Key Gene Network Related to Primary Ciliary Dyskinesia in Hippocampus of Patients with Alzheimer'S Disease Revealed by Weighted Gene Co-Expression Network Analysis

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Research Article

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Abstract

Background. Alzheimer's disease (AD) is closely related to aging, showing an increasing incidence rate for years. As one of the main organs involved in AD, hippocampus has been extensively studied due to its association with many human diseases. However, little knowledge is known on its association with primary ciliary dyskinesia (PCD).

Material and Methods. The microarray data of hippocampus on AD were retrieved from the Gene Expression Omnibus (GEO) database to construct the co-expression network by weighted gene co-expression network analysis (WGCNA). The gene network modules associated with AD screened with the common genes were further annotated based on Gene Ontology (GO) database and enriched based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. The protein-protein interaction (PPI) network was constructed based on STRING database to identify the hub genes in the network.

Results. Genes involved in PCD were identified in the hippocampus of AD patients. Functional analysis revealed that these genes were mainly enriched in ciliary tissue, ciliary assembly, axoneme assembly, ciliary movement, microtubule based process, microtubule based movement, organelle assembly, axoneme dynamin complex, cell projection tissue, and microtubule cytoskeleton tissue. A total of 20 central genes, e.g.,

DYNLRB2, ZMYND10, DRC1, DNAH5, WDR16, TTC25, and *ARMC4* were identified as hub genes related to PCD in hippocampus of AD patients.

Conclusion. Our study demonstrated that AD and PCD have shared metabolic pathways. These common pathways provide novel evidence for further investigation of the pathophysiological mechanism and the hub genes suggest new therapeutic targets for the diagnosis and treatment of AD and PCD.

Subjects Bioinformatics, Cell Biology, Molecular Biology, Neurology

Introduction

As the most common progressive neurodegenerative disorder associated with aging [1], Alzheimer's disease (AD) is the main cause of dementia in the elderly people [2]. Studies have found that AD is closely related to a variety of factors, such as oxidative stress [3], autophagy dysfunction [4], and diabetes [5]. Although the studies on AD have advanced greatly, the number of AD patients worldwide is still increasing dramatically. It is predicted that by 2050, there may be four times more patients with AD [6], causing significant inconvenience to patients and financial burden to the society [7]. Although some drugs have been developed to target intermediate products, e.g., aggregated Amyloid β-protein (A-β), involved in the known developmental mechanisms of AD, almost all drugs fail to significantly improve patients' symptoms [8]. Hippocampus is among the most important brain structures involved in memory, and is a critical site of pathogenesis in dementing illnesses such as AD [9]. The hippocampal formation was essential for the proper functioning of spatial and episodic memory in the early stages of AD [10].

Transcriptome analysis has revealed that the cause for the energy crisis in hippocampus neurons was related to the differentially expressed genes (DEGs) [11]. There are also studies on the correlation between hippocampal volume and AD, however, no explicit relationship was revealed [12]. In animal experiments, shRNA silenced Egr-1 in the hippocampus and improved the cognition of 3xTG AD mouse model [13]. Adult hippocampal neurogenesis (AHN) impairment contributes significantly to the cognitive decline in patients of AD [14], More importantly, hippocampus is also the main location for A-β deposition [15]. Therefore, it is crucial to understand the pathological status of hippocampus in AD patients in order to study the mechanisms of AD-related memory impairments [16].

Cilia are dynamic microtubule-based organelles present on the surface of many types of eukaryotic cells. Cilia defects underlie a growing list of human disorders, collectively called ciliopathies, with overlapping phenotypes such as developmental delays and cognitive and memory deficits [17]. It has been reported that Serotonin 5-HT6 receptors affect cognition in a mouse model of AD by regulating cilia function [18]. Microtubules are an important part of cilia, playing an important role in cell division (i.e., beating of cilia and flagella) and intracellular transport [19].

