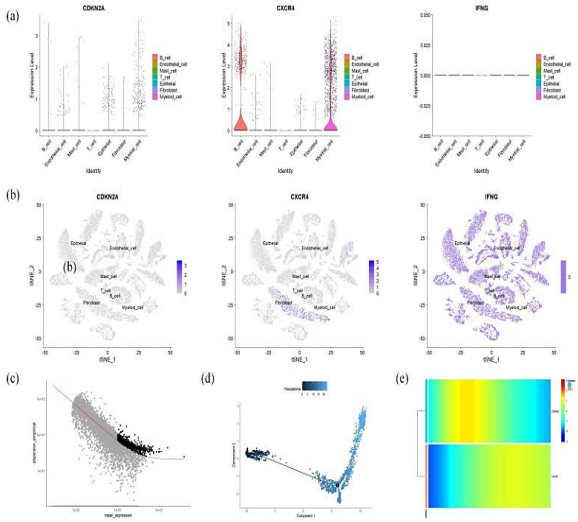
we generated a cellu- lar developmental time series plot (Figure 9d) and observed the expression changes of the key autophagy genes CDKN2A and CXCR4, which had relatively high expression levels, during the cell growth and development cycle (Figure 9e).

We found that the expression level of the CDKN2A gene increased initially and then decreased during the cell growth and devel- opment cycle, while the CXCR4 gene showed an increasing trend.

Through pseudo-temporal analysis based on single-cell genomics,

we can better understand the developmental trajec- tory of target genes in different cell types and the dynamic changes in cell states. This further reveals the dynamic changes of key autophagy genes in the process of Alzheimer's disease.

Figure 9:



(a) Violin plots showing the expression levels of CDKN2A, CXCR4 , and IFNG genes in different cell types.

(b) Heatmap illustrating the expression distribution of CDKN2A, CXCR4 , and IFNG genes in different cell types in t- SNE space.

(c) Scatter plot of genes after discrete analysis and filtering of single-cellomics data followed by reordering.

(d) Single-cell omics cell developmental timeline plot.

(e) Heatmap show- ing the expression changes of key autophagy genes CDKN2A, CXCR4 , and IFNG in the cell growth and development cycle in Alzheimer's disease.

Cell communication analysis results

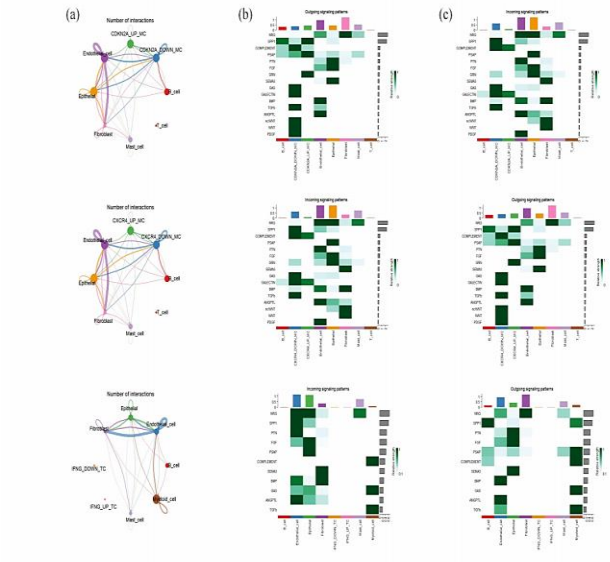
After localizing and pseudo-temporal analysis of key autophagy genes, it was found that the CDKN2A, CXCR4, and IFNG genes have different expression patterns in different types of cells (Figure 9a).

It was found that the CDKN2A and CXCR4 genes are highly expressed in myeloid cells compared to other cells, while the IFNG gene is mainly expressed in T cells with lower expression levels.

Taking the CDKN2A gene as an example, we divided the myeloid cell cluster data into two groups based on the differential expression of the CDKN2A gene (Figure 10a).

