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ORIGINAL ARTICLE

Construction of immune/Creutzfeldt–Jakob disease-related gene coexpression network to predict biomarkers

Xiaoou Hai[1] | Jiaming Zhou[2] | Guangyan Liu[1]

[1]Department of Pathogenic Biology, College of Basic Medical Sciences, Shenyang Medical College, Shenyang, China

[2]Franklin and Marshall College, Lancaster, Pennsylvania, USA

Correspondence
Guangyan Liu, Department of Pathogenic Biology, College of Basic Medical Sciences, Shenyang Medical College, No. 146, Huanghe North Street, Shenyang, 110034, Liaoning Province, China.
Email: canjian_85@163.com

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Abstract

Background and purpose: Creutzfeldt–Jakob disease (CJD) is a transmissible spongiform encephalopathy characterized by rapid onset and high mortality. Despite considerable progress in the treatment and diagnosis of CJD, patient prognosis remains poor. Many studies have found that the immune response is associated with the pathophysiology of CJD. However, few studies have reported coexpression correlations between genes associated with CJD and the immune response. This study was undertaken to construct a network of coexpressed immune- and CJD-related genes that may reveal new biomarkers and therapeutic targets for CJD.

Methods: Gene expression data from 11 CJD patients and 10 nonneurological controls were obtained from the Gene Expression Omnibus database. High-confidence protein–protein interaction (PPI) data were downloaded from the Human Protein Reference Database, and gene expression data of immune- and CJD-associated genes were downloaded from the AmiGo16 and DisGeNET databases, respectively. An immune/CJD-related expression network was constructed based on Pearson correlation coefficients and PPI networks, and a CJD-directed neighbour coexpression network was extracted, in which we compared the gene expression patterns and correlations between different groups. The samples were classified using CJD-specific modules, and differentially expressed genes (DEGs) between the CJD and nonneurological controls groups were identified within the CJD-specific modules. Further functional analysis was performed using Kyoto Encyclopaedia of Genes and Genomes (KEGG) enrichment analysis of genes in each CJD-specific module.

Results: We constructed an immune/CJD-related coexpression gene network comprising 2007 nodes and 5268 edges, with immune-associated genes occupying important positions in the network. In the CJD-directed neighbour coexpression network, immune-associated genes exhibited the highest coexpression level with their interacting genes. Results from Pearson correlation analysis showed that most of the CJD-associated genes were positively correlated with immune-associated genes. Screening for CJD-specific modules identified MAPK1, CASP3, APP, MAPT, SNCA, and YWHAH, indicating a close connection between CJD and the immune response. Analyses of coexpression status and expression level of CJD-specific genes revealed a very high coexpression pattern for any two genes, with most genes being DEGs. Finally, KEGG enrichment analyses of all CJD-specific genes showed that the pathophysiology of CJD is closely related to infection and the immune response.

Conclusions: Our coexpression network analysis revealed a close connection between CJD- and immune-associated genes, and we identified six CJD-specific modules. Biological function analysis of CJD-specific module genes revealed that immune responses are associated with CJD pathophysiology and may provide novel diagnostic and therapeutic biomarkers for this disease.

KEYWORDS
Creutzfeldt–Jakob disease, diagnostic biomarkers, immune response, therapeutic biomarkers

INTRODUCTION

Creutzfeldt–Jakob disease (CJD) is a transmissible central nervous system (CNS) disease that is fatal. It is characterized by rapid progressive dementia and focal lesions of the cerebral cortex, basal ganglia, and spinal cord. CJD is the most common human prion disease, with a mortality rate of up to 100% [1]. The main CJD neuropathies are cavernous changes, neuronal loss, astrocyte proliferation, microglial activation, and PrP^{Sc} (protease-resistant isoform of the host-encoded cellular prion protein (PrPC), named PrP^{Sc}) deposition [2,3]. CJD is a rare disease, with 1–2 cases per million people reported annually, and an average age of onset of 60.7 years [4]. CJD cases can be divided into sporadic CJD (sCJD), familial (characterized by an autosomal dominant mutation in the prion protein [*PRNP*] gene), iatrogenic, and variant. Of these, sCJD is the most common form, accounting for 85% of CJD cases [5]. Although CJD is rare, the lack of treatments for this disease and its rapid progression to death have a profound effect on patients and their families, and there is a clear need to identify therapeutic strategies to combat CJD.

