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Analysis and Identification Genetic Effect of SARS-CoV-2 Infections to Alzheimer's Disease Patients by Integrated Bioinformatics

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Abstract.
Background: COVID-19 pandemic is a global crisis which results in millions of deaths and causes long-term neurological sequelae, such as Alzheimer's disease (AD).
Objective: We aimed to explore the interaction between COVID-19 and AD by integrating bioinformatics to find the biomarkers which lead to AD occurrence and development with COVID-19 and provide early intervention.
Methods: The differential expressed genes (DEGs) were found by GSE147507 and GSE132903, respectively. The common genes between COVID-19 and AD were identified. Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG), and protein–protein interactions (PPI) network analysis were carried out. Hub genes were found by cytoscape. A multivariate logistic regression model was constructed. NetworkAnalyst was used for the analysis of TF-gene interactions, TF-miRNA coregulatory network, and Protein-chemical Interactions.
Results: Forty common DEGs for AD and COVID-19 were found. GO and KEGG analysis indicated that the DEGs were enriched in the calcium signal pathway and other pathways. A PPI network was constructed, and 5 hub genes were identified (*ITPR1, ITPR3, ITPKB, RAPGEF3, MFGE8*). Four hub genes (*ITPR1, ITPR3, ITPKB, RAPGEF3*) which were considered as important factors in the development of AD that were affected by COVID-19 were shown by nomogram. Utilizing NetworkAnalyst, the interaction network of 4 hub genes and TF, miRNA, common AD risk genes, and known compounds is displayed, respectively.
Conclusion: COVID-19 patients are at high risk of developing AD. Vaccination is required. Four hub genes can be considered as biomarkers for prediction and treatment of AD development caused by COVID-19. Compounds with neuroprotective effects can be used as adjuvant therapy for COVID-19 patients.

Keywords: Alzheimer's disease, bioinformatics, compound, COVID-19, differentially expressed genes, hub gene

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INTRODUCTION

Ever since cases of COVID-19 were first reported on 31 December 2019 in China, COVID-19 has rapidly spread through the whole world and caused millions of deaths [1, 2]. Several studies showed that COVID-19 can impair the central nervous system and result in long-term neurological sequelae, like Alzheimer's disease (AD) [3, 4]. As we know, the therapy and management of AD patients impose a substantial burden on society and families. In addition, research indicated that AD patients were more susceptible to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), and COVID-19 would cause their AD condition to worsen [3, 5].

Our paper studies the interaction between COVID-19 and AD in order to explore the effective control strategy for managing the diseases. We aimed to explain the central nervous system damage caused by COVID-19 in the population by using integrated bioinformatics analysis, especially concentrating on the potential molecular biological functions and pathways that cause and aggravate AD, thus benefiting future exploration of intervention and treatment strategies.

We screened differential expressed genes (DEGs) by using GSE147507 [6] and GSE132903 [7], respectively, and found the common DEGs between COVID-19 and AD. Then we carried out a series of bioinformatics analysis such as Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG), protein–protein interactions (PPI), hub gene identification, etc.

We found that common DEGs were enriched in calcium signaling and other pathways, among which the imbalance of calcium ion homeostasis is one of the most important pathogenesis of AD. We found 4 hub genes (*ITPR1, ITPR3, ITPKB, RAPGEF3*) that may play an important role in the occurrence and development of AD in COVID-19 patients through the identification of hub genes and the validation of the multivariate logistic regression model. The 4 hub genes can be considered as biomarkers for the occurrence and development of AD caused by COVID-19. In addition, we utilized the common hub genes of the two diseases to construct clinical diagnostic model (ROC_AUC = 0.757) in order to provide effective strategy and tools for early screening risk of AD. This study also indicated that vaccination is a relatively safe and economic intervention to achieve universal immunization. Finally, compounds with neuroprotective effects can be used as adjuvant therapy for COVID-19 patients.

METHODS

Dataset collections

Dataset (GSE147507: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE147507) [6] was selected for SARS-CoV-2 infection in human at transcriptional levels and dataset (GSE132903: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE132903) [7] was selected for the gene expression of AD analysis. Both datasets were acquired from GEO database (https://www.ncbi.nlm.nih.gov/geo/). Samples of lung biopsy for healthy negative control and lung sample from postmortem COVID-19 patient were selected from GSE147507 for analysis. Samples which composed of temporal gyrus samples from AD patients (AD = 97) and non-dementia controls (ND = 98) was selected from GSE132903. The study flow is shown as in Fig. 1.

DEGs identification and common gene identification between COVID-19 and AD

DESeq2 [8] and limma [9] package in R software (version 4.0.2) with adjusted P-value < 0.05 and $\log2|FC| > 1$ were used for DEGs identification between COVID-19 patients and health control from GSE147507. And limma packages in R software with $\log2|FC| > 0.5$, adj.p.val < 0.01 were used to obtain DEGs from GSE132903. The common DEGs between COVID-19 and AD was obtained by using R software.

GO and KEGG analysis

GO and KEGG enrichment analysis were carried out with the "clusterProfiler R" [10] package (v3.16.1) of R software.

PPI network analysis and hub genes identification

Common DEGs were used to construct a PPI network by STRING (https://string-db.org/) with a confidence score of ≥ 0.4. Hub genes of the PPI network were identified using degree algorithm from cytoHubba [11], a plugin in Cytoscape, and visualized using Cytoscape (v3.7.2).