Subsequently, we observed the communi- cation networks and outgoing/incoming signaling patterns be- tween the cells in these groups (Figure 10b, c) and plotted a bubble chart to display the signaling pathway of different cell groups (Figure 11a).

Figure 10:

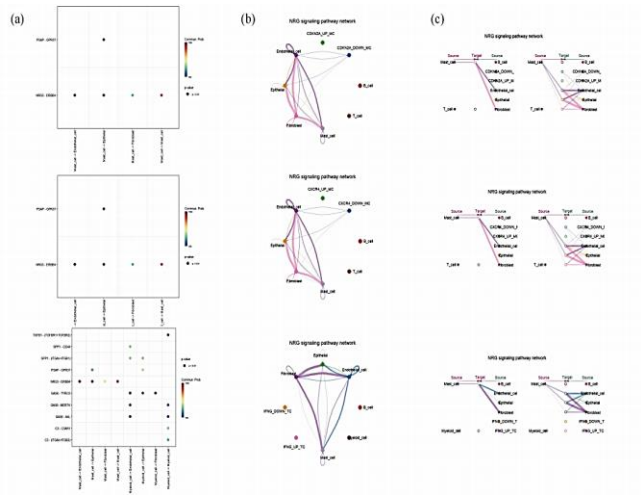


(a) Visualization depicting the overall communi- cation network between different grouped cells. (b) Heatmap displaying the outgoing signaling patterns between different grouped cells. (c) Heatmap illustrating both the incoming and outgoing signaling patterns between different grouped cells.

The NRG signaling pathway network was found to have a high occurrence frequency in the entirecell communication network.

By visualizing theinteractions be- tween different cell groups on theNRG signaling pathway (Figure 11b),

Figure 11:



(a) Bubble plot demonstrating the signaling path- way diagram corresponding to different grouped cells.

(b) Network of NRG signaling pathways.

(c) Visualization of NRG signaling pathways with Mast cells and T cells as signal- receiving cell clusters under different groups.

it was observed that there is a close connection be- tween the Mast cell and Endothelial cell groups in the NRG sig - naling pathway.

It is speculated that the changesin CDKN2A autophagy gene expression are related to the reciprocal interaction between the NRG signaling pathway and the Mast cell cell group and B cells.

The analysis methods for the other two genes are consistent with this.

Mendelian random sampling analysis results

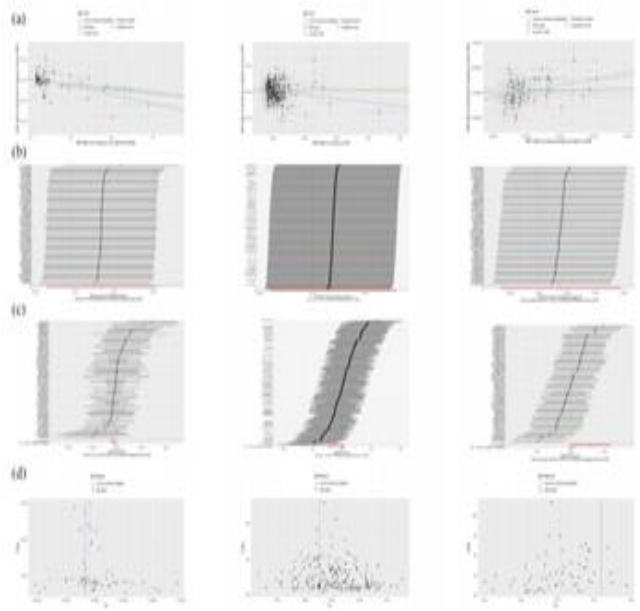
To explore the association between different exposure fac- tors and the onset of Alzheimer's disease and gain a deeper understanding of possible pathophysiological mechanisms, we adopted Mendelian randomization analysis using depression and vascular inflammation as exposure factors and Alzheimer's disease as the outcome variable.

Mendelian randomization analysis allows for more accurate assessment of causal relation- ships between influencing factors and outcome variables, thus assisting in the formulation of clinical decisions.

