American Journal of Alzheimer's Disease & Other Dementias®
1-6

DOI: 10.1177/1533317514537548
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Analysis of Differentially Expressed Genes Associated With Alzheimer's Disease Based on Bioinformatics Methods

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Abstract

Objective: To screen differentially expressed genes (DEGs) of Alzheimer's disease (AD). **Methods:** The gene expression profile (GSE26972) of AD was downloaded from Gene Expression Omnibus database. The DEGs were mapped to protein–protein interaction (PPI) data for acquiring the potential PPI relationship. The coexpressed significance of a gene pair in AD was determined. Then significantly enriched Gene Ontology (GO) terms and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways of DEGs were analyzed based on database for annotation visualization and integrated discovery tool. **Results:** The PPI network showed 7 upregulated genes and 4 downregulated genes that might play meaningful functional roles in AD. Meanwhile, 3 significantly enriched KEGG pathways as well as several significant GO terms (included α-actinin binding, interleukin 33 receptor activity, and telethonin binding) were identified. **Conclusions:** The screened DEGs have the potential to become candidate target molecules to monitor, diagnose, and treat AD.

Keywords

Alzheimer's disease, differentially expressed genes, protein–protein interaction network, functional enrichment analysis

Introduction

Alzheimer's disease (AD) was first described[1] by German neurologist Alois Alzheimerin in 1907. As one of the most common types of dementia, AD had characteristics such as progressive memory impairment, cognitive dysfunction, personality changes, language barriers, and other neuropsychiatric symptoms.[2] With the current increasing disease rate of AD, previous studies indicate the total number of people with AD dementia in 2050 to be 13.8 million.[3] Nowadays, clinical diagnostic criteria for AD, especially early diagnosis, are not sufficient. Besides, studies on pathogenesis of AD are still unclear, especially the genetic mechanisms of AD.

Considering the huge damage of AD, preventive measures are urgently needed. In the past decades, research of AD mostly focused on the molecular mechanism. There are 5 main pathogenesis theories about AD including cerebral accumulation of amyloid β (Aβ) protein,[4] central nervous cholinergic damage,[5] excitatory amino acids,[6] abnormal phosphorylation of τ protein,[7] and dysregulation of intracellular calcium.[8,9] Moreover, many genes have proved the association with AD. Amyloid protein precursor (*APP*),[10] *presenilin 1* (ps-1),[11] and *presenilin-2* (ps-2)[12] have been confirmed as virulence gene of familial AD, while apolipoprotein E (*apoE*)[13] has been proved tightly relevant to sporadic AD. Meanwhile, the mutation of *APP* is proved to cause an amino acid substitution of the carboxy terminus of the β-amyloid (*A*β) peptide.[10] Additionally, mutant *PS1* influences *APP* processing both in vitro and in vivo, and the elevated extracellular concentrations of amyloidogenic *A*β1-42 peptides are precipitated disease in PS1-linked familial AD.[14] Furthermore, *apoE* is now considered to have best-established genetic association with AD,[15] especially the *apoE ε4* which is considered as the only well-verified susceptibility gene. The effect of *apoE ε4* on AD is influenced by age and ethnicity.[16] Although previous studies have identified several potential genes and proteins as determinants of AD, the needs for more research to elucidate the mechanism of AD are highlighted.

In this article, differentially expressed genes (DEGs) between AD samples and normal samples were identified based on the data downloaded from Gene Expression Omnibus

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(GEO) database. Bioinformatics methods were used to construct a protein–protein interaction (PPI) network of DEGs. Meanwhile, function and pathway annotation of DEGs were performed based on Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways databases. The present study hoped to provide a new view and evidence for the mechanism of AD.

Materials and Methods

Derivation of Genetic Data

Gene expression profile (GSE26972)[17] was downloaded from a public functional genomics data repository GEO (http://www.ncbi.nlm.nih.gov/geo/) database, including 3 AD samples and 3 normal samples. Those data were analyzed based on Affymetrix Human Exton 1 S.T arrays.

Data Preprocessing

The raw genetic data were analyzed by the Oligo package on R-bioconductor, (http://www.bioconductor.org/).[18] while the normalization and calculation of expression value were performed by robust multiarray average algorithm.[19] was utilized for background correction, normalization, and calculation of expression value.

