Bioinformatics Approaches to Identify Novel Therapeutic Targets of Mixed Dementia

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Research

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Abstract

Background

Mixed dementia (MxD) refers to a patient's coexistence of Alzheimer's disease (AD) and vascular dementia (VaD). Until recently, pharmacological studies have generally focused on pure disease, AD or VaD, and have shed little light on the best therapeutic approaches to MxD. Therefore this study aimed to explore potential targets for MxD.

Methods

GSE36980 and GSE122063 datasets were downloaded from the Gene Expression Omnibus (GEO). GEO2R was utilized to identify the differentially expressed genes (DEGs) in GSE36980 between MxD and control groups. Then the genes in the top 50% of the median absolute deviation (MAD) with at least MAD greater than 0.01 and phenotypes of samples in GSE36980 were analyzed using R package “weighted gene co-expression network analysis” (WGCNA). Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) functional enrichment were analyzed on the selected dark green module genes. Protein-protein interactions analyses were performed in the STRING database and key genes were screened based on the maximal clique centrality (MCC) and maximum neighborhood component (MNC) algorithm of Cytoscape software. Key genes were then verified in external dataset GSE122063.

Results

There were 162 DEGs identified, including 121 up-regulated and 41 down-regulated genes. And the dark green module was the mostly related to MxD, among which, 43 genes were also DEGs in GSE36980 datasets. The most significantly enriched biological processes were the type I interferon signaling pathway (GO:0060337, $P = 1.79E-25$) and defense response to virus (GO:0051607, $P = 2.34E-22$). Due to the highest scores received, STAT1, IFIT3, OAS2, and MX1 may play crucial roles in MxD. Finally, test in external dataset GSE122063, STAT1, and IFIT3 were significantly up-regulated in AD patients.

Conclusions

In conclusion, STAT1 and IFIT3 may serve as potential targets for the pathogenesis of MxD, which should be further explored in the future.

Background

Alzheimer's disease (AD) and vascular dementia (VaD), the most prevalent types of dementia in the elderly, are likely to increase as the population ages[1, 2]. In medical practice, mixed dementia (MxD) refers to patients suffering from AD and VaD simultaneously, and clinically manifest both

neurodegeneration and cerebrovascular injury[3]. These two kinds of lesions had shared risks like hypertension, smoking, diabetes mellitus, a history of heart disease, and atrial fibrillation[4], each lesion could be regarded as sufficient to produce dementia on its own, or interact with each other to affect the progression of dementia. While stroke may accelerate deterioration in patients diagnosed with AD or prompt from the prior dementia stage to develop dementia[5, 6]. Correspondingly, neurodegenerative changes may also facilitate vascular injuries consisting of resting cerebral blood flow reduction and vasoconstriction mediated by β -amyloid, resulting in increased susceptibility to ischemia[7]. Reports from different autopsy-based studies showed 5%-35% of dementia patients exhibit mixed pathological features[8]. Therefore, the occurrence of MxD was probably to be underestimated.

Presently there are no disease-modifying treatments for any of the neurodegenerative dementias[9]. As the most classic theoretical hypothesis of AD, massive attempts have been made to conduct anti-A β immunotherapy, but they have not been successful so far. Among them, Aducanumab received the highest expectations because of its unique drug design. However, the FDA's latest report suggested that more than 40% of high-dose patients occurred amyloid-associated imaging abnormality, and caused 1 death due to cerebral edema[10]. It has been speculated before that a potential reason for the unsuccessful clinical trials of anti-A β immunotherapy against AD is the relatively high percentage of cerebrovascular comorbidities in the AD population[11]. Although immunotherapy resulted in a decrease in total A β levels, the final anti-A β immunotherapy did not improve cognitive function in AD/VaD mixed dementia model mice. In addition, anti-A β immunotherapy could further aggravate cerebral hemorrhage after treatment[11]. It seems to support the notion of unique pathogenic mechanisms of MxD. However, less attention has been paid to unique pathogenic mechanisms of MxD, or the shared mechanisms and targets of AD and VaD .

As an emerging discipline, bioinformatics provides new inspiration and methods to elucidate the pathological mechanism of diseases and to discover new disease targets[12, 13]. Up to date, attempts have been tried in identifying potential biomarkers of VaD or AD[14–16], or distinguishing mechanisms underlying VaD and AD[17], but not focusing on the shared pathological mechanism of MxD. Herein, we were dedicated to identifying and analyzing the candidate biomarkers or therapeutic targets in the expression profile dataset of MxD (GSE36980) overlapped by GEO2R and weighted gene co-expression network analysis (WGCNA). Then we performed further functional enrichment analysis of the most significant module genes in WGCNA. Subsequently, we constructed the protein-protein interaction (PPI) network and selected hub genes according to networks. Finally, hub genes were validated in an external dataset (GSE122063). The study design and workflow are presented in Fig. 1.