The diagnosis of CJD is one of the most challenging aspects of this disease, due to its diverse symptoms. Histopathological confirmation by brain biopsy is the gold standard for diagnosis. However, the risks involved in obtaining brain tissue have led to the development of a brain-free diagnostic method that combines imaging, analysis of cerebrospinal fluid (CSF) for biomarkers, and observation of clinical symptoms. However, discordant studies have led to controversies about the clinical value of some established surrogate biomarkers [6]. The identification of additional representative biomarkers will therefore help to improve the diagnosis and treatment of CJD.

Most CJD cases feature a large number of activated cell inflammatory responses. This inflammatory reaction in the brain, characterized by microglia strongly expressing major histocompatibility complex Class II and leukocyte antigen, represents a special form of the innate immune response [7]. Studies have revealed an increase in expression of pro- and anti-inflammatory cytokines and immune response mediators in the CSF of CJD patients [8]. In addition, the nuclear factor kappa B/IκB kinase and Janus kinase/signal transducer and activator of transcription signalling pathways are activated in sCJD mice [9]. Furthermore, a recent study found that prostaglandin-endoperoxide synthase 2 (known as PTGS2 or COX-2) is involved in prion-induced neuroinflammation and microglial activation in CJD patients [10,11]. It has thus become apparent that the pathogenesis of CJD includes activation of multiple immune-related signalling pathways, and investigating the status of immune-related genes in CJD may contribute to the diagnosis and treatment of this disease. So far, few studies have reported on the research of CJD and immune-related gene coexpression.

In this study, we used bioinformatic analysis to construct an immune/CJD-related coexpression gene network, with the aim of screening potential biomarkers for the diagnosis and treatment of CJD. The outcomes of this study help to address the dearth of information with respect to immune-related gene expression in CJD, and lay a theoretical foundation to expand clinical strategies for treating CJD.

MATERIALS AND METHODS

Data collections

We collected the transcriptome data of frontal cortices in CJD patients (10 samples) and nonneurological controls (11 samples) from the Gene Expression Omnibus (GEO) database (accession number: GSE124571). Moreover, the high-confidence protein–protein interaction (PPI) data were downloaded from the Human Protein Reference Database (HPRD; http://hprd.org/download) [12]. Next, we obtained 3279 immune-associated genes in *Homo sapiens* from AmiGo16 (http://amigo.geneontology.org/amigo/search/bioentity) by inputting the keywords "organism," "*Homo sapiens*," "type," and "protein" [13]. Furthermore, we downloaded the CJD-associated genes from the DisGeNET database (https://www.disgenet.org/search).

Construction of the immune/CJD-related coexpression network

To analyse the correlation of gene expression between CJD patients and nonneurological controls, Pearson correlation analysis of gene expression between any two gene pairs was performed in the R package "psych" (v1.9.12.31) [14]. Next, the genes with Pearson coefficient value > 0.7 and false discovery rate < 0.05 were screened to construct the initial gene coexpression network. In addition, to

further observe the coexpression correlation of genes, the initial gene coexpression network was mapped to the PPI network obtained from the HPRD, and only the common networks were preserved to construct an immune/CJD-related coexpression network. Meanwhile, all genes were divided into four groups—CJD-associated genes, immune- and CJD-associated genes (the intersection of CJD-associated genes and immune-associated genes), immune-associated genes, and other genes—and the number of genes in each group was determined. Finally, the common networks were visualized via Cytoscape (v3.8.0).