The *t* test was used to identify genes that were significantly differentially expressed between 3 AD samples and 3 normal samples. Then, the DEGs with fold change value of >2 or <0.5 and *P* value of <.05 were only selected. After that, cluster analysis was performed to guarantee the screened DEGs.

Function Annotation of DEGs

Gene Ontology function and enrichment analysis of KEGG pathways were performed based on database for annotation visualization and integrated discovery (DAVID)[20] online analytical tools. The GO terms and KEGG pathways with *P* value less than .05 were identified.

Construction of PPI Network

Ingenuity Pathway Analysis (IPA) database was used to obtain information about the PPI and protein–biomolecule interaction. After the PPI network construction based on the IPA database, the DEGs were mapped to the PPI data that have been collected from mint[21] and hprd[22] database. To determine the coexpressed significance of a gene pair in AD, Pearson's correlation coefficient (PCC)[23] test was used to calculate the relationship between nodes and edges in the PPI network. Finally the PPI network of AD with the |PCC| >0.6 and each edge linked with at least 1 DEG was constructed.

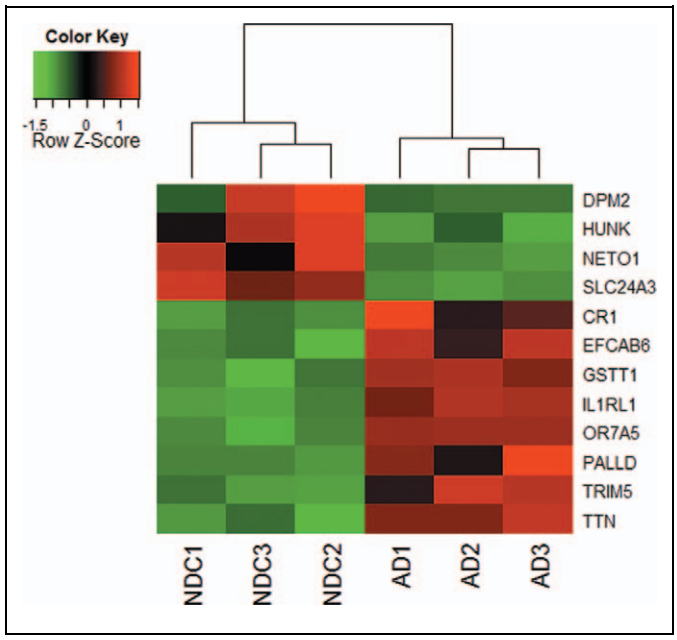

Figure 1. Dendrogram of differentially expressed genes (DEGs) by cluster analysis. The below abscissa axis represents specimen: NDC represents normal sample, AD represents sample of Alzheimer's disease. The above abscissa axis represents the clustering of specimens. The right longitudinal axis represents genes. Red shows upregulated genes while green shows downregulated genes.

Results

Analysis of DEGs

A total of 12 DEGs were identified (Figure 1). Clusters analysis of DEGs showed that the upregulated DEGs included dentin matrix protein 2 (*DMP2*), hormonally upregulated Neu-associated kinase (*HUNK*), neuropilin and tolloid-like 1 (*NETO1*), and solute carrier family 24, member 3 (*SLC24A3*), while the downregulated DEGs included complement component (3b/4b) receptor 1 (*CR1*), EF-hand calcium binding domain 6 (*EFCAB6*), glutathione S-transferase theta 1 (*GSTT1*), interleukin 1 receptor-like 1 (*IL1RL1*), olfactory receptor, family 7, subfamily A, member 5 (*OR7A5*), palladin, cytoskeletal associated protein (*PALLD*), tripartite motif containing 5 (*TRIM5*), and titin (*TTN*).

Construction of PPI Network

The PPI network was constructed based on IPA database (Figure 2A). To further investigate the potential function of significant DEGs and their related proteins, the nodes and lines of top 5 DEGs were abscised (Figure 2B). The nodes of top 5 DEGs were ubiquitin C (*UBC*), β-*estradiol*, *APP*, interferon gamma (*IFNG*), and Ca^{2+}. Moreover, 8 upregulated genes including *TTN*, *CR1*, *IL1RL1*, *EFCAB6*, *PALLD*, *OR7A5*, *GSTT1*, and *TRIM5* as well as 4 downregulated genes including *HUNK*, *DMP2*, *SLC24A3*, and *NETO1* were revealed in this PPI network.