Methods

DEGs identification

GSE36980 which contained 33 frontal cortex samples and 29 temporal cortex samples, was downloaded from the Gene Expression Omnibus (GEO) database. Among these samples, 3 samples were given a

pathological diagnosis of AD and meantime had been clinically diagnosed as having VaD. Then GSE36980 was downloaded and analyzed by the online tool GEO2R from the GEO database. And R program was utilized to identify the differentially expressed genes (DEGs), with a P-value < 0.05 and $|\log_2FC| \geq \log_2(1.5)$ as cutoff values as reported[18]. DEGs were shown in the Volcano plot by using “ggplot2” package.

WGCNA

WGCNA is a systems biology method used to describe gene association patterns between different samples and relations between genes and phenotypes to identify candidate biomarkers or therapeutic targets[19]. The genes in the top 50% of the median absolute deviation (MAD) with at least MAD greater than 0.01 and phenotypes of samples were analyzed using R package “WGCNA”. To ensure that the results of network construction are reliable, the cut height of sample tree was set as 50 to remove abnormal samples. The optimal soft threshold for adjacency computation was then graphically determined. The filtered genes would be hierarchically clustered into different modules by calculating topological overlap measure (TOM) and those with higher similarity would be classified into the same module. Then the module eigengene (ME) values of each module were calculated. Additionally, the correlations coefficient between the ME values and phenotypes of disease were calculated.

Module Enrichment

The Database for Annotation, Visualization and Integrated Discovery (DAVID, <https://david.ncifcrf.gov/>) provides systematic, comprehensive biological annotation information for large-scale genes or proteins, and provides the most significantly enriched biological annotations[20]. Gene ontology (GO) and Kyoto encyclopedia of genes and genome (KEGG) pathway enrichment of genes in WGCNA dark green module were carried out by DAVID, then the bubble plot of biological process, cellular component, molecular function, and pathways were drawn by <http://www.bioinformatics.com.cn>, a free online platform for data analysis and visualization.

DEGs-module gene overlapping

Venn diagram was used to screen overlapped genes compatible with both filter modes of DEG and WGCNA by “VennDiagram” package. Then, the PPI network was constructed by the shared genes.

PPI network construction

The STRING database (<https://string-db.org/>) can be used to analyze the interactions between proteins and proteins[21]. In our study, the species was limited to “Homo sapiens”, the lowest interaction score was set to high confidence (0.700), then discrete targets were hidden and the remaining parameters kept the default settings. Subsequently, the PPI network obtained in the STRING database was visualized and further analyzed using the Cytoscape software.

Hub-module-DEGs selection

The top 10 scores of target protein in string network were output, which was ranked by the maximal clique centrality (MCC) and maximum neighborhood component (MNC) method using Cytohubba plug-in.

Validation in GSE122063

GSE122063 which contained 8 VaD, 11 CON, and 12 AD patients, was downloaded and analyzed by GEO2R from the GEO database. And R program was utilized to visualize the results, with a P-value < 0.05 and $|\log_2FC| \geq \log_2(1.3)$ as cutoff values. Expression data of GSE122063 were shown in Volcano plot with labels indicated hub genes by using “EnhancedVolcano” package in the R program.

Results

Identification of DEGs in mixed dementia

MxD indicated patients who were given pathological diagnosis of AD and also had been clinically diagnosed as having VaD. All cortex samples of mixed dementia and no dementia controls from the database GSE36980 were selected for DEG identification. Based on significant p-values and absolute $\log_2FC$ values, 162 DEGs were identified, including 121 up-regulated and 41 down-regulated genes (Fig. 1).

Identification of potential genes related to mixed dementia via WGCNA analysis

All cortex samples of GSE36980 except for 1 patient diagnosed with lewy body dementia were selected for WGCNA analysis. The square of the correlation coefficient, 0.85, was set as the standard to choose the soft threshold. Meanwhile, the suitable soft threshold (β) for network construction was selected as 8 (Fig. 3A). Thus the gene co-expression network conformed to an unsigned network. A total of 25 modules were constructed, of which the dark green module contained 54 genes. Among all the modules, the dark green module was the most correlated with MxD. The correlation index was 0.523 and the P-value was 0.003 (Fig. 3D).