Construction of the CJD-directed neighbour coexpression network

First, the first neighbour of CJD-associated genes and immune- and CJD-associated genes, which belong to CJD-related genes, was selected for the CJD-directed neighbour coexpression network. Second, correlation between two genes in each group was calculated to obtain the coexpression correlation coefficients. Based on the correlation coefficients between two genes, the cumulative distribution function (CDF) was performed in the four groups to analyse the gene expression pattern and the correlation of different groups. In addition, the Wilcoxon rank-sum test was used to compare the coexpression correlation coefficients between two gene groups. Furthermore, the CJD-related genes and their interacting genes were collected to construct the network, and a heatmap was used show the degree of correlation via the R package "heatmap" [15].

Screening of the CJD-specific modules

To identify the important modules associated with CJD, the CJD-directed neighbour coexpression network was used as the input file to perform clustering analysis using the GraphWeb tool. The modules whose central node is CJD-related genes were used as output modules. Next, the R package "Consensus ClusterPlus" (v1.52.0) [16] was used to carry out the synonymous cluster analysis based on the expression of modules genes, and the parameters were set to maxK = 8, reps = 1000, and clusterAlg = hc. Moreover, we selected the appropriate *K* value according to the clustering results and extracted their expression data in the GSE124571 dataset. Additionally, the R package "pheatmap" (v1.0.12) was used to draw the expression heatmap.

Gene expression patterns in CJD-specific modules

To explore the genes' correlation in CJD-specific modules, the Pearson correlation coefficients between each gene pair in the module were calculated, and a correlation heatmap was drawn via the R package "pheatmap." Then, the gene expression patterns between CJD patients and nonneurological controls in each module were analysed. That is, the differentially expressed genes (DEGs) in each module were identified between different samples (CJDs vs. nonneurological controls) with the threshold of absolute log2 fold-change > 1 and a $p < 0.05$ with the R package "limma" [17].

Kyoto Encyclopaedia of Genes and Genomes pathway enrichment analysis

The enrichment analysis of genes in each module were performed in the R package "clusterProfiler" (v3.16.0) [18] with the threshold of $p = 0.05$ and $q = 0.05$.

