Bioinformatics-Based Acquisition of Alzheimer's Disease Hub Genes

Meng-Ting Hou, Xi-Yu Li, and Juan Bao[✉]

School of Public Health, Hubei University of Medicine, Shiyan 442000, China
bao_0203@126.com

Abstract. OBJECTIVE: To analyze the functions and pathways of differentially expressed genes (DEGs) between Alzheimer's disease (AD) patients and normal controls using bioinformatics methods, and to screen Hub genes to provide theoretical support for the study of AD pathogenesis and therapeutic targets. METHODS AD-related data microarrays (GSE197505) were obtained from the GEO database, and the data were processed using GEO2R to obtain DEGs. The screened DEGs were enriched for Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) signaling pathways, using the Matascape platform. Cytoscape software was utilized to map the PPI network and screen the Hub genes. RESULTS A total of 142 DEGs were screened as down-regulated genes in this study. GO/KEGG analysis showed that these DEGs were involved in biological processes such as positive regulation of cell cycle protein-dependent serine/threonine kinase activity, and PI3K-Akt signaling pathway. Eight Hub genes were finally screened by the PPI network, four of which were validated by the literature. CONCLUSION The results of the bioinformatics network analysis revealed the Hub genes of AD, contributing to a better understanding of the mechanisms of AD and facilitating the discovery of new therapeutic targets.

Keywords: Alzheimer's Disease · Bioinformatics · Differentially Expressed Genes · Hub Genes

1 Introduction

The 2015 Global Alzheimer's Disease Report, "Dementia's Impact on the World," has reported that with the trend of population aging, China has the largest number of Alzheimer's disease patients in the world, up to 9.5 million [1]. AD is a neurodegenerative disease characterized by chronic cognitive impairment with atrophy of the cerebral cortex, deposition of β-amyloid, neuronal fibrillary tangles, and the formation of senile plaques. AD is the most common type of dementia, accounting for 50–70% of all types of dementia [2]. The World Alzheimer's Disease Report 2019, published by Alzheimer's Disease International, states that there were an estimated 50 million people globally living with dementia in 2019, and the number is projected to reach 152 million in 2050. Additionally, the cost associated with treating dementia is currently one trillion dollars per year and is projected to double by 2030 [3]. The insidious onset of AD, the slow and irreversible course of the disease, and the increasing burden of the

disease pose serious challenges to the prevention and treatment of AD. Regarding the diagnosis of AD, the more established markers are amyloid and Tau proteins, and the presence of markers in the cerebrospinal fluid is indicative of a patient with AD. Genetic data can also provide an important basis for diagnostic aid for researchers. It has been found that the apolipoprotein E4 gene and the clumping protein gene can control the process of amyloid clearance, and the lack of these genes will result in AD disease [4]. In this study, bioinformatics methods were utilized to identify and screen Hub genes that may be involved in the occurrence and development of AD, to explore the mechanism of action of these genes, and to provide new targets and a basis for the diagnosis and treatment of AD.

2   Tools and Methods

2.1   Tools

GEO Database and GEO2R. The GEO Database is a public repository for storing and sharing gene expression data and is the most comprehensive public gene expression database available today [5]. GEO2R is an online tool available in the GEO database that analyzes differentially expressed genes using the R language.

STRING and Cytoscape. The STRING database integrates protein information data from multiple sources to create a comprehensive protein interaction network. Cytoscape is an open source software platform for visualizing molecular interaction networks and biological pathways.

GO and KEGG. GO is a standardized classification system widely used in gene function annotation and bioinformatics research. KEGG is a comprehensive bioinformatics database and resource that provides information on gene signaling pathways and more.

Metascape. Metascape is an online bioinformatics analysis platform that integrates more than 40 bioinformatics knowledge bases, including GO, KEGG, UniProt and DrugBank, enabling pathway enrichment as well as bioprocess annotation.