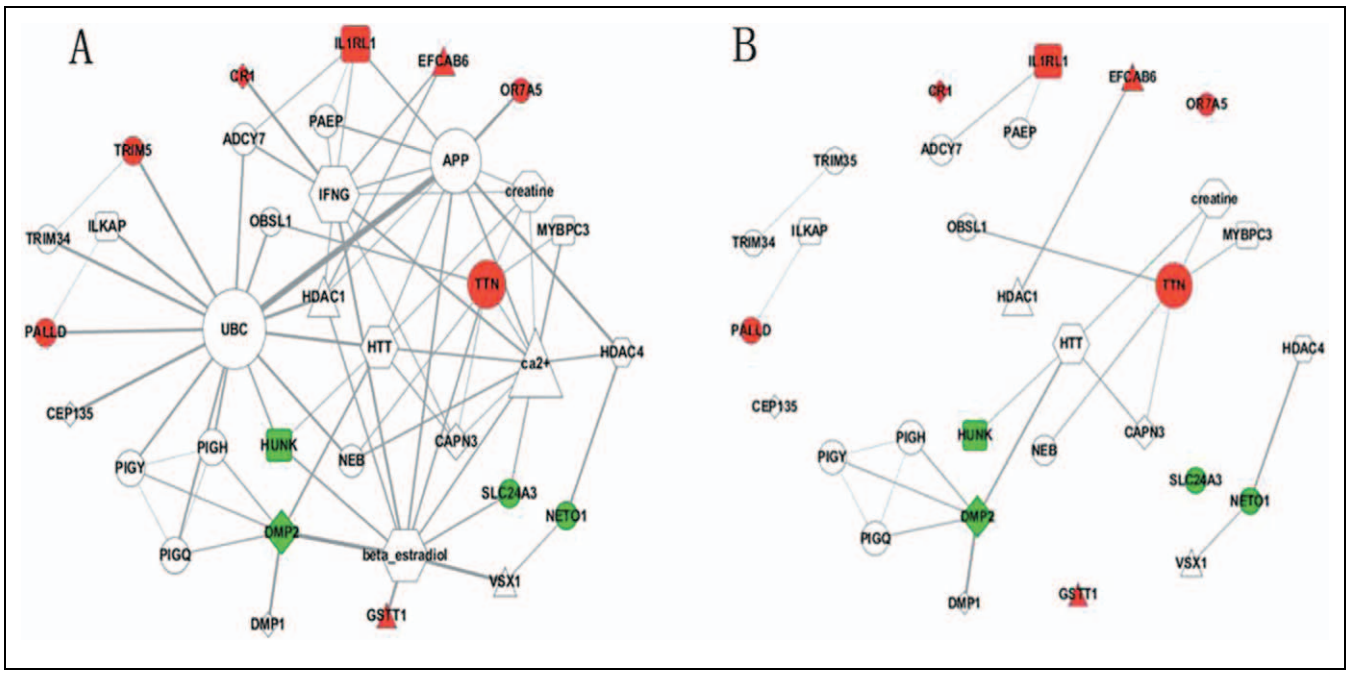

Figure 2. Protein–protein interaction (PPI) network of different expression genes (DEGs) based on Information-technology Promotion Agency Japan (IPA) database. A, PPI network of DEGs based on IPA database. B, PPI network after nodes and lines of top 5 were abscised. The red points stand for upregulated genes, the green points stand for downregulated genes, and the white points stand for genes which have PPI with DEGs. The size of node corresponds to the degree of this node. The thickness of the lines represents the ligation: the thicker the line is, the more important this connection is.

Function and Pathway Enrichment Analysis of DEGs

Gene Ontology functional enrichment indicated that DEGs were significantly enriched into several GO terms, such as α-actinin binding, interleukin 33 receptor activity, and telethonin binding. Top 10 of GO terms are listed in Table 1. Besides, the result of KEGG analysis revealed 3 enriched KEGG pathways: glycosylphosphatidylinositol (GPI)-anchor biosynthesis, N-glycan biosynthesis, and glutathione metabolism (Table 2).