Furthermore, aberrant interaction between the microtubule-associated protein Tau and the filamentous actin is connected to synaptic impairment in AD [20]. PCD is the most representative disease of ciliary dysfunction. It is a rare hereditary disease characterized by abnormal movement of cilia in human body [21]. When the cilia function normally, they beat together, helping to push the mucus through the respiratory system to the throat area, ultimately expelled by coughing. This process is very important for the human body to resist infection [22]. Because cilia play important roles in both AD and PCD, we hypothesize that AD and PCD share similar pathogenesis. It has been reported that there are more than 20 kinds of ultrastructural abnormalities in cilia, most of which are dynein arm defects and microtubule defects. Next generation sequencing has enhanced the gene identification, and mutations in more than 40 genes have been reported to cause PCD, with many other genes likely to be discovered [23]. Although studies have demonstrated that genes related to PCD such as dynein axonemal intermediate chain 1 (*DNAI1*) play an important role in AD [24], no studies in-depth have been conducted. To date, studies on the association between AD and PCD are sparse. Therefore, it is necessary to study the relationship between AD and PCD, especially at the molecular and genetic levels.

To date, the analysis of weighted gene co-expression network (WGCNA) is a well-established advanced method applied to investigate the molecular mechanism of genes and reconstruct gene co-expression network through transforming the adjacency matrix into a topological overlap matrix [25]. In this study, we have identified the gene set related to PCD in the hippocampus of AD patients based on WGCNA analysis and further investigated the association between PCD and AD.

Materials & Methods

Data Preparation

Gene expression profiles of AD were downloaded from Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/) [26]. The dataset of GSE48350 [27] based on GPL570 [HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array contained microarray data from normal controls (aged 20-99 years) and AD cases from 4 brain regions, including hippocampus, entorhinal cortex, superior frontal cortex, and post-central gyrus. Expression levels of synaptic and immune related genes were assessed to investigate the age-related changes, AD-related changes, and region-specific patterns of changes. A total of 19 samples of AD and 43 samples of normal controls of hippocampus tissues were included in this study.

Differential Gene Analysis

All differential gene analyses were performed by R foundation for statistical computing (2020) version 4.0.3. The dataset used for differential gene analysis was retrieved from GEO database with the format of MINIML. Limma package (version: 3.40.2) of R software was used to investigate the differential expression of mRNAs. The effect of remove the batch was performed by using the 'remove Batch Effect' command in Limma package. The adjusted *P*-value was analyzed to correct the false positive results in GEO datasets. The parameters "Adjusted $P < 0.05$ and Log (Fold Change) >1 or Log (Fold Change) < −1" were defined as the thresholds for the screening of differential expression of mRNAs. The box plot was generated by the R software package ggplot2. The heatmap was displayed by the R software package pheatmap.

GO Annotation and KEGG Pathway Enrichment Analysis

To further confirm the underlying functions of potential target genes identified by differential gene analysis, these genes were analyzed by functional enrichment based on Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) databases [28]. GO is a widely used tool for annotating genes with functions in three categories, including molecular function (MF), biological pathways (BP), and cellular components (CC). KEGG is a practical resource for analytical study of gene functions and associated high-level genome functional information. To better understand the carcinogenesis of mRNAs, Cluster Profiler package (version: 3.18.0) in R was employed to further analyze the GO functions of potential target genes and the enrichment of the KEGG pathway. In the enrichment results, $P < 0.05$ or false discovery rate (FDR) < 0.05 was considered to be enriched to a meaningful pathway.

Gene Set Enrichment Analysis

Gene Set Enrichment Analysis (GSEA, https://www.gseamsigdb.org/gsea/index.jsp) was applied to identify the significant pathways in dataset GSE48350. This method determines whether a priori defined set of genes show statistically significant differences or not between two biological states (e.g. phenotypes) [29]. The coefficients of Spearman correlation between genes and sample labels were defined as the weight of genes [30]. Statistical significance was assessed by comparing the enrichment score with the enrichment results generated from 1000 random permutations of the gene sets to obtain nominal P values. The significant level of pathways was determined by the levels of normalized enrichment score (NES) ≥ 1.0, false discovery rate (FDR) ≤ 0.25, and $P \leq 0.05$.