2.2   Methods

Dataset Download Source and Extraction. Enter the search keyword Alzheimer's disease in the GEO database with the search formula: ("alzheimer disease"[MeSH Terms] OR ("alzheimer disease"[MeSH Terms] OR Alzheimer's disease[All Fields])) AND "Homo sapiens"[organism] AND "NORMAL", select SERIES. Download gene chip GSE197505 from the GEO database.

Differential Expression Genes Screening. The t-test was performed using GEO2R on the genes of AD patients' brain tissues and normal brain tissues from GSE197505 gene chips, and the DGEs were screened out by statistical methods. Define $\log_2 FC > 1$ as up-regulated differentially expressed gene "UP" and $\log_2 FC < -1$ as down-regulated differentially expressed gene "DOWN".

GO and KEGG Signaling Pathway Enrichment Analysis. Enter the Gene ID of the DEGs in Metascape, select Homo sapiens for the sample type, select Custom Analysis for the analysis method, and then perform GO and KEGG enrichment analysis.

Screening for Hub Genes. In the STRING database, select multi-protein analysis, enter the gene ID of the DEGs, and select Homo sapiens for the sample type to obtain the key gene PPI network of the differentially expressed gene. Download the relevant data into Cytoscape software, visualize the data, and then use the Mcode plug-in to screen and map the PPI network of Hub genes.

3   Results

3.1   GeneChip Characterization

The AD GeneChip GSE197505 selected for this study contains frontal cortical isolated extracellular vesicles from 8 AD patient donors and 10 normal control frontal cortical isolated extracellular vesicles (EVs) [6]. EVs are carriers of nucleic acids, lipids and proteins and play an important role in the pathogenesis of neurodegenerative diseases.

3.2   Differentially Expressed Genes Screening

Differentially expressed genes were screened by GEO2R on GeneChip GSE197505, with screening thresholds set: $|\log_2 FC| > 1$ and P.adj < 0.05, and after probe de-duplication, a total of 142 DEGs were read, all of which were down-regulated genes. A volcano map of differentially expressed genes was drawn (see Fig. 1). The multidimensional data of this data set was down-analyzed (see Fig. 2), and using the Uniform Manifold Approximation and Projection (UMAP, consistent popular approximation and projection) method, it can be seen that the data set was separated into high expression group and ground expression group.
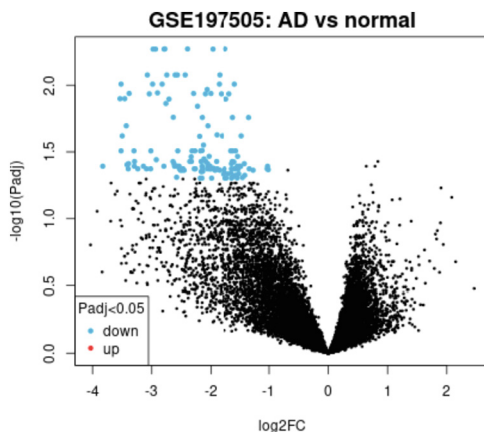

Fig. 1. Volcano plot of differentially expressed genes. This figure shows that the differentially expressed genes are all down-regulated genes (blue dots are down-regulated genes and black dots are genes with insignificant differential expression). (Color figure online)
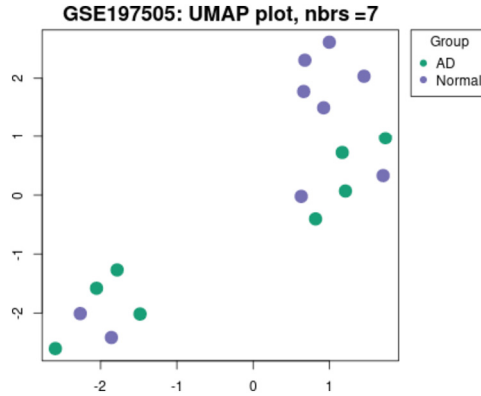
Fig. 2. UMAP diagram of GSE197505.