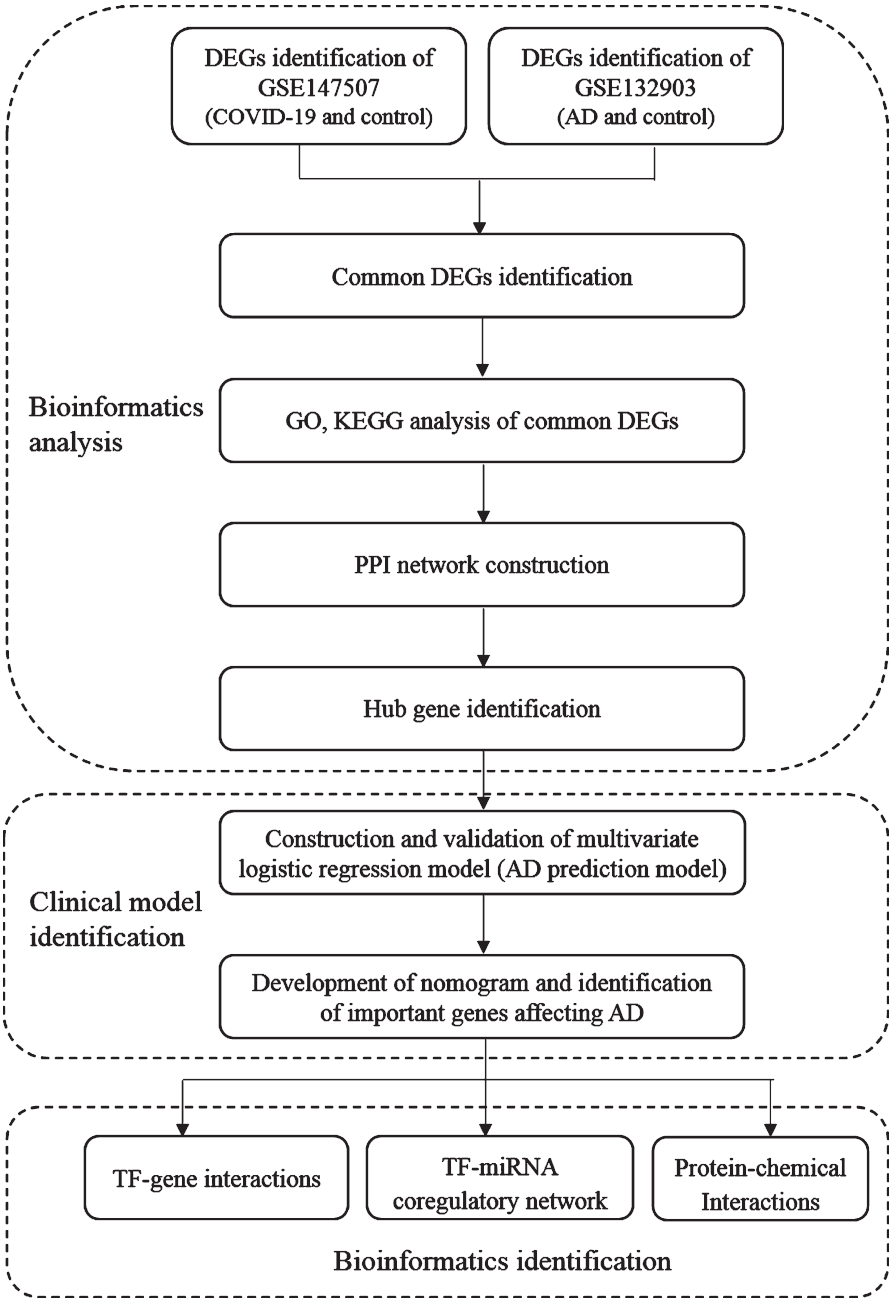

Fig. 1. Study flowchart. AD, Alzheimer disease; DEGs, differential expressed genes; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes.

Model construction

Sex, age, and hub gene expression profiles from GSE132903 were integrated to analyze the correlation between these factors and AD using univariate logistic regression. The baseline data was shown as in Table 1. Then the data was divided into a training set (70%) and a test set (30%), the difference between the two sets was then verified. In the training set, a multivariate logistic regression model was constructed by incorporating the features of $p < 0.05$ in the results of univariate logistic regression analysis. ROC_AUC was performed to validate the model in the test set.

Table 1
Baseline data of AD and the control in GSE132903

	AD	ND	*p*
NO.	97	98	
ITPR1 (mean(sd))	9.39 (0.92)	10.03 (0.82)	6.30E-07
ITPR3 (mean(sd))	8.63 (0.61)	8.04 (0.55)	1.40E-10
ITPKB (mean(sd))	9.88 (0.82)	8.98 (0.74)	7.90E-14
RAPGEF3 (mean(sd))	8.34 (0.77)	7.52 (0.53)	5.10E-14
MFGE8 (mean(sd))	10.42 (0.6)	9.89 (0.54)	2.40E-09
Age (mean(sd))	85.02 (6.75)	84.98 (6.9)	0.97
Sex (%)	*N* = 97 (100(%))		
Female	48 (49.48%)	48 (48.98%)	
Male	49 (50.52%)	50 (51.02%)	1
AD_diagnosis (%)	*N* = 97 (100(%))		
AD	97 (100%)	0 (0%)	
Normal	0 (0%)	98 (100%)	0

$p < 0.05$ is considered to be statistically significant.

Development and validation of a multigene containing nomogram

Nomograms include several lines corresponding to certain clinical parameters and have been widely used to predict the incidence of patients in a clinical environment [12]. A multigene containing nomogram was constructed according to the multivariate logistic regression model in the training set.

Validation of hub genes by Alzdata

The hub gene expression profiles between AD and control brain tissues were determined using AlzData (http://www.alzdata.org). AlzData is a database that provides human brain gene expression profiling [13, 14].

TF-gene interactions

NetworkAnalyst [15] (https://www.networkanalyst.ca/) was used to find the TF-gene interaction with identified hub genes. TF-gene interaction analysis with integration of common AD risk genes [16, 17] (*APP, PSEN1, PSEN2, APOE, SORL1, ABCA7, TREM2, PLCG2, BDNF*) and hub genes was also performed by NetworkAnalyst.

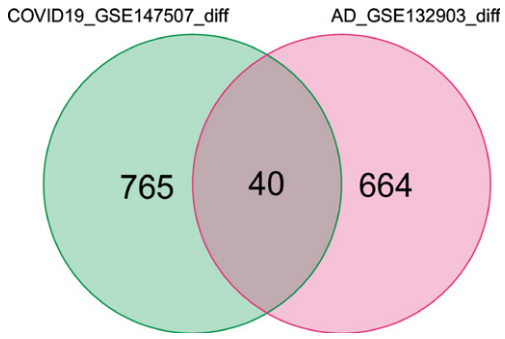

Fig. 2. Common DEGs are represented by a Venn diagram. 40 genes were found as common DEGs from 805 DEGs of COVID-19 and 704 DEGs of AD patients.

TF-miRNA coregulatory network

TF-miRNA coregulatory network was constructed with the identified hub genes using NetworkAnalyst tool [15].

Protein-chemical interactions

An important component of the study also included using NetworkAnalyst to identify compounds that interact with hub genes [15].

RESULTS

DEGs identification and common DEGs identification between COVID-19 and AD

We obtained 805 DEGs from GSE147507 and 704 DEGs from GSE132903. Then we took the intersection of 805 DEGs for COVID-19 and 704 DEGs for AD and determined 40 common DEGs, this was then visualized with a Venn diagram (Fig. 2).

GO and KEGG analysis

The 40 common DEGs between AD and COVID-19 were used for GO and KEGG analysis. GO analysis showed that genes were enriched in molecular function of inositol 1,4,5 trisphosphate binding and calcium-release channel activity (Fig. 3). KEGG