The appli- cation of this method is expected to provide important scien- tific evidence for more effective prevention and intervention of Alzheimer's disease.

After conducting sensitivity analysis (Figure 12b), we found that the central values for vascular inflammation and mood fluc- tuations were greater than 0, indicating a more reliable re- lationship between vascular inflammation and mood fluctua- tions. Further analysis was conducted on the exposure factors, and a forest plot was generated to reflect the increased risk of Alzheimer 's disease with worsening mood fluctuations un - der the IVW (Inverse Variance Weighted) calculation method (while the unreliable nature of the results generated by depres- sion can be seen from the leave-one-of the results, balances potential random errors and biases through random grouping, ensuring that the differences between the ex- perimental and control groups are caused by the treatment vari- able rather than other factors. It also enhances the effectiveness of statistical inference, establishes causal relationships between exposure factors and outcome variables, and conducts statistical hypothesis testing Carter et al. (2023).

Figure 12:



(a) Scatter plots depicting depression, vas- culitis, and emotional fluctuations as exposure factors,with Alzheimer's disease as the outcome variable. (b) Leave- one- out plots for each exposure factor after heterogeneity and multi- effectiveness testing. (c) Forest plot showing depression, vas- culitis, and emotional fluctuations as exposure factors, with Alzheimer's disease as the outcome variable. (d) Funnel plot displaying depression, vasculitis, and emotional fluctuations as exposure factors, with Alzheimer 's disease as the outcome variable.

DISCUSSIONS

For the final selection of genes:

The relationship between upregulation of the CDKN2A gene and Alzheimer's disease deserves further discussion. Genes regulated by CDKN2A play important roles in cell cycle reg- ulation and apoptosis, and have also been found to be closely associated with the autophagy pathway Budina-Kolomets et al.(2013).

In Alzheimer's disease,the aggregation of abnormal proteins and disrupted cel- lular metabolism are important components of the pathogenic mechanism, and the upregulation of the CDKN2A gene may lead to out plot, and the lower sig- nificance of the results related to vascular inflammation can be observed from the box plot) (Figure 12a , c) .

Additionally, the funnel plot indicates that the Mendelian randomization analysis satisfies the requirements for Mendel's second law of random- ization (Figure 12d) .

Mendelian randomization analysis can effectively help con- trol confounding factors in experiments and minimize the ran - dom differences between experimental and control groups.

This approach reduces the impact of confounding factors on exper- imental results, improves the reliability and comparability dysregulation of the autophagy pathway, thereby affect- ing the clearance of abnormal proteins and ultimately exacer- bating neuronal dysfunction and death.

Furthermore, CXCR4, as a chemokine receptor, is involved in neuronal survival, migration, and synapse formation in the nervous system, and is also associated with the autophagy path- way Hu et al.(2018).

Its upregulation may affect neuronal survival or worsen the disease by influencing the stability of neural cir- cuits.

IFNG, as an important immune regulatory factor, plays a key role in inflammation response and immune function Deng et al.(2023) . Pre- vious studies have shown that abnormal activation of inflamma- tion and the immune system is closely related to the pathogenic mechanisms of Alzheimer's disease Aisen et al.(1997).

The downregulation of IFNG may lead to changes in specific immune functions in patients, affecting the inflammatory response of brain tissue and the ability to clear abnormal proteins, thereby exacerbating neu- ronaldamage and neurodegeneration.

The Alzheimer's disease key genes selected through tran - scriptomics play critical roles in the regulation of the autophagy pathway, neuronal metabolism, and immune function. Addi- tionally, the complementary information provided by single- cell transcriptomics allows for further exploration of therela- tionship between Alzheimer 's disease and the autophagy path- way. This may help reveal the pathogenic mechanisms of the disease and provide a theoretical basis for the development of new therapeutic strategies.

Afterwards, we conducted Mendelian randomization analy - sis and found a reliable positive correlation between mood fluc- tuations and Alzheimer's disease. We will discuss this from multiple perspectives.