3.3   Functional Analysis of Differentially Expressed Genes

Metascaped was used to annotate the differentially expressed genes with gene ontology markers and related metabolic pathways and biological processes. GO enrichment analysis showed that the molecular functions of the differentially expressed genes in the GeneChip GES197505 mainly included cytokine binding, calcium binding, antigen binding, etc., with cellular sublocalization mainly in the cellular matrix, and involved in biological processes mainly in positive regulation of The differentially expressed genes were mainly involved in the positive regulation of cell cycle protein-dependent serine/threonine kinase activity, vascular morphology, response to organophosphorus, and peptide lysine modification, etc. (see Fig. 3). KEGG enrichment analysis showed that the major enrichment pathways of the differentially expressed genes included oocyte meiosis, PI3K-Akt signaling pathway, and the synthesis of thyroid hormones (see Fig. 4). Among them, the PI3K-Akt pathway plays an important role in the survival, proliferation and functional maintenance of neuronal cells. Abnormal activation or impairment of this pathway is associated with neurological disorders such as Alzheimer's disease, Parkinson's disease, and neurodegenerative diseases [7].
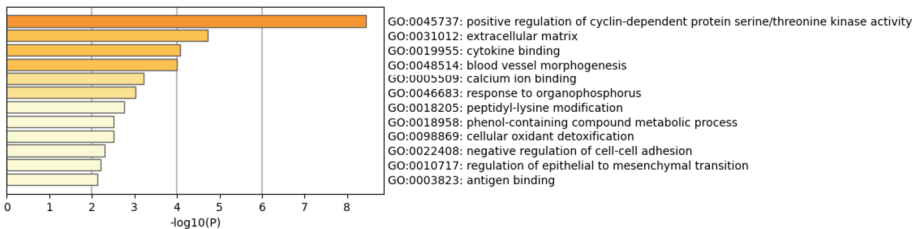

Fig. 3. Bar graph of GO enrichment analysis of differentially expressed genes.
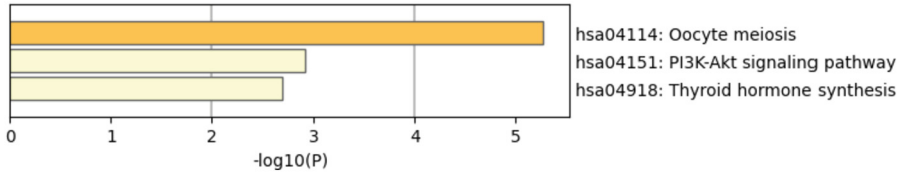
Fig. 4. Bar graph of KEGG pathway enrichment analysis of differentially expressed genes.

3.4 Relationship of Hub Genes in Protein-Protein Interaction Networks

The data related to protein-protein interaction information were obtained from the STRING database and imported into Cytoscape to analyze and visualize to construct a PPI network (see Fig. 5). This PPI network has 89 nodes and 176 edges. By KEGG analysis, these key genes were mainly closely related to progesterone-mediated oocyte maturation and oocyte meiosis. Eight Hub genes that play a pivotal role in this PPI network were screened using the MCODE plug-in (see Fig. 6), namely, LRRC32, ITPR3, SLC26A6, NOS3, ADAM33, PIEZO1, GSDMB, IL4R.
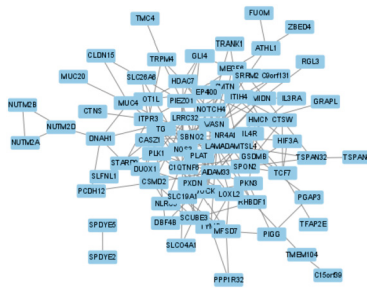
Fig. 5. PPI network of differentially expressed genes.

Fig. 6. Hub genes in the PPI network.