Functional and Pathway Enrichment for genes related to mixed dementia

Based on 54 genes of mixed dementia obtained from the dark green module via WGCNA analysis, the top 10 biological processes, molecular function, cellular components were screened out by GO biological process enrichment analysis. As plotted in Fig. 4A-C, the most significantly enriched biological process were type I interferon signaling pathway (GO:0060337, $P = 1.79E-25$), defense response to virus (GO:0051607, $P = 2.34E-22$), the most significantly enriched molecular function were double-stranded RNA binding (GO:0003725, $P = 8.80E-07$), NAD + ADP-ribosyltransferase activity (GO:0003950, $P = 7.15E-05$), the most significantly enriched cellular component were cytosol (GO:0005829, $P = 1.84E-07$), cytoplasm (GO:0005737, $P = 1.12E-05$). The most significantly enriched pathways were herpes simplex infection, measles, etc (Fig. 4D).

PPI network construction and hub genes screening

The overlapping 43 genes via the venn diagram were selected for PPI network construction. There were 41 nodes and 280 edges created in the PPI network, and the average node degree was calculated as 13.7. Then the PPI network data was imported into Cytoscape, and the top 10 hub genes were selected by using the descending order of degree in the Cytohubba plug-in (Fig. 5C-D). Due to the highest scores received, STAT1, IFIT3, OAS2, and MX1 may play crucial roles in mixed dementia.

Validation of crucial genes

The top 10 hub genes was finally test in external dataset GSE122063. STAT1, IFIT3 were significantly up-regulated in AD patients.

Discussion

Dementia is more likely to be progress when vascular lesions and neurodegenerative changes coexist which are quite common in aging populations. Available medications like cholinesterase inhibitor therapy or memantine only provide modest clinical benefits once a patient has developed MxD[22]. Until now studies have generally focused on pure disease, either AD or VaD, and have shed little light on the best therapeutic targets to MxD as a whole entity[23]. Thus we studied the gene expression datasets employing WGCNA and DEG approaches to identify novel therapeutic targets of MxD. Due to the highest scores received, STAT1, IFIT3, OAS2, and MX1 may play crucial roles in mixed dementia. Besides the notion of converging pathogenic mechanisms, the concept of MxD solely understood as the coexistence of different pure forms of dementia is still far from being abandoned[3]. We verified our results in another microarray gene expression dataset which contains samples of independent VaD and AD. This dataset had strict filtering conditions, as AD cases should have no infarcts in the autopsied hemisphere and VaD cases must have low Braak staging. In our test, OAS2 and MX1 were not significantly up-regulated either in AD or VaD.

Signal transducer and activator of transcription 1 (STAT1) is known to play a key role in inflammatory responses and could be directly or indirectly involved in multiple inflammatory diseases[24, 25]. Neuroinflammation, triggered by BBB dysfunction, plays an important role in the pathogenesis of AD and VaD[26, 27]. STAT1 was up-regulated in MxD cases and verified in AD samples in our study. It is reported that downregulate STAT1 could rescue hTau-induced suppression of NMDAR expression with amelioration of synaptic functions and memory performance[28]. And knockout of STAT1 could abolish AD pathologies induced by δ -secretase-generated APP and Tau fragments[29]. STAT1 has been also identified as a point of convergence for the cross-talk between the pro-atherogenic IFN- γ , TLR4 and IL-6 activated pathways in immune as well as vascular cells, as such amplifying pro-inflammatory signals[30]. Although in our results, STAT1 was not up-regulated in external VaD cases. However, it was reported that STAT1 was significantly activated 2 hours after middle cerebral artery occlusion and reperfusion in mice. And compared with wild-type mice, STAT1-knockout mice had reduced numbers of TUNEL-positive neurons, smaller infarct volume, but higher neurological scores[31]. Inhibition of

JAK2/STAT1-mediated neuroinflammation signaling pathway could ameliorate memory impairment in ischemia/reperfusion stroke rats[32], indicating the important role of JAK2/STAT1 pathway in VaD treatment.