Discussions

In the present study, a gene expression profile downloaded from GEO was used to explore the possible candidate genes of AD. A total of 12 DEGs (4 upregulated genes and 8 downregulated genes) were identified between the normal samples and samples with AD.

Protein–protein interaction network showed that *TTN*, *CR1*, *IL1RL1*, *EFCAB6*, *PALLD*, *OR7A5*, *GSTT1*, and *TRIM5* were upregulated genes and *HUNK*, *DMP2*, *SLC24A3*, and *NETO1* were downregulated genes. As a kind of protein degradation pathway,[24] ubiquitin proteasome pathway (UPP) dysregulation could induce the overphosphorylation of τ and degradation dysfunction of *A*β in AD.[25,26] The *TRIM* proteins consist of an N-terminal *E3* ubiquitin ligase Really Interesting New Gene domain which has been demonstrated to possess *E3* ubiquitin ligase activity in vitro allowing self-polyubiquitylation[27] and played roles in the polyubiquitylation and turnover of the protein.[28] The present study showed that *TRIM5* was overexpressed in samples with AD group which inferred that the overexpression of *TRIM5* might activate the UPP and increase decomposition of paraprotein. Besides, as *TTN* is reported to modulate Ca^{2+} homeostasis,[29] and Ca^{2+} is an important factor in AD,[30] there is no surprise that *TTN* is differently changed in AD. Moreover, previous study indicates that *NETO1* is associated with neurological diseases by the effects on the maintenance of neural circuitry and synaptic plasticity.[31] Drugs that bind to or modulate the activity of protein encoded by *PALLD* gene are suggested for the treatment of AD.[32] Genetic dysregulation of the *IL1RL1* axis appeared to be involved in conferring predisposition to AD,[33] and the IL1RL1 pathway plays an important role in AD.[34] Consistent with the above-mentioned studies, our results indicated that *NETO1*, *PALLD*, and *IL1RL1* might be involved in AD. Furthermore, an association between AD risk and markers spanning *CR1* has been observed,[35] and human erythrocytes, which abundantly expressed *CR1*, was able to sequester *A*β[36,37] and to favor its clearance via the C3b-mediated adherence to erythrocyte *CR1*.[37] In a word, *CR1* played a protective role via the generation and binding of *C3b*, which might contribute to *A*β clearance.[38] Therefore, we speculated that *CR1* might play a role in AD via effecting on *A*β. There was no literature drawn that *EFCAB6* and *OR7A5* were related to AD. Thus, the present study might add a new candidate to the list of genes involved in AD. The PPI network also showed that β-estradiol might enhance the expression of *DMP2*, *HUNK*, and *scl24A3* and decrease the expression of *GSTT1*. Figure 1 shows *DMP2*, *HUNK*, and *SLC24A3* are low expressed, and *GSTT1* is overexpressed in samples with AD. Previous studies indicate that the

Table 1. Significantly Enriched Gene Ontology Terms of Differentially Expression Genes (Top 10).[a]