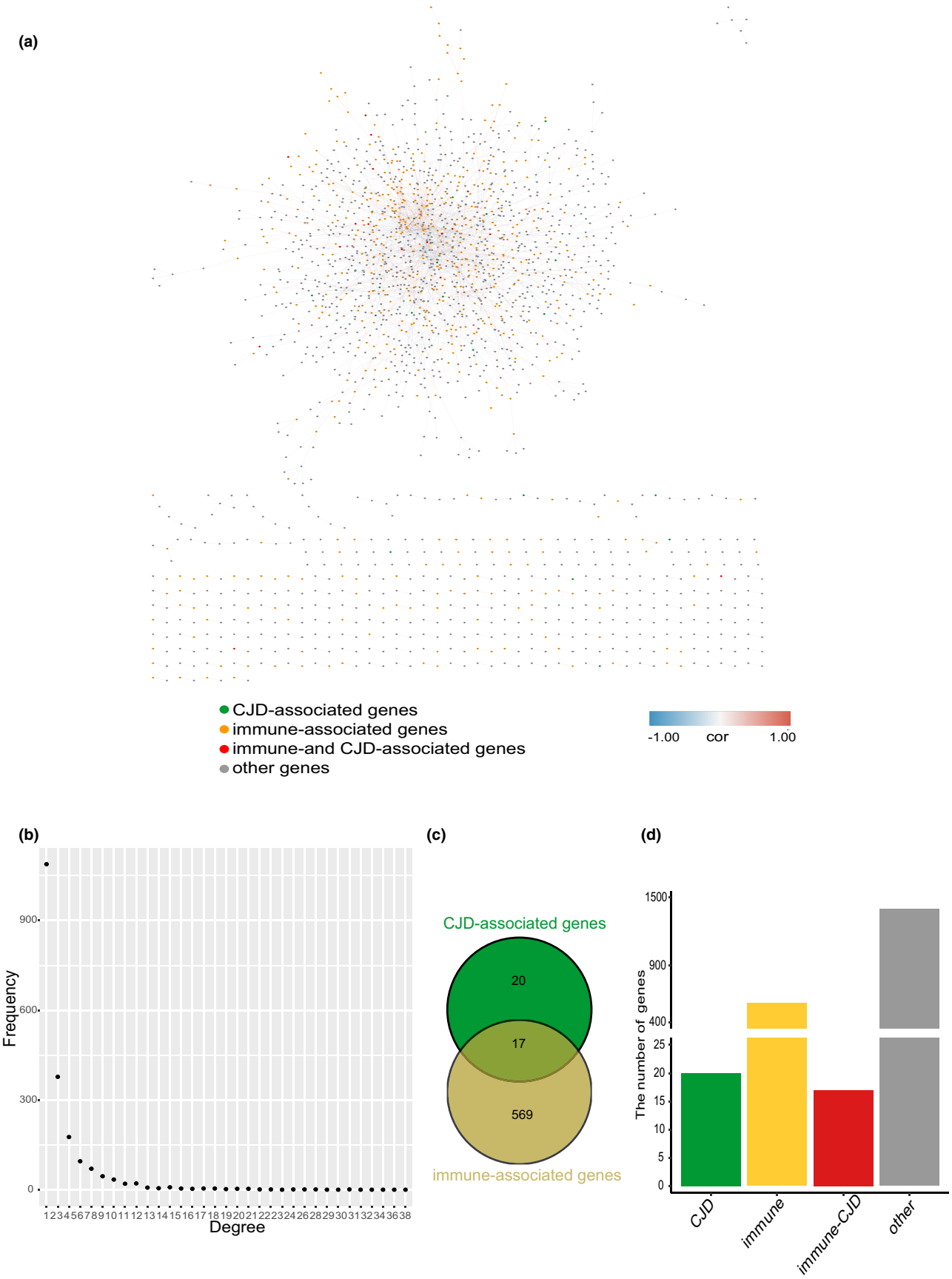
Statistical analysis

All statistical analyses were performed in R (v3.5.2), and $p < 0.05$ was considered statistically significant. Pearson chi-squared test and Wilcoxon rank-sum test were employed for comparison of variables.

RESULTS

Immune-associated genes may play a key role in CJD

Many studies have found that the immune response is associated with the pathophysiology of CJD. Based on the importance of the immune response in CJD, immune-associated genes were analysed. A total of 3279 immune-associated genes were identified from the Amigo database, including *KLF2*, *ARRB2*, *LGALS7*, *PRDX2*, *RABL3*, *RAB44*, *JAK1*, *BDKRB1*, *IKBKE*, and *PHPT1*. Similarly, a total of 137 CJD-associated genes were identified from the DisGeNET database, including *PRNP*, *MSL3P1*, *SNORA16B*, *CPED1*, and *ALDH1A1*. Using these data, we constructed an immune/CJD-related coexpression network based on the initial gene coexpression and PPI networks, which is shown in Figure 1a. In the network, the green, orange, red, and grey nodes represent CJD-associated genes, immune-associated genes, immune- and CJD-associated genes, and other genes, respectively. The network contained a total of 2007 nodes and 5268 edges. Our analysis of the degree distributions of all genes revealed that they were scale-free, with an R^2 value of 0.9946 (Figure 1b). Furthermore, our network analysis indicated that there were 17 genes in common between the immune- and CJD-associated gene groups, such as *MAPT*, *SNCA*, *APP*, *CASP3*, *MAPK3*, *MAPK1*, and *TREM2*. Twenty CJD-specific genes and 569 immune-specific genes were identified based on the Venn diagram (Figure 1c). In addition, we found that the numbers of CJD-associated genes and immune- and CJD-associated genes were similar to each other, whereas groups comprising the immune-associated and other genes were much higher (Figure 1d). The top five genes with the highest degree were *FYN*, *MAPK1*, *YWHAB*, *CALM1*, and *LYN*. Four of these genes are immune-associated genes, suggesting that the immune response may play an important role in CJD.