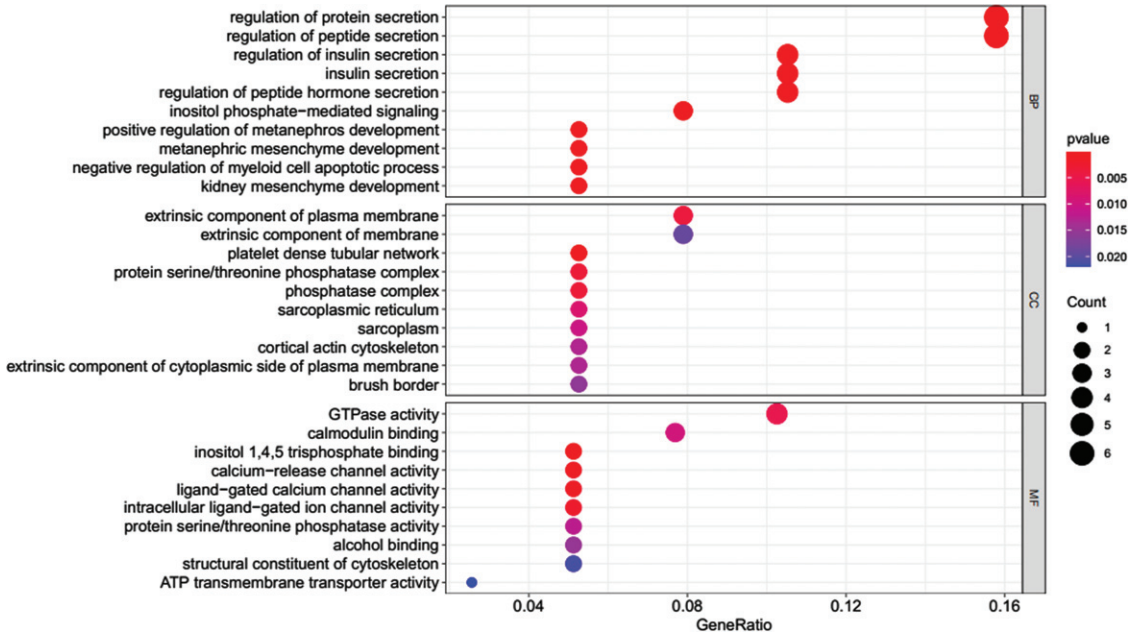

Fig. 3. GO enrichment analysis for the common DEGs. BP, biological process of GO analysis; CC, cellular component of GO analysis; MF, molecular function of GO analysis.

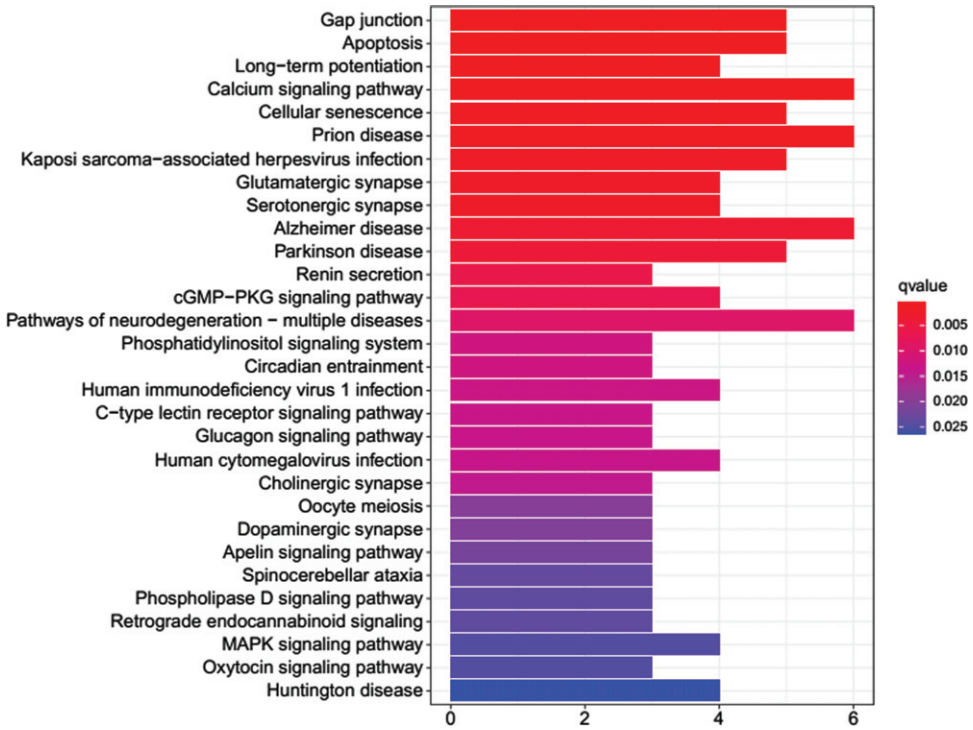

Fig. 4. KEGG enrichment analysis for the common DEGs.

analysis demonstrated the genes were enriched on pathways of apoptosis, long-term potentiation, calcium signaling, cellular senescence, glutamatergic synapse, AD, etc. (Fig. 4).

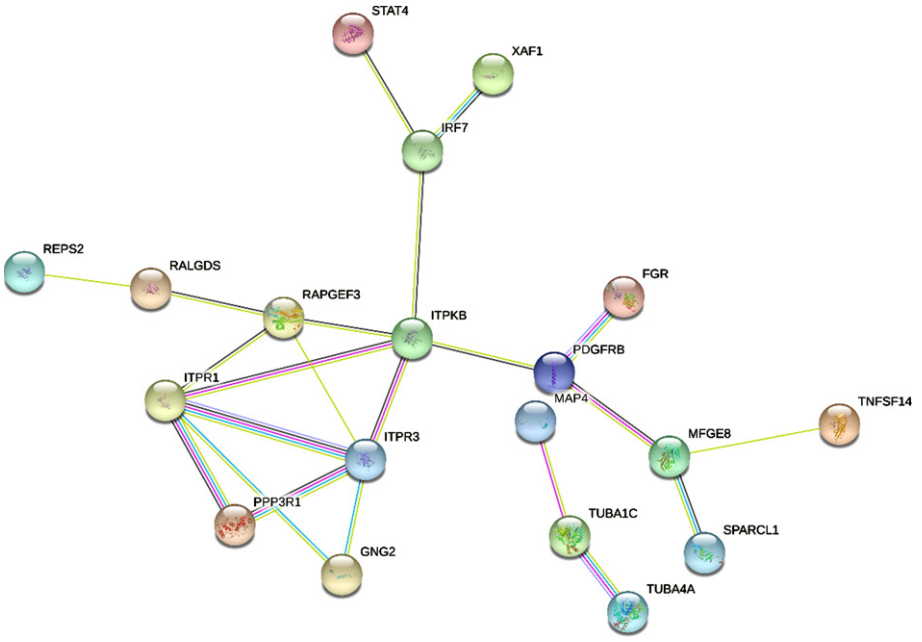

Fig. 5. PPI network for common DEGs which are shared by COVID-19 and AD.

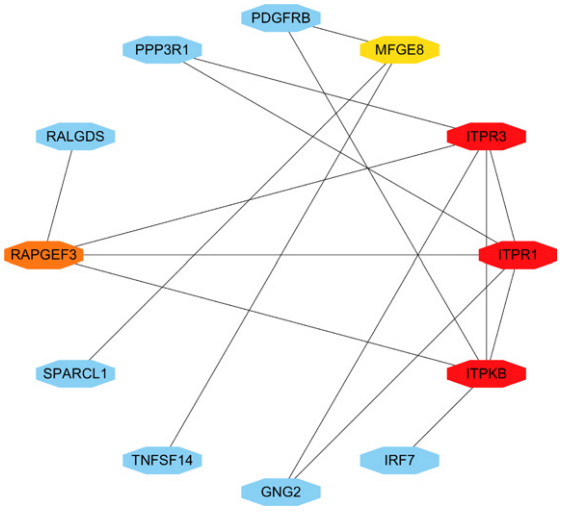

Fig. 6. Identification of hub gene from the PPI network. According to the degree value, the highlighted 5 genes (*ITPR1, ITPR3, ITPKB, RAPGEF3, MFGE8*) are considered as hub genes.

PPI network analysis and hub genes identification

We entered the 40 common DEGs into STRING and imported generated files into Cytoscape (https://cytoscape.org/) for the visualization of PPI network analysis (Fig. 5). Then we used cytoHubba to identify the 5 hub genes *ITPR1, ITPR3, ITPKB, RAPGEF3,* and *MFGE8* (Fig. 6).