Weighted Gene Co-expression Network Analysis

Weighted Gene Co-expression Network and co-expression modules were constructed by Weighted Gene Co-expression Network Analysis (WGCNA), which was performed using the WGCNA package in R [31]. The hippocampus regions of the microarray data of GSE48350 were applied as a primary source of data for the WGCNA analysis. The network construction started by calculating robust correlations between all genes across in all relevant samples. The correlation adjacency matrix was increased to the power $\beta = 18$ based on scale-free topology criterion. The power parameter was selected to amplify the strong connections between genes and to penalize the weak connections. The first principal component was considered as the module eigen gene (ME), representing the highest percent of variance for all the genes in a module. Module membership (kME) measured the correlations between each gene and each ME. The within-module connectivity (kin) for each gene was determined by summing the connectivity of that gene with each of the other gene set in the same module [32, 33], showing significant correlations with MEs and high within-module connectivity, and were considered as hub genes of the modules. The hub genes were verified by using Cytoscape's cytoHubba plugin [34]. In order to analyze the correlation between module and phenotype, we transformed the classified variables into numerical variables. Specifically, female was defined as 0 and male as 1; non-AD patients under 80 years old were defined as 0 and non-AD patients above 80 years old as 0.5; and AD positive as 1. In this study, a total of 3 phenotypes were analyzed using Spearman method to calculate the correlation.

Protein-Protein Interaction (PPI) Analysis

All common genes from the selected modules were further analyzed by the online Search Tool for the Retrieval of Interacting Genes (STRING) database (Version 11.0; http://string-db.org/) to establish the network through protein-protein interaction (PPI) analysis [35]. A combined score of more than 0.4 was applied to build the PPI network, which was visualized by the Cytoscape software (version 3.8.2, http://cytoscape.org/) [36]. The common genes in networks were screened by the degree of the gene nodes. The genes with the most interactions were considered as hub genes, which may play important roles in the disease's pathogenesis.

Gene Analysis by Venn Map

Venn diagrams are used to show logical connections between different groups of things (sets). In addition to Hub genes, the other two sites involved are Genecards database (https://www.genecards.org) and Disgenet database (https://www.disgenet.org/).

Gene Interaction Heatmap

Gene correlation can be used to observe the relationship between different genes and to study the relationship between an unknown gene and a known gene in order to predict the function of the unknown gene. The negative and the positive correlations between two genes suggest that these genes may cooperate or antagonize with each other, respectively.

Statistical Analysis

GraphPad Prism (version 8.0.0) was utilized to perform the statistical analysis. The normality test and homogeneity of variance test were performed on data extracted from GEO datasets. Data that passed these two tests underwent t-testing for comparisons between two groups. Spearman test was used to investigate the correlation between module and phenotype in WGCNA analysis. The Gene Interaction Heatmap was used to assess the correlation of gene set between AD and PCD. Venn graph was applied to find the similarity between data sets. *P* values less than 0.05 were considered statistically significant.

Results

Screening of Differentially Expressed Genes (DEGs)

The gene expression data (GSE48350) on 19 patients with AD were compared with those of 43 control samples (CTs) from GEO database (Tables S1). All samples were hippocampus tissues. After the run of 'remove Batch Effect' command in Limma package, the expression level of genes was basically at the same level, which was selected for downstream difference analysis (Fig. 1A, Table S2). Based on the cutoff criteria, a total of 88 DEGs (24 up-regulated and 64 down-regulated) were identified in AD samples (Fig. 1B). Heatmap was used to show gene expression levels (Fig. 1C). According to the absolute value of Log, top ten genes included *LTF, SLC17A6, CFAP126, TMEM155, CALB1, SLC47A2, ANKIB1, NWD2, CP*, and *MRAP2.*

GO Annotation and KEGG Pathway Enrichment Analysis

To further confirm the underlying functions of potential target genes, DEGs were analyzed by functional enrichment (Fig. 2, Table S3). The up-regulated genes were significantly enriched in KEGG pathways of complement and coagulation cascades and pertussis, and in the GO functions of negative regulation of endopeptidase activity, negative regulation of peptidase activity, response to molecule of bacterial origin, and negative regulation of proteolysis. The down-regulated genes were significantly enriched in the KEGG pathways of nicotine addiction and neuroactive ligand-receptor interaction, and in the GO functions of vesicle-mediated transport in synapse, synaptic vesicle cycle, neurotransmitter transport, and synapse organization.