(a)
CJD-associated genes
immune-associated genes
immune-and CJD-associated genes
other genes
-1.00
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(b)
Frequency
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20
17
569
immune-associated genes
(d)
The number of genes
CJD
immune
immune-CJD
other

FIGURE 1 Identification of immune-/Creutzfeldt–Jakob disease (CJD)-related genes in CJD. (a) Construction of the immune- or CJD-directed neighbour coexpressed network. Different genes are represented by different colours (green: CJD-associated genes; orange: immune-associated genes; red: immune- and CJD-associated genes; grey: other genes). (b) Degree distribution of all nodes in the immune/CJD-related coexpression network. (c) Venn diagram of overlapped genes between CJD-associated genes and immune-associated genes. (d) Distinct gene numbers of different gene types

Strong coexpression pattern between immune- and CJD-associated genes

We next constructed a CJD-directed neighbour coexpression network to further analyse the correlation between CJD- and immune-associated genes (Figure 2a). The CJD-directed neighbour coexpression network is a subnetwork of the immune/CJD-related coexpression network, created by extracting CJD-associated genes and directly interacting partners. This subnetwork comprised 20 CJD-associated genes, 51 immune-associated genes, 17 immune- and CJD-associated genes, and 114 other genes. The levels of interactions between different groups were detected using the CDF, which showed that the CJD-associated genes and immune- and CJD-associated genes exhibited similar coexpression levels, whereas immune-associated genes showed the highest co-expression level with their interacting genes (Figure 2b). Similarly, the immune-associated gene group presented the maximum Pearson correlation value (Figure 2c). We also discovered that the gene expression correlations were significantly distinct between the immune-associated and the other gene groups. We subsequently constructed a coexpression network comprising only CJD-associated genes and their directly interacting genes (Figure 2d). Twenty CJD-associated genes were extracted from the network, and most of them interacted with immune-associated genes, such as *NEFL*, *CHGB*, *ATF2*, and *YWHAH*. Notably, *NEFL* showed strong coexpression with immune-associated genes, including *VIM*, *PKN1*, and *SPTAN1*. Moreover, the results of Pearson correlation analyses suggested that most of the CJD-associated genes were positively correlated with immune-associated genes, and a small number of CJD-associated genes were negatively correlated with immune-associated genes (Figure 2e). These results demonstrate that there may be complex interactions and patterns of expression between CJD- and immune-associated genes.

CJD-specific modules are special classifiers in CJD

Although we obtained one CJD-directed neighbour coexpression network, we did not know which genes and their interacting genes were closer to CJD. Using GraphWeb, we identified six modules from the network: MAPK1, CASP3, APP, MAPT, SNCA, and YWHAH. All of the key nodes in each of the six modules were CJD-related genes, including both CJD-associated genes and immune- and CJD-associated genes (Figure 3a). The number of genes in each module was variably distributed, and 37, 19, 19, 18, 17, and 10 genes were identified, respectively (Figure 3b). Moreover, all of the key nodes in the six modules interacted with immune-associated genes, especially in the MAPK1 module. Furthermore, all genes in the MAPK1, CASP3, APP, MAPT, and SNCA modules belonged to the immune- and CJD-associated genes, which further demonstrated that the immune system plays a vital role in CJD.