4   Discussions

4.1 Significance of Hub Genes for Alzheimer's Disease

With its insidious onset, slow and irreversible course, and increasing disease burden, AD has become one of the greatest global public health and social challenges facing humanity today and in the future [8]. In this study, combining with GEO, STRING and other related

databases, we screened 8 Hub genes related to AD (LRRC32, ITPR3, SLC26A6, NOS3, ADAM33, PIEZO1, GSDMB, IL4R) using bioinformatics technology by annotating the gene functions, molecular metabolic pathways, and the establishment of PPI networks. Finally, the relationship between Hub genes and AD is analyzed in the light of domestic and international studies and literature, which will provide new ideas for the study of the pathogenesis of AD and help in the early diagnosis and treatment of AD.

4.2   Mechanisms of Hub Genes Action in AD

NOS3.   NOS3 belongs to the nitric oxide synthase family, and in 1999, Dahiyat [9] conducted a study investigating the association between the NOS3 gene and AD, suggesting that changes in the nitric oxide synthase system may influence AD-related pathogenesis, and that NOS3 gene expression induces neuronal and glial degeneration in the brain. Superoxide free radicals react with NO to produce peroxynitrite, which promotes lipid peroxidation, which in turn further accelerates degenerative changes and causes AD [10]. The arginine metabolic pathway produces the gaseous signaling molecule NO mainly through NOS, and the NO produced by eNOS is essential for maintaining normal cerebral blood flow [11], and previous studies have verified that the formation of plaques and tangles in the AD brain is closely related to the reduction of capillary eNOS expression [12, 13]. NO from nNOS has been found to play an important role in synaptic plasticity and learning memory, while NO from iNOS is pro-inflammatory [14]. It is directly associated with 1- glutamate and NMDA receptors in the CNS, and therefore with long time-range potentiation (LTP), which is considered a major cellular mechanism of learning and memory, and synaptic transmission is enhanced by repeated stimulation of presynaptic terminals. Therefore, large amounts of Ca2 + are expressed through NMDA receptors located on the postsynaptic membrane, and calcium/calmodulin-mediated regulation of nNOS/NO shows a potential induction of LTP [15]. The results of GO enrichment analysis in this study also showed that the molecular functions of the differentially expressed genes in GeneChip GES197505 included calcium ion binding. Further studies on the mechanism of action of NOS3 are still needed.

Piezo1.   Alzheimer's disease is associated with beta-amyloid deposition (Aβ). The mechanosensitive ion channel Piezo1 is increased in microglia in response to stiff stimulation of Aβ fibers. Upregulation of Piezo1 in Aβ plaque-associated microglia was observed in AD mouse models and human patients. Piezo1-deficient microglia interfered with microglia aggregation, phagocytosis, and Aβ plaque compaction, leading to exacerbation of Aβ and neurodegenerative lesions in AD. In contrast, activation of Piezo1 ameliorated cerebral Aβ load and cognitive deficits in 5xFAD mice [16]. Another study demonstrated that the mechanosensitive cation channel Piezo1 plays a key role in translating ultrasound-related mechanical stimuli through its trimeric propeller-like structure, but the importance of Piezo1-mediated mechanotransduction in brain function has not been sufficiently emphasized. In addition to mechanical stimulation, Piezo1 channels are strongly modulated by voltage. Fangxuan C et al. [17] hypothesized that Piezo1 may play a role in the conversion of mechanical and electrical signals to induce phagocytosis and degradation of Aβ and β-phosphodiester. To this end, a transcranial magnetoacoustic stimulation (TMAS) system was designed and applied to 5xFAD mice

to assess whether TMAS could alleviate the symptoms of AD mouse models by activating Piezo1. Finally, it was confirmed that Piezo1 can convert TMAS-related mechanical and electrical stimuli into biochemical signals, and it was determined that the favorable effects of TMAS on synaptic plasticity in 5xFAD mice are mediated by Piezo1. This could lead to the use of Piezo1 as a candidate target for AD therapy and provide new ideas for AD treatment.