Gene Set Enrichment (GSEA) Analysis

We further analyzed the microarray data with the software 'Gene Set Enrichment Analysis'[29, 37]. Three up-regulated gene sets in AD including *CHR2Q13*, *CHR19P12*, and *KRAS.PROSTATE_UP. V1_DN* and one down-regulated gene set *CHR4P14* were justified. No pathway associated with PCD was identified (Fig. 3, Figs. S1-S4). Three positional gene sets (i.e., *CHR2Q13*, *CHR19P12*, and *CHR4P14*) were enriched in different locations of different chromosomes. As the oncogenic signature gene set, the *KRAS.PROSTATE_UP. V1_DN* could be down-regulated in epithelial prostate cancer cell lines over-expressing an oncogenic form of KRAS gene.

WGCNA and Key Module Identification

All genes in the array were used to conduct WGCNA (Figs. 4 and 5). By setting the soft-threshold power as 18 (scalefree R^2 = 0.9, blockSize = 7000, minModuleSize = 20, deepSplit = 2, mergeCutHeight = 0.25, hub_cut = 0.9, net_threshold = 0, slope = –0.96; Fig. 4A), we acquired a total of 12 modules (Fig. 5A; Table S4). The Tom diagram of the relationship between gene clustering and modules in each module of WGCNA was shown in Fig. 4B. The relationship between modules and genes in the modules was shown in Fig. 4C. The correlation coefficient between grey module and gene expression in the module was the lowest. The number of genes in each module was provided in Table1. Based on the heatmap of module-trait correlations, the key module containing a total of 160 genes was identified as the most positively correlated with AD (correlation coefficient = 0.40, P = 0.01; Fig. 5B).

Table 1
Module and the number of genes in each module

The color of module	Number of genes in the module
Grey60	27
Greenyellow	71
Purple	74
Magenta	154
Lightcyan	160
Pink	174
Red	266
Green	302
Yellow	343
Brown	551
Turquoise	7,090
Grey	11,337

Selection of Hub Genes

All the common genes from the selected modules were further analyzed by the online STRING database to construct the network. After the PPI analysis, cytoscape was used for visualization and the plug-in cytohubba was used to screen hub genes. The top 20 genes identified were defined as hub genes (Fig. 6; Table 2).

Table 2
The Hub genes related to PCD

Gene	Full Name	DPI	Score
DNAI1	dynein axonemal intermediate chain 1	0.692	0.6
DNAAF3	dynein axonemal assembly factor 3	0.731	0.59
CCDC114	coiled-coil domain containing 114	0.5	0.53
CCDC65	coiled-coil domain containing 65	0.5	0.52
DNAH5	dynein axonemal heavy chain 5	0.692	0.4
RSPH1	radial spoke head component 1	0.5	0.36
CCDC39	coiled-coil domain containing 39	0.615	0.35
ZMYND10	zinc finger MYND-type containing 10	0.731	0.35
DNAI2	dynein axonemal intermediate chain 2	0.5	0.34
DNAAF1	dynein axonemal assembly factor 1	0.654	0.33
ARMC4	armadillo repeat containing 4	0.615	0.32
DRC1	dynein regulatory complex subunit 1	0.654	0.32
FOXJ1	forkhead box J1	0.577	0.32
HYDIN	HYDIN axonemal central pair apparatus protein	0.538	0.31
TTC25	tetratricopeptide repeat domain 25	0.538	0.31
TEKT1	tektin 1	0.308	0.01
Notes.			
Data retrieved from the Disgenet database (https://www.disgenet.org/).			
PCD: Primary Ciliary Dyskinesia.			
DPI: Disease Pleiotropy index for the gene			
Score: Gene-Disease Association Score			