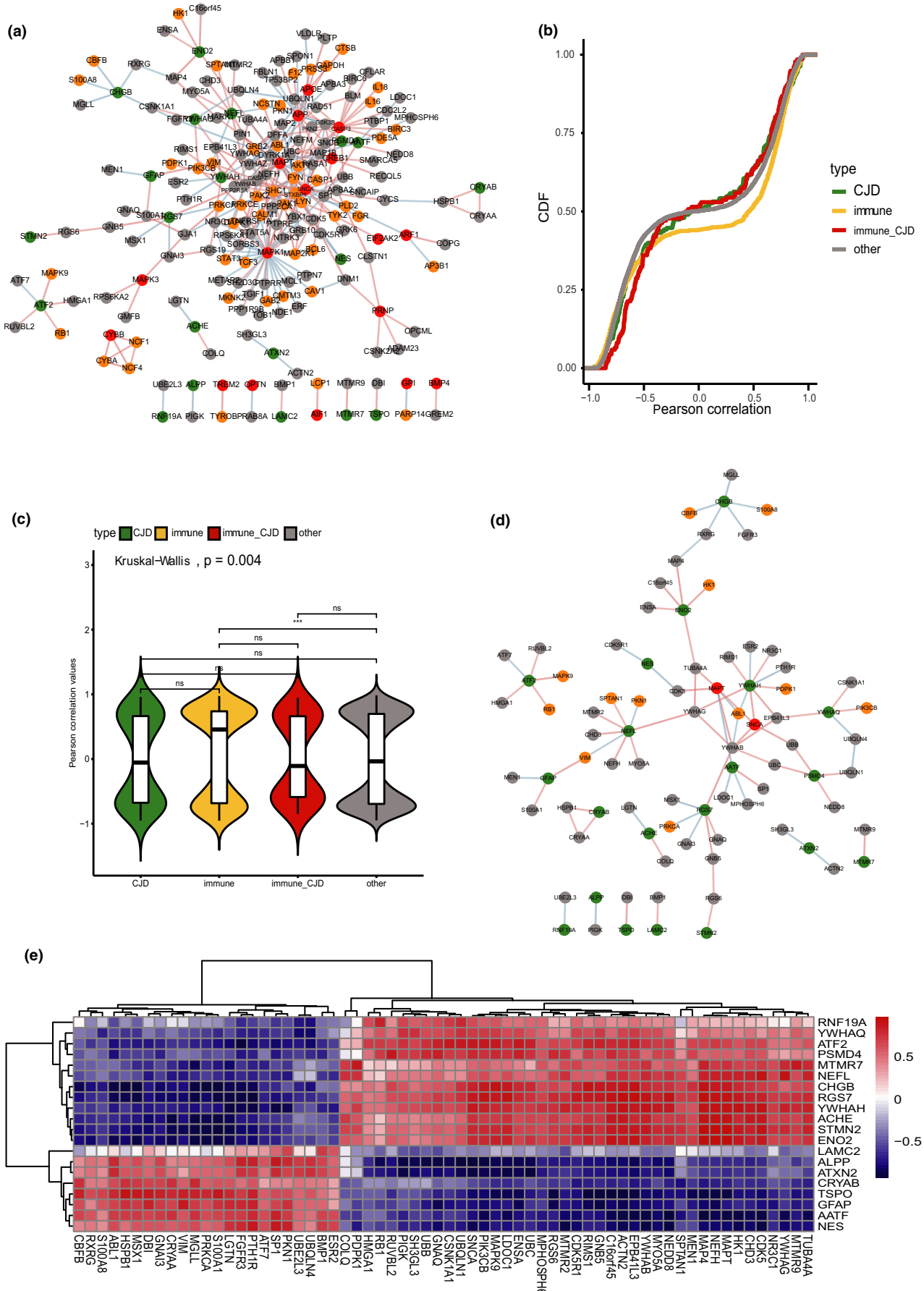
To further explore the significance of the genes associated with each module, we carried out a synonymous cluster analysis based on the gene expression data. We determined the optimum number of groups based on the CDF values and the relative change in the area under the curve of the CDF plot. Interestingly, the results showed that the samples in the MAPK1, MAPT, and SNCA modules were divided into three subtypes. The samples in the APP and YWHAH modules were each divided into four subtypes, and the CASP3 module included five subtypes (Figure 3c–f, Figure S1).