GSDMB. GSDMB is a member of the GSDM protein family. Zhou et al. [18] found that granzyme A cleaved GSDMB, releasing the GSDMB-N fragment, which caused perforation of the cell membrane and led to cellular juxtaposition in GSDMB-expressing cancer cells. To explore the role of GSDMB in nonclassical juxtaposition, Chen et al. [19] specifically knocked down GSDMB in THP-1 cells and found that the induced nonclassical juxtaposition was inhibited. In contrast, the high expression of GSDMB induced using lentiviral system showed promotion of nonclassical juxtaposition. It was verified that GSDMB was involved in nonclassical juxtaposition. In addition, the team found that GSDMB promoted the enzymatic activity of caspase-4 by combining with caspase-4, which led to the cleavage of GSDMD by caspase-4, thus releasing GSDMD-N-terminal protein leading to cellular juxtaposition, which led to the release of inflammatory factors in large quantities, resulting in inflammatory diseases. Microglia focal death caused by neuroinflammatory response is closely related to the pathogenesis of many neurological pathologies (e.g., stroke, depression, neurodegenerative diseases, etc.), and it is the main cell type in which focal death of cells occurs [20]. Liang et al. [21] found that microglia were able to secrete ASCs under the action of Aβ fibers, a major neurotoxic component of Alzheimer's disease patients, and to produce NLRP3-dependent IL-1β inflammatory factor, which induces cellular pyroptosis and further amplifies the inflammatory response; thus confirming that the deletion of the ASC gene and the blockade of antibodies play an inhibitory role in the recruitment of Aβ fibers, suggesting that inhibition of cellular pyroptosis-released ASC may slow down the progression of Alzheimer's disease. The team also found that the brains of AD model mice highly expressed the cellular focal death-related molecules NLRP3 inflammatory vesicles and Caspase-1, and that deletion of the NLRP3 or Caspase-1 genes greatly improved spatial memory and enhanced Aβ clearance in the mice. These studies reveal novel mechanisms of GSDMB-mediated nonclassical cellular focal death associated with AD, providing potential therapeutic strategies and targets.

ADAM33. The ADAM33 gene is localized on the short arm of chromosome 20, 20p13, and belongs to the ADAM gene superfamily, which consists of eight structural domains. The gene is widely expressed in the human body, but is highly expressed in tissues such as the brain and lungs. The proteins encoded by the ADAM gene superfamily have metalloprotease activity, and this activity has been associated with the development of a variety of diseases [22]. The protease activity of ADAM has a protective function for the organism. For example, ADAM9, -10, and -17 have α-secretase activity, and in patients with Alzheimer's disease, α-secretase cleaves amyloid precursor protein (APP) from the middle of amyloid β peptide (Aβ), attenuates the deposition of β-peptide amyloid plaques and promotes the release of neuroprotective agent, sAPPα. Therefore, the α-secretase activity of ADAM molecules provides a a new tool for the treatment of Alzheimer's disease [23]. The ADAM33 gene can be a candidate gene for asthma, and its inhibitors

are expected to be used in the treatment of asthma [24]. And further studies on the relationship between ADAM33 and AD are needed.

In summary, PIZO1 and GSDMB may provide targets for the treatment of AD, NOS3 and ADAM33 can be further investigated as candidate genes, whereas IL4R,Lrrc32,ITPR3, and SLC26A6 have been less studied in AD, and their mechanisms of action need to be further investigated.

5   Conclusion

This study utilized a series of bioinformatics methods to screen multiple Hub genes, some of which have been shown to serve as therapeutic targets for AD. The newly screened Hub genes will provide ideas and rationale for understanding the pathogenesis of AD and exploring new therapeutic options.