Gene Analysis by Venn Map

In order to verify the reliability of the predicted results, we used the Venn diagram to intersect the DEGs, the related genes from WGCNA analysis, and the genes related to AD in Genecards database. The results showed that there were 59 repeats between DEGs and AD related genes in Genecards and 57 repeats between genes in lightcyan module and AD related genes in Genecards, indicating that the diagnostic efficiency of the two methods was comparable (Fig. 7A, B). Therefore, we used the method of WGCNA to identify the gene module related to AD and PPI to identify the hub genes. Then, we used Venn diagram to

intersect the hub genes and the genes related to PCD in Disgenet database. The results showed that 16 out of the 20 hub genes were duplicated, indicating that these hub genes were closely related to PCD. In order to investigate the functions of these hub genes, we further annotated these genes based on GO and KEGG databases. Results of GO annotation showed that these genes were closely associated with the axoneme and cilium (Fig. 7C, Table S5). The results of the enrichment analysis based on KEGG database showed that these hub genes were closely related to Huntington's disease, which shares many medical similarities with AD.

Gene Interaction Heatmap

Because of the application of different chip platforms in investigating the expression of genes, many genes were not detected in GPL570 [HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array. Therefore, the top 30 genes were selected and defined as the genes most closely related to AD. These results suggested that hub genes showed varied degrees of correlation with genes related to AD (Fig. 8; Table S6).

Discussion

As people's average life expectancy has increased due to the further improvement of modern medicine and science, the number of patients with AD will be increased as well [38]. Genome-wide association studies have identified numerous genomic loci associated with AD, while the causal genes and variants were still being continuously identified [39]. Most of the conventional methods of differential gene analysis focus on the differential expression of a single gene, i.e., the greater the difference of a single gene expression, the more important the role the single gene plays [40]. In our study, we identified the genes with the highest changes in the expression in the hippocampus of AD patients. We further investigated these genes with functional enrichment analysis. Studies have shown that *LTF* increased the α-Secretase-Dependent amyloid precursor protein processing via the ERK1/2-CREB and HIF-1α pathways in a mouse model of AD [41]. Furthermore, loss of *SLC17A6* was correlated with cognitive decline in AD [42]. Although cilia and flagella associated protein (CFAP) was essentially important for sperm flagellum biogenesis [43], no association was revealed between *CFAP126* and AD. In the APP/PS1 mouse model of AD, showing anxiety-like behavior, the photo stimulating the pBLA-vCA1 circuit ameliorated the anxiety in a Calb1-dependent manner [44]. Similar to these genes, many of the DEGs identified in our study were related to AD with only one gene related to cilia. The KEGG functional enrichment analysis revealed that these DEGs were involved in pathways of nicotine addiction, neuroactive ligand-receptor interaction, vesicle-mediated transport in synapse, synaptic vesicle cycle, neurotransmitter transport, and synapse organization. The KEGG pathway of nicotine addiction is associated with neurological diseases [45], and other pathways were related to synapse, whereas no association with cilia was identified.

GSEA used the pre-defined gene set to rank the genes based on the degree of differential expression. This analysis has been used to identify key transcriptome biomarkers in AD [46]. The results of our GSEA analysis showed that the genes have implications for disease were enriched in four gene sets, i.e.,

CHR2Q13, *CHR19P12*, *CHR4P14*, and *KRAS.PROSTATE_UP. V1_DN*. The gene *TUBAL3* related to cilia was identified only in *KRAS.PROSTATE_UP. V1_DN*.

With the development of bioinformatics technology, more and more advanced and adequate methods have been developed and applied in life sciences [47]. At present, WGCNA is a well established method and applied in various studies of human diseases [29]. In our study, the results of the WGCNA analysis showed that 12 modules were related to AD, and the lightcyan module showing the highest correlation with AD contained a total of 160 genes. Based on the PPI network, 20 hub genes were identified to play important roles in the network associated with PCD [48–51]. We ranked the hub genes following the Score obtained in the Disgenet database, and the results revealed that the top four genes included *DNAI1*, *DNAAF3*, *CCDC114*, and *CCDC65*. Studies have shown that *DNAI1* was strongly linked to the ciliary beat pattern variations [48], while the *DNAAF3* variation in respiratory cilia was found uniformly immotile due to their defected dynein arms [49]. *CCDC114* was located at the basal body of a cilium and the knockdown of *CCDC114* could affect the formation of cilia in hRPE1 cells [50]. *CCDC65* was a central hub gene for assembly of the nexin-dynein regulatory complex and other regulators of ciliary and flagellar motility [51]. The results of both the GO and KEGG analyses showed that these hub genes were not only related to the regulation of axonemal dynein complex assembly and cilium movement, but also played an important role in Huntington's disease. It was worth noting that both the axonemal dynamic complex and cilium movement were relevant to neurological diseases [52].