GO ID	*P* Value	Count	Term
GOTERM_BP_ALL			
GO:0031033	.002108	1	Myosin filament assembly or disassembly
GO:0018406	.002108	1	Protein amino acid C-linked glycosylation via 2'-α-mannosyl-L-tryptophan
GO:0018103	.002108	1	Protein amino acid C-linked glycosylation
GO:0018211	.002108	1	Peptidyl-tryptophan modification
GO:0035269	.002108	1	Protein amino acid O-linked mannosylation
GO:0030241	.002108	1	Muscle thick filament assembly
GO:0048739	.00281	1	Cardiac muscle fiber development
GO:0030240	.003511	1	Muscle thin filament assembly
GO:0055003	.004212	1	Cardiac myofibril assembly
GO:0045087	.005142	2	Innate immune response
GOTERM_MF_ALL			
GO:0051393	.000721	1	α-actinin binding
GO:0002114	.000786	1	Interleukin 33 receptor activity
GO:0031433	.000786	1	Telethonin binding
GO:0008273	.001572	1	Calcium, potassium–sodium antiporter activity
GO:0051371	.001572	1	Muscle α-actinin binding
GO:0004875	.002357	1	Complement receptor activity
GO:0004582	.003141	1	Dolichyl-phosphate β-D-mannosyltransferase activity
GO:0004908	.005491	1	Interleukin 1 receptor activity
GO:0043621	.007836	1	Protein self-association
GO:0015491	.013288	1	Cation–cation antiporter activity
GOTERM_CC_ALL			
GO:0031501	.002058	1	Mannosyltransferase complex
GO:0000506	.002058	1	Glycosylphosphatidylinositol-N-acetylglucosaminyltransferase (GPI-GnT) complex
GO:0000932	.015004	1	Cytoplasmic mRNA processing body
GO:0005884	.019735	1	Actin filament
GO:0030018	.023101	1	Z disk
GO:0000794	.025116	1	Condensed nuclear chromosome
GO:0030176	.027797	1	Integral to endoplasmic reticulum membrane

Abbreviations: GO, Gene Ontology; mRNA, messenger RNA.
[a] *P* value less than .05 were consider to be significantly different.

Table 2. The Enriched KEGG Pathways.

KEGG ID	*P* Value	Count	Term
563	.024489	1	Glycosylphosphatidylinositol (GPI)-anchor biosynthesis
510	.042779	1	N-Glycan biosynthesis
480	.048497	1	Glutathione metabolism

Abbreviation: KEGG, Kyoto Encyclopedia of Genes and Genomes.

expression of *HUNK* may be regulated by β-estradiol,[39] and *GSTT1* is involved in estrogen metabolism.[40] The changed expression of *DMP2*, *HUNK*, *scl24A3*, *GSTT1*, and β-estradiol might result in the interactions between β-estradiol and these genes, which needed further studies. The PPI network result also showed that *UBC*,[41] β-estradiol,[10] *APP*,[42] *IFNG*,[43] and Ca^{2+} were[30] important factors in AD, which have been reported to have important relevance with the pathogenesis of AD. These 5 factors might be involved in the 5 pathogenesis theories of AD which was mentioned earlier.[4-9] Besides, β-*estradiol* has already been used as a potential therapeutic drug for AD,[44] while *APP* has been considered as the criteria for the diagnosis of AD.[45] This study indicated that DEGs that were related to *UBC*, *IFNG*, and Ca^{2+} pathways might be the important potential therapeutic targets for AD.

Additionally, the analysis of KEGG in the present study revealed 3 enriched pathways: GPI-anchor biosynthesis, N-glycan biosynthesis, and glutathione metabolism. Amyloid β, which is thought to be one of the causes of AD, is a cleaved fragment from N-glycans of amyloid precursor protein, and the number of N-glycans having a bisecting *GlcNAc* residue is proved in AD brains.[46] Considering the above-mentioned information, we hypothesized that N-glycan biosynthesis might be involved in the pathogenesis of AD. Besides, research that focused on glutathione metabolism demonstrated the induction of glutathione reductase and glutathione peroxidase messages in the AD hippocampus[47] and showed that decreased glutathione content might be involved in AD pathology in humans.[48] However, there is no evidence to support that GPI-anchor biosynthesis is involved in AD.

In conclusion, the DEGs of AD were analyzed by computational bioinformatics approaches. The present study indicated that *TTN*, *CR1*, *IL1RL1*, *EFCAB6*, *PALLD*, *OR7A5*, and *GSTT1* were upregulated genes and *HUNK*, *DMP2*, *SLC24A3*, *NETO1*

were downregulated genes that might play meaningful roles in AD. Meanwhile, the enriched pathway revealed several pathways including GPI-anchor biosynthesis pathway, N-glycan biosynthesis pathway, and glutathione metabolism pathway. The present study indicated several potential target molecules to treat the AD and might shed new light on the mechanism of AD.

Acknowledgments

This work was supported by Science and technology project of Binzhou medical university and Science and technology project of Colleges and universities in Shandong Province.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The authors disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by Science and technology project of Binzhou medical university and Science and technology project of Colleges and universities in Shandong Province.

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