References

1. Prince, M.: The Global Impact of Dementia an analysis of prevalence, incidence, cost and trends. World Alzheimer Report 2015 (2015)
2. China dementia and cognitive disorders guideline writing group.: cognitive disorder disease specialized committee of neurologists branch of Chinese physicians association. 2018 China dementia and cognitive disorders diagnostic and treatment guidelines (I): diagnostic criteria for dementia and its classification. Chin. Med. J. **98**(13), 965–970 (2018)
3. Alzheimer's Disease International.: World Alzheimer Report 2019: Attitudes to dementia. Alzheimer's Disease International, London (2019)
4. Ruoqi, Z.: Progress in molecular imaging of pathological markers of Alzheimer's disease. Shanxi Med. J. **50**(3), 381–384 (2021)
5. Barrett, T.: NCBI GEO: archive for high-throughput functional genomic data. Nucleic Acids Res. **37**(Database issue), D885-D890 (2019)
6. Dan, L.: Long RNA profiles of human brain extracellular vesicles provide new insights into the pathogenesis of Alzheimer's disease. Aging Dis. **14**(1), 168–178 (2023). PMID: 36818567
7. Manish, K.: Implications of phosphoinositide 3-kinase-akt (PI3K-Akt) pathway in the pathogenesis of Alzheimer's disease. Mol. Neurobiol. **59**(1), 52–64 (2021)
8. Blennow, K.: Evolution of Abeta42 and Abeta40 levels and Abeta42/Abeta40 ratio in plasma during progression of Alzheimer's disease: a multicenter assessment. J. Nutr. Health Aging **13**(3), 232–236 (2009)
9. Dahiyat, M.: Association between Alzheimer's disease and the NOS3 gene. Ann. Neurol. **46**(4), 664–667 (1999)
10. Carmen, G.: Increased susceptibility to plasma lipid peroxidation in Alzheimer disease patients. Curr. Alzheimer Res. **1**(2), 103–109 (2004)
11. Bergin, D.: Altered plasma arginine metabolome precedes behavioural and brain argi nine metabolomic profile changes in the APPswe/PS1ΔE9 mouse model of Alzheimer's disease. Transl. Psychiatry **8**(1), 108 (2018)

12. Jeynes, B.: Significant negative correlations between capillary expressed eNOS and Alzheimer lesion burden. Neurosci. Lett. **463**(3), 244–248 (2009)
13. John, P.: Neurofibrillary tangles and senile plaques in Alzheimer's brains are associated with reduced capillary expression of vascular endothelial growth factor and endothelial nitric oxide synthase. Curr. Neurovasc. Res. **5**(3), 199–205 (2008)
14. Robert, F.: NO/cGMP-dependent modulation of synaptic transmission. Handb. Exp. Pharmacol. **184**, 529–560 (2008)
15. Harikesh, D.: Alzheimer's disease: a contextual link with nitric oxide synthase. Curr. Mol. Med. **20**(7), 505–515 (2020)
16. Jin, H.: Microglial piezo1 senses Aβ fibril stiffness to restrict Alzheimer's disease. Neuron **99**(1), 122–135 (2022)
17. Fangxuan, C.: Transcranial magneto-acoustic stimulation attenuates synaptic plasticity impairment through the activation of piezo1 in Alzheimer's disease mouse model. Research **6**(3), 58–67 (2023)
18. Zhiwei, Z.: Granzyme A from cytotoxic lymphocytes cleaves GSDMB to trigger pyroptosis in target cells. Science **368**(6494), 1260–1263 (2020)
19. Chen, Q.: Molecular mechanism of GSDMB enhancing the enzymatic activity of cysteoaspartase caspase-4 and thus promoting nonclassical cellular pyrokinesis. Nanjing University (2018)
20. Chunkai, J.: Progress of cellular focal death in stroke and depression. J. Stroke Neurol. Dis. **40**(05), 463–467 (2023)
21. Fei, L.: High-intensity interval training and moderate-intensity continuous training alleviate β-amyloid deposition by inhibiting NLRP3 inflammasome activation in APPswe/PS1dE9 mice. NeuroReport **31**(5), 425–432 (2020)
22. Primakoff, P.: The ADAM gene family: surface proteins with adhesion and protease activity. Trends Genet. **16**(2), 83–87 (2000)
23. Masashi, A.: Putative function of ADAM9, ADAM10, and ADAM17 as APP alpha-secretase. Biochem. Biophys. Res. Commun. **301**(1), 231–235 (2003)
24. Zhiguang, S.: Progress in the study of ADAM33 gene. Genetics **04**, 636–640 (2005)