Gene interaction heatmap showed that the 19 hub genes were associated with AD related key genes. Gene interaction is used to predict the function of the unknown gene [53]. Our results indicated a relationship between PCD and AD.

The role of A-β in AD development has been widely recognized with its deposition as one of the main symptoms of AD. Studies showed that the A-β inhibition of mitochondrial axonemal transport was associated with the early pathophysiology of AD [54–56]. Furthermore, it was reported that the A-β was transmitted through neuronal connections on the axon membrane [57]. PCD was a multiple inherited disorder caused by ciliary structural defects [58]. Evidently, PCD was directly related to ciliary movement. Moreover, studies have shown that the motor protein of axons was related to PCD [59, 60]. These results suggest that AD and PCD are linked by the functions of cilia and axons.

For the first time, we have applied not only the conventional methods of differential gene analysis, but also the GSEA and WGCNA to analyze the experiment data and to identify the gene sets related to PCD in the hippocampus of AD patients. We have withdrawn the following conclusions. First, most genes obtained by conventional differential gene analysis have been confirmed by our results. The GO and KEGG analyses further verified that these genes were related to AD. However, both the enrichment and GSEA analyses failed to identify any association between AD and PCD. Second, PCD related modules in hippocampus of AD patients were discovered by WGCNA. Third, the gene interaction heatmap showed that hub genes were bound to AD related key genes. These results indicated that the hub genes were involved in the regulation of axonemal dynein complex assembly and the regulation of cilium movement,

both of which played important roles in AD as well. These results strongly suggest that both AD and PCD were related in the functions of cilia and axons.

Conclusion

Based on the results of establishing the key gene network through WGCNA, our findings suggested that AD and PCD may share the pathogenesis, mainly reflected in the functions of cilia and axons. These commonalities indicated strongly the association between AD and PCD, providing theoretical foundations for further exploration of the pathogenesis and treatment of these two human diseases.

Abbreviations

AD	Alzheimer's Disease
PCD	Primary Ciliary Dyskinesia
AHN	Adult Hippocampal Neurogenesis
DEGs	Differentially Expressed Genes
GEO	Gene Expression Omnibus
GO	Gene Ontology
KEGG	Kyoto Encyclopedia of Genes and Genomes
MEs	Module Eigengenes
MM	Module Membership
PPI	Protein-protein Interaction
WGCNA	Weighted Gene Co-expression Network Analysis
CTs	Control Samples
NES	Normalized Enrichment Score
FDR	False Discovery Rate
A-β	Amyloid β-protein

Declarations

Ethics approval and consent to participate

Not necessary.

Consent for publication

Not applicable.

Availability of data and materials

The raw data of this study are derived from the GEO data portal (https:// www. ncbi. nlm. nih. gov/ geo/), GSE48350/ GSE28146 which are publicly available databases.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Pengcheng Xia and Jing Chen conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, and approved the final draft.

Ming Li and Le Wang conceived and designed the experiments, authored or reviewed drafts of the paper, and approved the final draft.

Xiaohui Bai1and Zhiming Lu conceived and designed the experiments, authored or reviewed drafts of the paper, and approved the final draft.

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Figures

Figure 1

Differential gene analysis of GSE48350. (A) Box plots based on standardized data. (B) Volcano plots constructed using fold-change values and adjusted P values. The red dots represent the over-expressed mRNAs and the blue dots indicate the down-expressed mRNAs with statistical significance. (C) Hierarchical clustering analysis of differentially expressed mRNAs between AD and normal tissues.