Interferon-induced protein with tetratricopeptide repeats 3 (IFIT3), which belongs to the IFIT family and induced by type I interferon, is an inflammatory-associated gene regulated through the JAK/STAT signaling pathway[33]. Electroacupuncture pretreatment partially decreased BBB permeability, reduced brain infarction volume, and improved neurological outcome by up-regulating the expression of antiviral and immunity activity-associated genes (such as Ifit1, Ifit3, Irf7, and Oasla)[34]. Besides, high expression of IFIT3 is correlated with poor survival in cancer patients[35]. But to our knowledge, no study has correlated expression of IFIT3 genes with AD or VaD disease thus far, warranting investigations of its roles in MxD pathology in the future.

Nevertheless, our research is based on secondary mining and analysis of published datasets, there are certain limitations as the number of samples for disease-associated targets identification is low. Thus further in vitro and preclinical experiments to properly evaluate the biological significance of the candidate targets are needed.

Conclusions

Integrated the results of DEGs and WGCNA, we found 43 candidate targets. These genes were significantly enriched in type I interferon signaling pathway. And further screened by the PPI network and verified by external datasets, STAT1 and IFIT3 may play crucial roles in MxD. In conclusion, our findings identified potential targets for MxD that may provide more new aspects in development and progression of MxD.

List Of Abbreviations

Alzheimer's disease: AD;

vascular dementia: VaD;

mixed dementia: MxD;

weighted gene co-expression network analysis: WGCNA;

protein-protein interaction: PPI;

Gene Expression Omnibus: GEO;

differentially expressed genes: DEGs;

median absolute deviation: MAD;

topological overlap measure: TOM;

module eigengene: ME;

Database for Annotation, Visualization and Integrated Discovery: DAVID;

Gene ontology: GO;

Kyoto encyclopedia of genes and genome: KEGG;

maximal clique centrality: MCC;

maximum neighborhood component: MNC;

Signal transducer and activator of transcription 1: STAT1;

Interferon-induced protein with tetratricopeptide repeats 3: IFIT3;

blood-brain barrier: BBB

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

ZJ conceived and designed the experiments; ZJ and HQF performed the research; both ZYT and ZJ wrote the manuscript with input from all authors and supervised the findings of this work. All authors read and approved the final manuscript.

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Figures

Figure 1

Study design and workflow

Figure 2

The volcano plot depicted DEGs in GSE36980 datasets. The 121 blue dots indicated the up-regulated genes while the 41 red ones represent the up-regulated ones. Horizontal axis: the $\log_2$ FC value; vertical axis: $-\log_{10}$ (P.Value). Abbreviations: DEG, differentially expressed gene

Figure 3

Identification of important modules by WGCNA. (A) Determination of soft-threshold power in the WGCNA. (B) Clustering dendrogram of samples for analysis. (C) Dendrogram of MAD genes clustered based on the measurement of dissimilarity. The color band shows the results obtained from the automatic single-block analysis. (D) Heatmap of the correlations between the MEs and clinical traits of MxD. Abbreviations: WGCNA, weighted gene co-expression network analysis; MAD, median absolute deviation; ME, module eigengene; MxD, mixed dementia.

Figure 4

Significantly enriched GO terms and KEEG pathways. (A) Representative bubble plots of biological process analysis of dark green module genes. (B) Representative bubble plots of cellular components of dark green module genes. (C) Representative bubble plots of molecular function of dark green module genes. (D) Representative bubble plots of KEEG analysis. Horizontal axis: the number of enriched genes; vertical axis: the corresponding GO terms or KEEG pathways; P values were represented by altered colors. Abbreviations: Gene ontology: GO; KEEG, Kyoto Encyclopedia of Genes and Genomes.

Figure 5

Construction of PPI network and selection of core targets. (A) Venn diagram of DEGs and dark green module genes screened via WGCNA in GSE36980. (B) PPI network of overlapping genes in the STRING database. (C) Core targets of overlapping genes conducted by MNC algorithm of Cytoscape. (D) Core targets of intersecting genes conducted by MCC algorithm of Cytoscape. Abbreviations: DEG, differentially expressed gene; PPI, protein-protein interaction; MCC, maximal clique centrality; MNC, maximum neighborhood component.

Figure 6

Validation of crucial targets in external datasets GSE122063. (A) The volcano plot depicted DEGs in AD compared to healthy control. The red dots represent the DEGs. (B) The volcano plot depicted DEGs in VaD compared to healthy control. The red dots represent the DEGs. Labels indicate hub genes. Horizontal axis: the $\log_2$ FC value; vertical axis: $-\log_{10}$ (P.Value). Abbreviations: DEG, differentially expressed gene; AD, Alzheimer's disease; VaD, vascular dementia.