Table 2
Univariate logistic regression of risk factors in AD

	characteristics	R val	*P* val
1	*ITPR1*	–0.82	0
2	*ITPR3*	1.69	0
3	*ITPKB*	1.42	0
4	*RAPGEF3*	1.97	0
5	*MFGE8*	1.57	0
6	Age	0	0.97
7	Sex	–0.02	0.94

$p < 0.05$ is considered to be statistically significant.

Model construction and validation

The training set and test set was verified, and there was no significant difference between the two sets (Supplementary Table 1). Univariate logistic regression was used to analyze features which would affect the occurrence of AD, including *ITPR1, ITPR3, ITPKB, RAPGEF3,* and *MFGE8* ($p < 0.05$) (Table 2). Multivariate regression analysis indicated that 4 hub genes (*ITPR1, ITPR3, ITPKB, RAPGEF3*) were important factors affecting AD ($p < 0.05$) (Table 3). The result was validated using ROC_AUC with a score of 0.757 in the test set (Fig. 7).

Table 3
Multivariate logistic regression of Risk Factors in AD

Intercept and Variable	β	Odds Ratio (95CI)			p
(Intercept)	–62.8404185				1.24E-05
ITPR1	2.2525753	9.51E + 00	(2.95E + 00	to	3.54E + 01)	0.000341
ITPR3	1.9249126	6.85E + 00	(1.63E + 00	to	3.24E + 01)	0.010611
ITPKB	0.9699832	2.64E + 00	(1.06E + 00	to	6.86E + 00)	0.0398
RAPGEF3	3.5058907	3.33E + 01	(7.21E + 00	to	2.03E + 02)	3.14E-05
MFGE8	–1.1648198	3.12E-01	(7.38E-02	to	1.22E + 00)	0.099675

p<0.05 is considered to be statistically significant.

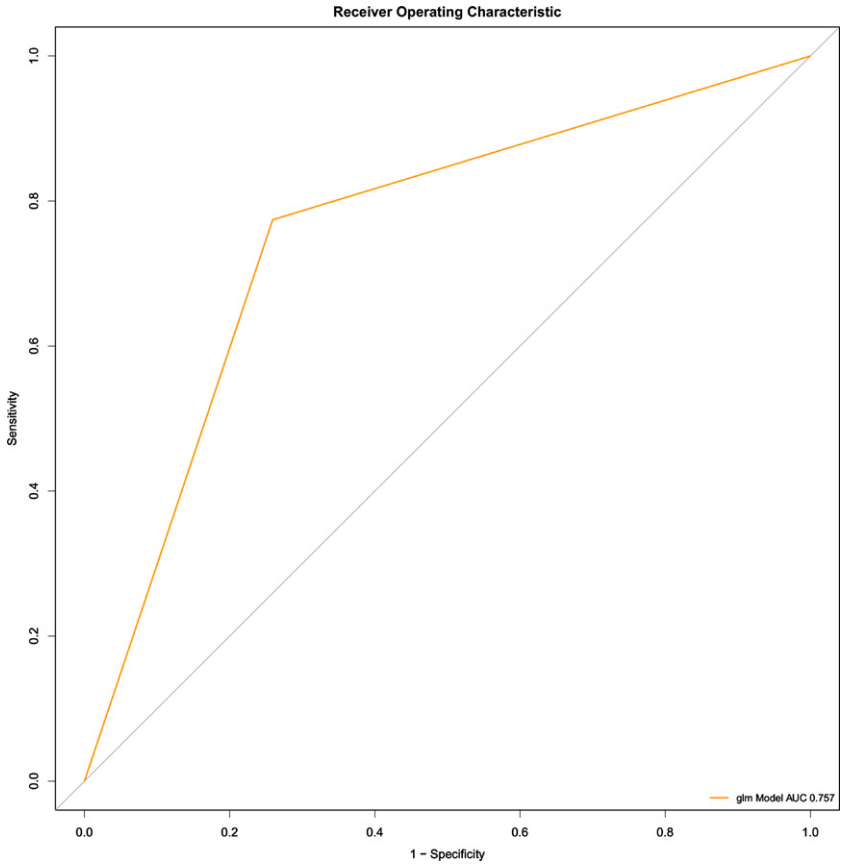

Fig. 7. ROC-AUC of the multivariate logistic regression model for AD prediction (test set)

Development of a multigene containing nomogram

A multigene containing nomogram was constructed according to the multivariate logistic regression model in the training set. For example, a sample (GSM3896060) from GSE132903 has an AD incidence probability of 0.883 and requires active treatment (Fig. 8).

Validation of hub genes by Alzdata

The 4 genes *ITPR1, ITPR3, ITPKB, and RAPGEF3* have significant differences in gene expression between AD and normal groups (Fig. 9). *RAPGEF3* has a high correlation with Aβ and tau, while *ITPR3* has a correlation with Aβ. *RAPGEF3* and APP and APOE interact in the PPI network (Supplementary Table 2).

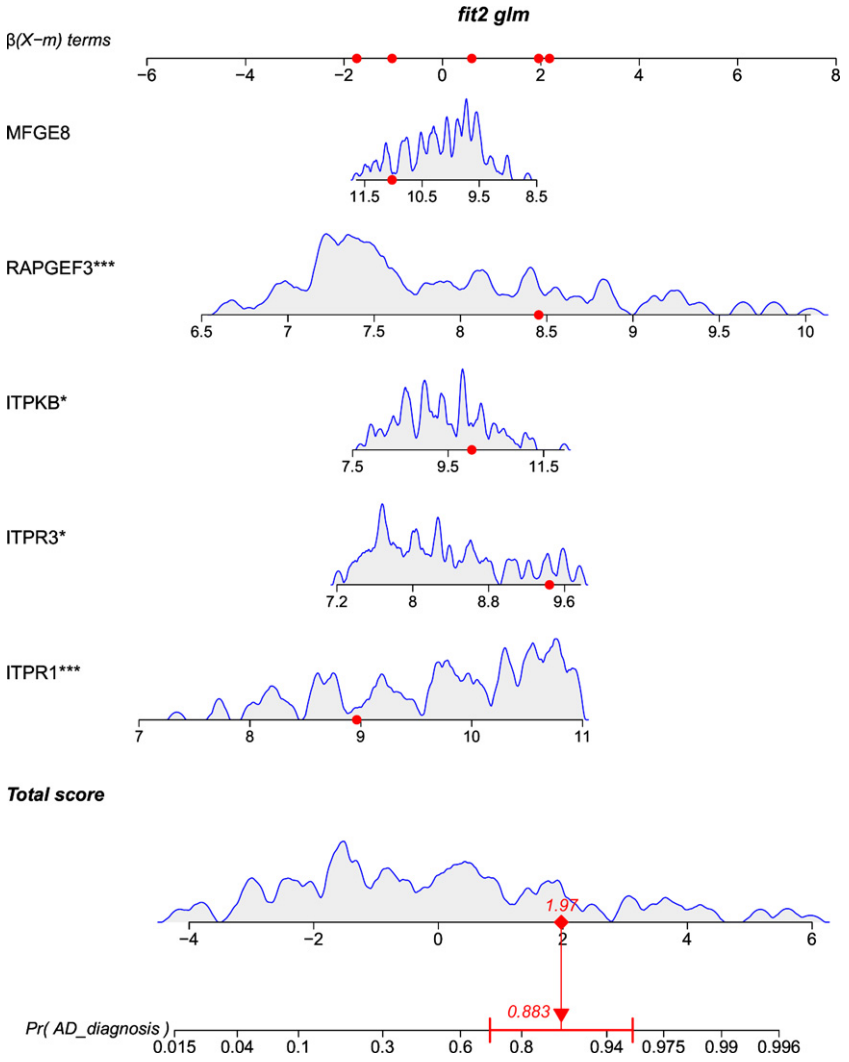

Fig. 8. Multigene based nomogram by 4 hub genes predicting the probability with Alzheimer's disease (GSE132903).