Ossenkoppele et al. (2015) Neurobiological perspective: Recent research suggests that mood fluctuations, especially persistent negative emotions, are closely related to changes in brain structure and function Yang et al. (2010). Mood fluctuations may lead to changes in hormone levels, such as increased cortisol and adrenaline levels, which can have negative effects on neurons, synaptic transmission, and neural networks Hammar et al. (1995).

This impact may be related to protein aggrega- tion and neuronal damage associated with Alzheimer's disease. Poncet et al.(2011) Inflammation and immune perspective: Mood fluctua- tions, particularly those caused by stress, can lead to inflam- matory responses and activation of the immune system Pereira et al.(2021). Chronic inflammation and immune system dysregulation have been found to be associated with the onset and progression of Alzheimer 's disease Li et al.(2023) .

This inflammatory response may af- fect the health of brain neurons and play a crucial role in the pathophysiology of Alzheimer's disease.

Mahmoudi et al. (2014) Genetic and environmental interaction perspective: Some studies suggest that an individual's genotype, particularly genes related to stress coping, may interact with mood fluctuations and the risk of Alzheimer 's disease Corbo et al.(2007) .

Environmental stress and mood fluctuations may exacerbate genetic susceptibility, thereby increasing the risk of Alzheimer 's disease Stuart et al.(2017).

Dohler et al. (2014) Clinical and epidemiological perspective: Large- scale epidemiological studies have shown that individuals exposed to long-term negative emotions may be more susceptible to Alzheimer 's disease Chen et al. (2017). These findings provide important ref- erences for further clinical practice and intervention measures. For example, emotion management and stress relief may be- come key strategies for preventing Alzheimer's disease.

Based on the reviewed literature, it can be concluded that the relationship between mood fluctuations and Alzheimer's dis- ease involves multiple aspects, such as neurobiology, inflamma- tion and immune response, genetic and environmental interac- tion, etc. Deeply examining this relationship from different an- gles can help us better understand the underlying mechanisms and provide a theoretical basis for the prevention and treatment of Alzheimer's disease, as well as provide valuable insights for related research and clinical practice.

Autophagy is a process that breaks down and clears damaged or aging organelles and proteins to maintain cellular homeosta- sis. In recent years, the importance of autophagy in various diseases has attracted widespread attention and plays a critical role in neurodegenerative diseases and cancer Ulamek-Koziol et al.(2013) .

In neurodegenerative diseases, abnormal regulation of the au- tophagy pathway is closely associated with neuronal degenera- tion and death. Many studies have shown that imbalanced regu- lation of autophagy can lead to the occurrence and development of neurodegenerative diseases, including Alzheimer's disease, Parkinson 's disease, and Huntington 's disease Schmukler et al.(2020) . Therefore, targeting the regulation of the autophagy pathway may become a potential strategy for the treatment of these diseases.

Further validation from different experimental perspectives is required to verify the above conclusions. Due to various lim- itations imposed by external factors, we are not able to conduct more in- depth experiments at this time. In summary, the au- tophagy pathway holds great promise in the treatment of neu- rodegenerative diseases and cancer, and personalized and effec- tive targeted drugs are expected to be developed. Furthermore, modulating autophagy could help overcome drug resistance and improve treatment outcomes. Therefore, in-depth research on the mechanism of autophagy can provide a theoretical basis for the prevention and treatment of related diseases and important guidance for the development and clinical application of new- generation therapeutic drugs.

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DECLARATIONS

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Funding Declaration

This study was conducted without funding and without any conflict of interest.

Data Availability Declaration

The data used in this research is sourced from the publicly available GEO database repository.

Competing Interest declaration

The authors declare no competing interests in this research.

Supporting Information

All relevant materials have been shown in the article.

Ethics approval

This research has been conducted following the ethical guidelines and has received approval from the relevant ethics committee.

Consent to participate

All participants involved in this study have provided their informed consent to participate.

Consent for publication

Written consent for the publication of any identifiable information or images has been obtained from all individuals included in this study.

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