Figure 2

The enriched KEGG signaling pathways and GO analysis demonstrating the primary biological functions of major potential mRNAs. (A) The KEGG pathway of up-regulated genes. (B) The GO function of up-regulated genes. (C) The KEGG pathway of down-regulated genes. (D) The GO function of down-regulated genes.

Figure 3

Gene Set Enrichment Analysis (GSEA) of all genes in the array showing the results of the top four GSEA terms based on normalized enrichment scores in the AD. (A) Enrichment plot of *CHR2Q13* (NES = 1.9267, nominal p-value = 0.0, FDR q-value = 0.0867). (B) Enrichment plot of *CHR4P14* (NES = 1.9218, nominal p-value = 0.0036, FDR q-value = 0.0121). (C) Enrichment plot of *CHR19P12* (NES = 1.7553, nominal p-value = 0.0154, FDR q-value = 0.2317). (D) Enrichment plot of *KRAS.PROSTATE_UP.V1_DN* (NES = 1.8564, nominal p-value = 0.0, FDR q-value = 0.0482).NES, Normalized Enrichment Score; AD, Alzheimer's disease; CN, cognitively normal.

Figure 4

Cluster dendrogram of genes identified by WGCNA. (A) Clustering of samples to detect outliers. Scale-free topology model (left) and mean connectivity (right) were applied to identify the soft-thresholding power. The power selected is 18. (B) Tom diagram of module relationship. (C) The relationship between modules and genes in the modules, the horizontal axis represented the correlation coefficient of gene and module,

which was mainly used to observe the distribution of correlation coefficient between gene expression in each module and within the module.

Figure 5

Key modules correlated with AD identified by WGCNA. (A) Clustering of all modules. The red line indicates the height cutoff (0.25). (B) Heatmap showing the relationships between different modules and clinical traits. Non-clustering DEGs in the grey module.

Figure 6

Hub gene set of the key module and interaction network of genes in the key module. Green circles represent genes in the lightcyan module. Red circles represent hub genes in the module.

Figure 7

Venn map of different gene sets and Path analysis diagram of hub genes. (A) Venn map of DEGs showing genes in lightcyan Model and AD related genes in Genecards database. (B)Venn map of hub genes and PCD genes in Disgenet database. (C) GO analysis diagram of hub genes.

Figure 8

Gene interaction heatmap between hub genes and key genes related to AD. The hub genes identified (two genes with incomplete information were deleted) were used as the vertical axis and the top 30 genes with the highest correlation with AD in Disgenet database was set as the horizontal axis. and the color bar represents correlation coefficients with red representing positive correlation, blue negative correlation, and darker color strong correlation. Asterisks represent levels of significance (* $p < 0.05$; ** $p < 0.01$). (A) Gene Interaction Heatmap of GSE48350. (B) Gene Interaction Heatmap of GSE28146.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- FigureS1TheheatmapofgenesetsCHR2Q13analyzedbyGSEA.png
- FigureS2TheheatmapofgenesetsCHR4P14analyzedbyGSEA.png

- FigureS3TheheatmapofgenesetsCHR19P12analyzedbyGSEA.png
- FigureS4CHR2Q13126.png
- FigureS4TheheatmapofgenesetsKRAS.PROSTATEUP.V1DN736analyzedbyGSEA.png
- FigureS5CHR4P14186.png
- FigureS6CHR19P12132.png
- FigureS7KRAS.PROSTATEUP.V1DN736.png
- TableS1SequencingofhippocampaldataindatasetGSE48350.xlsx
- TableS2DifferentiallyexpressedgenesofTableS1.csv
- TableS3GOandKEGGanalysisofdifferentiallyexpressedgenesinTableS2.xlsx
- TableS4ModuleinfoofWGCNAanalysis.xlsx
- TableS5GOanalysisdiagramofhubgenes.xlsx
- TableS6GeneInteractionHeatmapdata.csv
- TableS8Moduleinfo.xlsx
- TableS9GOanalysisdiagramofhubgenes.xlsx
- TableS10GeneInteractionHeatmapdata.csv