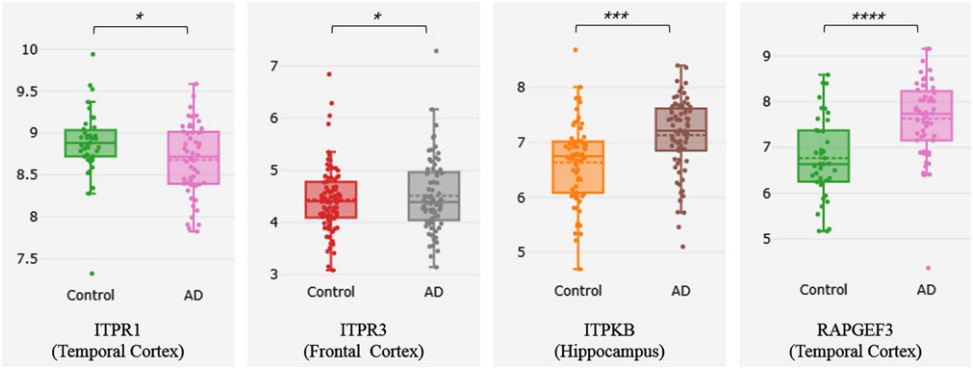

Fig. 9. Differential expression of Cross platform normalized data (AlzData)

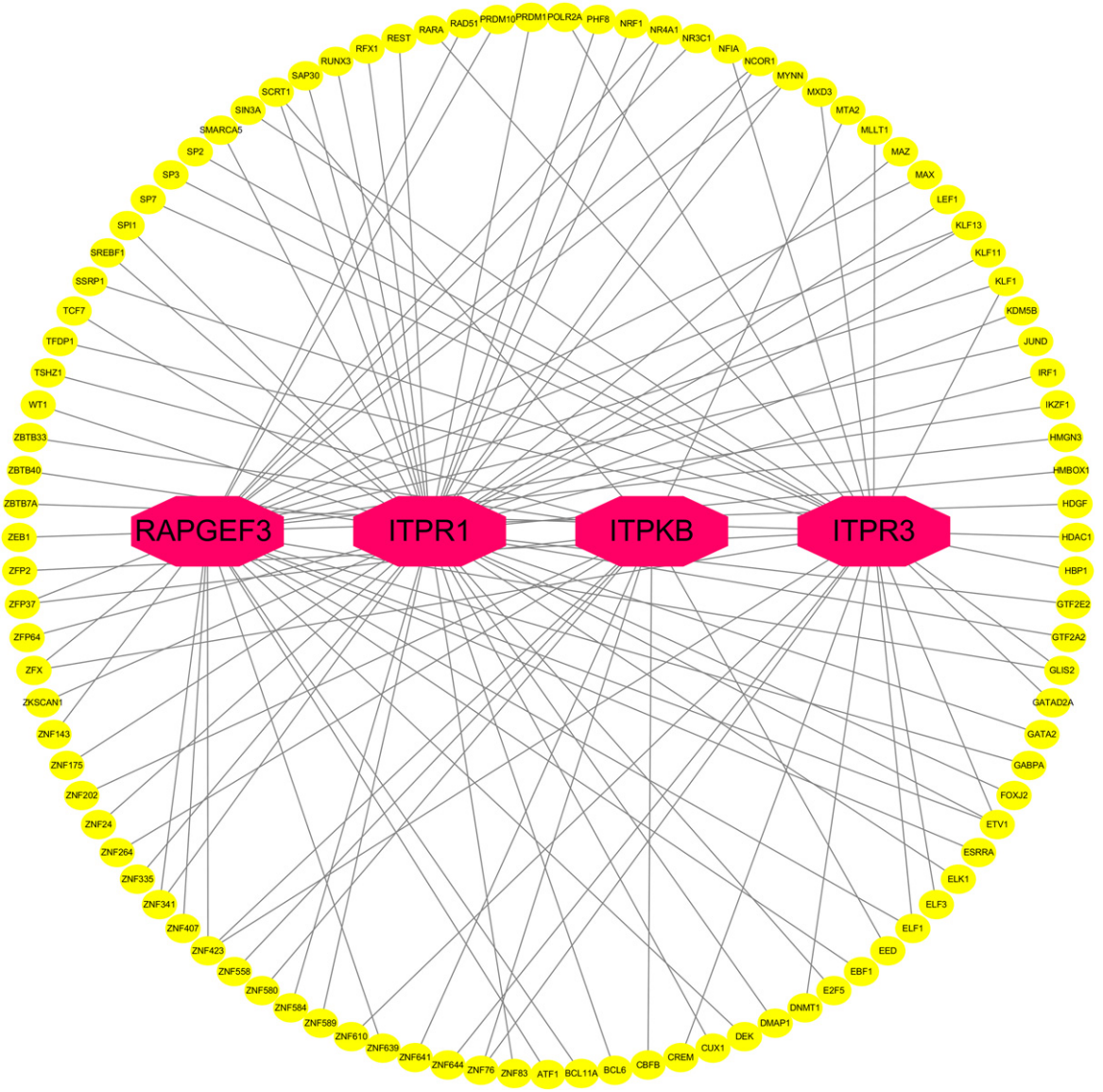

Fig. 10. Network of TF-gene interactions with 4 hub genes. The red node represents the hub genes, yellow node represents TF-genes.

TF-gene interactions

TF-gene interactions showed the interaction of TF genes and 4 hub genes (Fig. 10, Supplementary Table 3). *ITPR1, ITPR3, ITPKB, and RAPGEF3* were regulated by 43, 28, 14, and 30 TF genes, respectively. A TF-gene regulatory network of 4 hub genes and common AD risk genes was constructed. Figure 11 and Supplementary Table 4 represent the interaction network between the 7 AD risk genes (*ABCA7, APOE, PSEN1, PSEN2, SORL1, PLCG2, BDNF*) and the 4 hub genes (*ITPR1, ITPR3, ITPKB, RAPGEF3*).

TF-miRNA coregulatory network

TF-miRNA coregulatory network showed the interaction of TF genes and miRNAs with 4 hub genes (Fig. 12, Supplementary Table 5).

Protein-chemical interactions

We found that resveratrol, genistein, and quercetin were compounds that could interact with most hub genes. Since these compounds were detected against common hub genes, these compounds represented

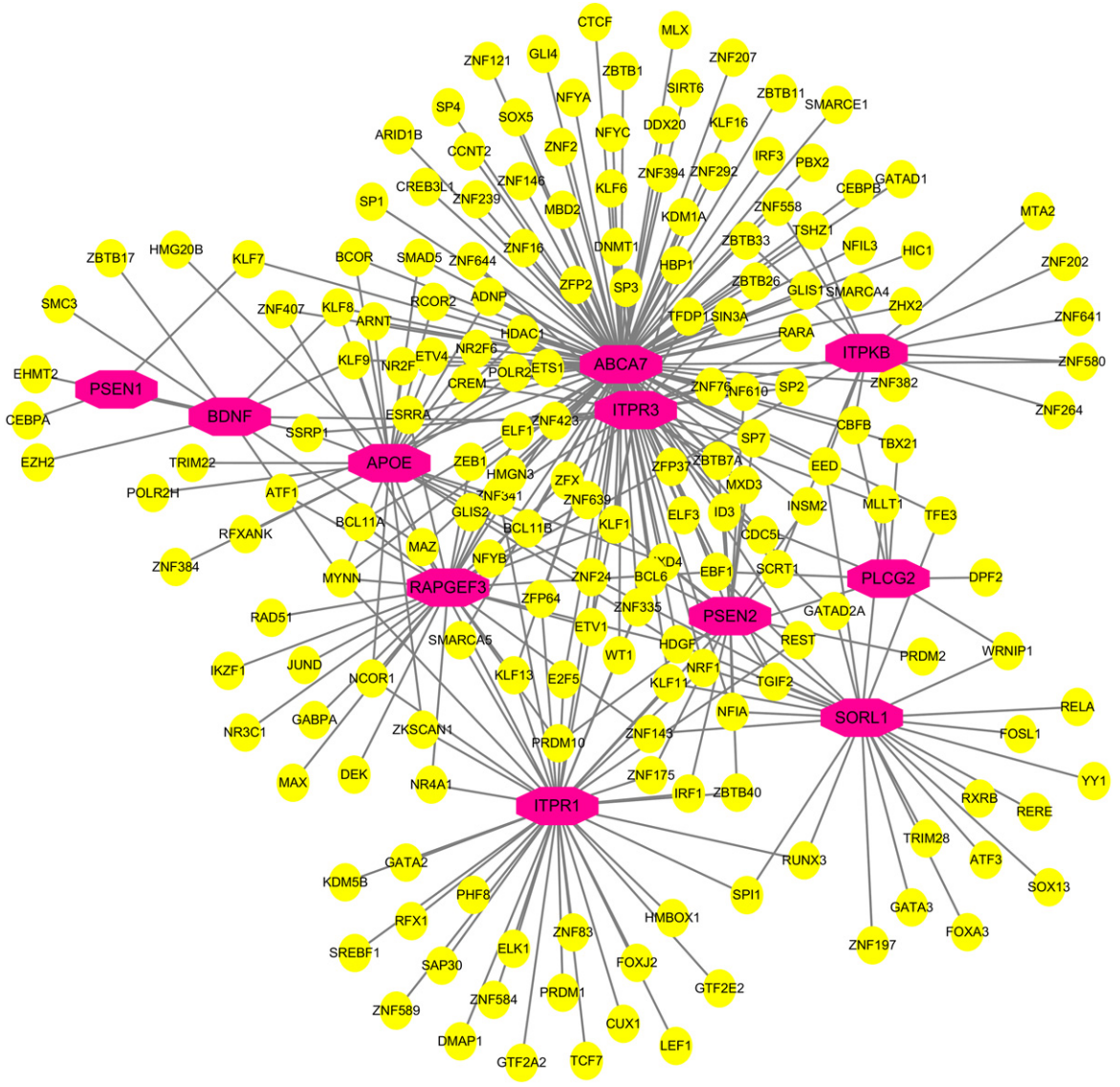

Fig. 11. Network of TF-gene interactions with 4 hub genes and common AD risk genes. AD risk genes: *PSEN1, PSEN2, APOE, SORL1, ABCA7, PLCG2, BDNF.* Hub genes: *ITPR1, ITPR3, ITPKB, RAPGEF3.* The red node represents the AD risk genes and hub genes, yellow node represents TF-genes.

common compounds in COVID-19 and AD (Fig. 13, Supplementary Table 6). Top 20 of the compounds which interact hub genes were listed in Table 4.

DISCUSSION

COVID-19 could lead to neurologic sequelae such as AD [3]. Since many studies have reported the pathogenesis of AD in association with COVID-19 [3, 18, 19], AD is of particular concern. We tried to explore the common molecular biology functions and pathways between COVID-19 and AD in order to find biomarkers of AD progression by COVID-19 and provide early intervention.

In this study, we have identified the DEGs of COVID-19 and AD, respectively. Then we found forty common DEGs of AD and COVID-19 and performed bioinformatics analysis. GO indicated that common DEGs were enriched in molecular function of inositol 1,4,5 trisphosphate binding and calcium-release channel activity. KEGG showed that common DEGs were enriched in pathways of apoptosis, long-term potentiation, calcium signaling,

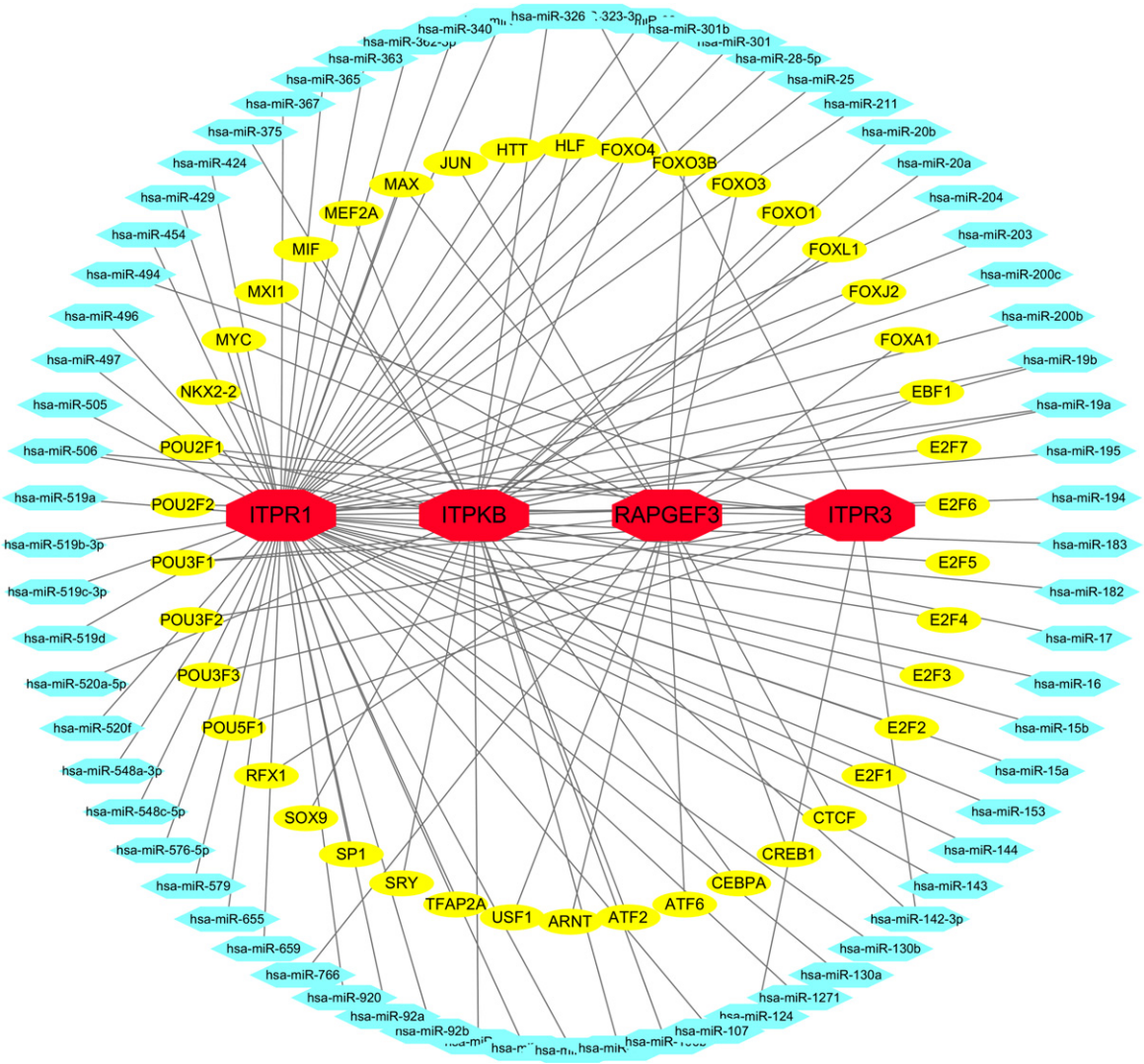

Fig. 12. TF-miRNA coregulatory network. The network presents the interactions between TF-genes, miRNA and 4 hub genes. The red color nodes represent the hub genes, yellow nodes are TF-genes and blue nodes indicate miRNA.

cellular senescence, glutamatergic synapse, and AD. The inositol 1,4,5-trisphosphate receptor (IP_3R) can mediate calcium-release channel activity and dysfunction of IP_3R may play a role in the pathogenesis of AD [20]. The calcium signaling pathway involved in the analysis of KEGG is consistent with the current studies which have confirmed calcium ionic homeostasis imbalance as one of the key mechanisms of AD pathogenesis [21–24]. Common DEGs enriched in pathways of long-term potentiation [25, 26] and glutamatergic synapse [27] may explain to some extent the cause of memory loss in some COVID-19 patients [28], as memory loss is widely known as a clinical manifestation of AD. Then we performed PPI network analysis on common DEGs and identified 5 hub genes (*ITPR1, ITPR3, ITPKB, RAPGEF3, MFGE8*).

By integrated the gender, age and the expression profiles of 5 hub genes in GSE132903 which included AD patients and the control group, we established a multivariate logistic regression model and found 4 hub genes (*ITPR1, ITPR3, ITPKB, RAPGEF3*) that were the most important factors affecting AD. *ITPR1* (Inositol 1,4,5-Trisphosphate Receptor Type 1) takes part in regulating calcium homeostasis in the endoplasmic reticulum and induces Ca^{2+} release into the cytosol and may be a potential target for treatment of AD [20, 29]. One study indicated that *ITPR3*

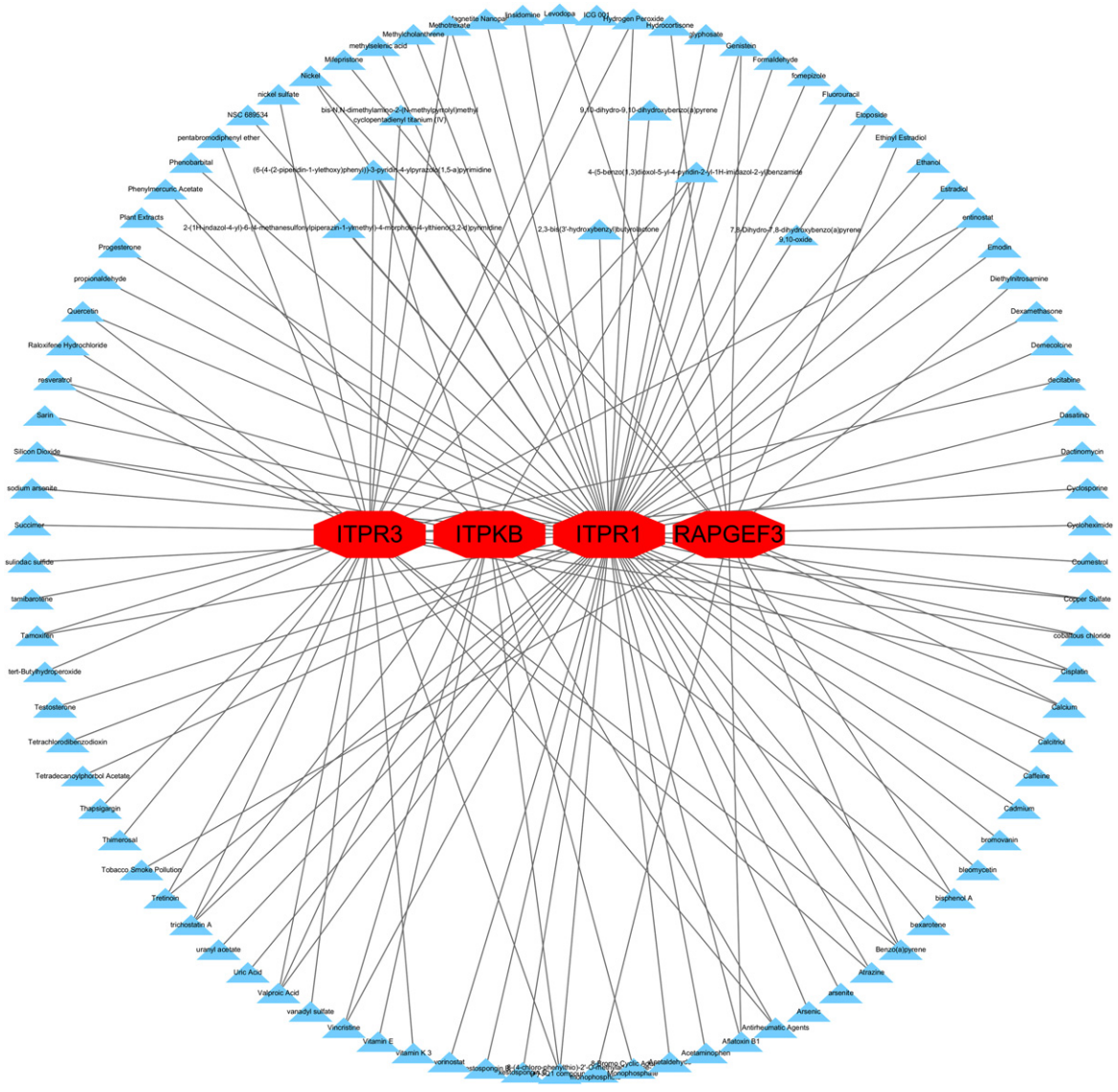

Fig. 13. Protein-chemical Interactions by NetworkAnalyst. Suggested compounds that interact with hub genes.

(Inositol 1,4,5-Trisphosphate Receptor Type 3) is particularly important at mitochondrial calcium and apoptosis modulation [30, 31]. In SH-SY5Y cells, knockout of *STIM1* can downregulate the expression of *ITPR3* which leads to the reduce of free calcium ion concentration of mitochondria, and finally results in energy metabolism disorders [32]. *ITPKB* (Inositol-Trisphosphate 3-Kinase B) is necessary for mature T cell and B cell functions [33, 34]. And in AD mouse models, inhibition of *ITPKB* can reduce the neuroinflammation in microglia [35]. It was also reported that melatonin can attenuate scopolamine-induced memory loss by rescuing EPACs/miR-124/Egr1 pathway [36], with EPAC being an alias for *RAPGEF3* gene. EPAC activation in neuronal cells has been confirmed to promote apoptosis [37]. EPAC is also involved in secretion of an amyloid precursor protein which is widely known to be an important mechanism leading to AD [38].

Our bioinformatics analysis and current literature suggests that imbalance in calcium homeostasis would be a shared mechanism to COVID-19 and AD. We speculate that the imbalance of calcium homeostasis in COVID-19 patients may eventually lead to the occurrence and development of AD as calcium homeostasis plays an important role in

Table 4
Suggested top 20 compounds for AD

Id	Compound	Degree	Betweenness	Genes
D001564	Benzo(a)pyrene	3	239.74	*ITPR1, ITPR3, RAPGEF3*
C459179	4-(5-benzo(1,3)dioxol-5-yl-4-pyridin-2-yl-1H-imidazol-2-yl)benzamide	3	110.7	*ITPR1, ITPR3, ITPKB*
C516138	(6-(4-(2-piperidin-1-ylethoxy)phenyl))-3-pyridin-4-ylpyrazolo(1,5-a)pyrimidine	3	110.7	*ITPR1, ITPR3, ITPKB*
C561695	(+)-JQ1 compound	3	110.7	*ITPR1, ITPR3, ITPKB*
C012589	trichostatin A	3	110.7	*ITPR1, ITPR3, ITPKB*
D014635	Valproic Acid	3	110.7	*ITPR1, ITPR3, ITPKB*
D016604	Aflatoxin B1	2	106.21	*ITPR1, RAPGEF3*
D002118	Calcium	2	106.21	*ITPR1, RAPGEF3*
D019833	Genistein	2	106.21	*ITPR1, RAPGEF3*
D009532	Nickel	2	106.21	*ITPR1, RAPGEF3*
D002945	Cisplatin	2	80.61	*ITPR3, RAPGEF3*
D001280	Atrazine	2	52.91	*ITPR1, ITPR3*
C018021	cobaltous chloride	2	52.91	*ITPR1, ITPR3*
D019327	Copper Sulfate	2	52.91	*ITPR1, ITPR3*
C118739	entinostat	2	52.91	*ITPR1, ITPR3*
D006861	Hydrogen Peroxide	2	52.91	*ITPR1, ITPR3*
D008727	Methotrexate	2	52.91	*ITPR1, ITPR3*
D011794	Quercetin	2	52.91	*ITPR1, ITPR3*
C059514	resveratrol	2	52.91	*ITPR1, ITPR3*
D013629	Tamoxifen	2	52.91	*ITPR1, ITPR3*

AD pathogenesis [39]. Recent studies suggested the sequelae of the nervous system in COVID-19 may be due to SARS-CoV-2 infection of central nervous system [40]. The latest study demonstrated virus-induced senescence can be the pathogenic trigger of COVID-19-related cytokine escalation and organ damage [41]. Another research demonstrated COVID-mediated cytokine storm can cause systemic inflammation, including neuroinflammation [42]. Neuroinflammation can then cause an increase in cytokines and reactive oxygen species by activating microglia, and then imbalance of calcium homeostasis in neurons will lead to neuronal necrosis and apoptosis, with a clinical symptom of memory loss [43]. Activation of microglia by calcium homeostasis dysfunction exacerbated disease progression [44]. Researchers found that the interaction between neuroinflammation and neuronal calcium dysregulation may synergistically lead to memory deficits [43, 45]. Since *ABCA7* is involved in the phagocytosis and clearance of amyloid-β by microglia [46], the interaction of ABCA7 with ITPR1, ITPR3, and ITPKB shown in Fig. 11 confirms the relationship between neuroinflammation and calcium homeostasis with the development of AD from the perspective of bioinformatics.

In addition, studies have found that since COVID-19 may exacerbate neuroinflammation and calcium homeostasis dysregulation in AD patients, COVID-19 infection can worsen AD conditions [3]. Recent studies showed that *APOE4* can aggravate the synaptic loss and neurodegeneration of brain organoids derived from iPSC in AD patients [47], which means that AD patients carrying *APOE4* are prone to disease progression after being infected by SARS-CoV-2. The latest research shows that neuronal *APOE4* can drive the occurrence and development of AD pathology by influencing immune response genes [48], and the inflammation and immune response that occur in COVID-19 infection are likely to become the inducement for *APOE* to participate in the pathogenesis of AD. In other words, even in the normal population, COVID-19 infection may induce AD due to the imbalance of calcium homeostasis and neuroinflammation. We speculate that the normal population carrying *APOE4* may be more susceptible to AD. Figure 11 showed a network interaction relationship between *APOE* and other genes such as *ABCA7, ITPR1, ITPR3*, and *ITPKB*, which also indicates that *APOE* is involved in the occurrence and development of AD. This study indicated that *APOE* is one of the important factors driving AD among the normal population and AD patients with COVID-19 infection. This may also explain to some extent why some COVID-19 patients are more likely to develop AD, and find a reasonable explanation for why the *APOE4* genotype population is more likely to suffer from AD.

In this study, we developed an AD risk diagnosis model (ROC_AUC = 0.757) using the common hub genes of COVID-19 and AD. This provides effective

strategies and tools for early screening AD risk in COVID-19 patients. which is valuable for the prognosis of COVID-19. We found that the 4 hub genes are not only the DEG of COVID-19, but also participate in the pathogenesis of AD. Therefore, these 4 genes can be considered as biomarkers for predicting the occurrence and development of AD in COVID-19 patients.

Finally, we found the interaction between several compounds and hub genes by NetworkAnalyst, among which resveratrol, genistein, and quercetin were already confirmed to have a neuroprotective effect on AD [49-54]. These compounds could be considered for the prevention and treatment of patients with COVID-19. But we also believe that taking individual protection and vaccination to avoid SARS-CoV-2 infection is the best strategy for individuals.

Conclusion

This paper developed an AD risk diagnosis model based on the common hub gene of COVID-19 patients and AD patients. According to the model and current research, COVID-19 patients are at high risk to develop AD. We highlighted that vaccination is effective and economic for preventing COVID-19. In addition, we found 4 hub genes (*ITPR1, ITPR3, ITPKB, RAPGEF3*) that can be considered as biomarkers to predict the occurrence and development of AD in COVID-19 patients. This model provides valuable strategies and tools for the prevention and treatment of COVID-19 and AD. Compounds with neuroprotective effects can be used as adjuvant therapy for COVID-19 patients.

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SUPPLEMENTARY MATERIAL

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