Coexpression status and expression level of CJD-specific genes

We further dissected the coexpression status of each module, and the results showed a very high coexpression pattern for any two genes. We then screened for gene pairs with absolute coexpression values of >0.5. In addition, we selected for coexpression correlation coefficients of >0.7 in each module, with the minimum proportion being 32.6% in the APP module and the maximum proportion being 48.95% in the CASP3 module. The SNCA and YWHAH modules showed the most similar coexpression patterns (Figure 4a,b, Figure S2).

Next, we identified the DEGs between patients with CJD and nonneurological controls, and we found that the gene expression profile differed between CJD and control samples in each module. The majority of genes in the SNCA and YWHAH modules were downregulated (Figure 4c,d, Figure S2).

To determine which DEGs in the six CJD-specific modules might play an important role in the disease, we mapped the neighbour co-expression network including CJD-associated genes, immune- and CJD-associated genes, and immune-associated genes (Figure 4e). The results showed that *SNCA* was coexpressed with three immune- and CJD-associated genes, three immune-associated genes, and one CJD-associated gene, and five, four, three, and three genes were coexpressed with *MAPT*, *APP*, *MAPK1*, and *FYN*, respectively. Interestingly, among the genes coexpressed with *SNCA*, *MAPT*, *APP*, *MAPK1*, and *YWHAH* were key nodes in the six modules, and they were all downregulated. The above analysis suggests that the key nodes of the six CJD-specific modules may be potential biomarkers of CJD.


(a)
(b)
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Pearson correlation
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(c)
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(d)
(e)
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YWHAQ
ATF2
PSMD4
MTMR7
NEFL
CHGB
RGS7
YWHAH
ACHE
STMN2
ENO2
LAMC2
ALPP
ATXN2
CRYAB
TSPO
GFAP
AATF
NES

FIGURE 2 Coexpression of immune- and Creutzfeldt–Jakob disease (CJD)-associated genes in CJD. (a) Construction of the CJD-directed neighbour coexpressed network (CDNC network). Different genes are represented by different colours (green: CJD-associated genes; orange: immune-associated genes; red: immune- and CJD-associated genes genes; grey: other genes). (b) Cumulative distribution curves of Pearson correlations for different gene types. (c) Violin plots of Pearson correlations for different gene types; ***$p < 0.001$. (d) The subnetwork of CJD-associated genes and their directly interacted genes was extracted from the CDNC network. (e) Heatmap of correlation between CJD-associated genes and immune-associated genes. CDF, cumulative distribution function; ns, not significant

Biological function analysis of CJD-specific modules shows that it is closely related to infection and immune response

To further investigate the biological functions of key nodes and their interacting genes in CJD-specific modules, we performed Kyoto Encyclopaedia of Genes and Genomes (KEGG) enrichment analyses using the R package "clusterProfiler." The results revealed the following conjoined pathways to be enriched in the MAPK1 and APP modules: "ErbB signalling pathway" (p = 2.91 E-07, p = 1.08 E-05, respectively), "neurotrophin signalling pathway" (p = 4.08 E-05, p = 4.09 E-05), "chronic myeloid leukaemia" (p = 4.55 E-06, p = 0.0003), and "growth hormone synthesis, secretion, and action" (p = 4.08 E-05, p = 0.001). The term "Alzheimer disease" (p = 4.33 E-08, p = 3.26 E-06, p = 3.17 E-05) was enriched in the APP, MAPT, and SNCA modules, respectively; "Parkinson disease" (p = 0.001, p = 3.31 E-06), "hepatitis C" (p = 0.003, p = 0.0002), and "oocyte meiosis" (p = 3.60 E-06, p = 7.33 E-05) were enriched in MAPT and SNCA; and "cell cycle" (p = 0.002, p = 0.0003) was enriched in the MAPT and YWHAH modules (Figure 5a–f). Interestingly, we

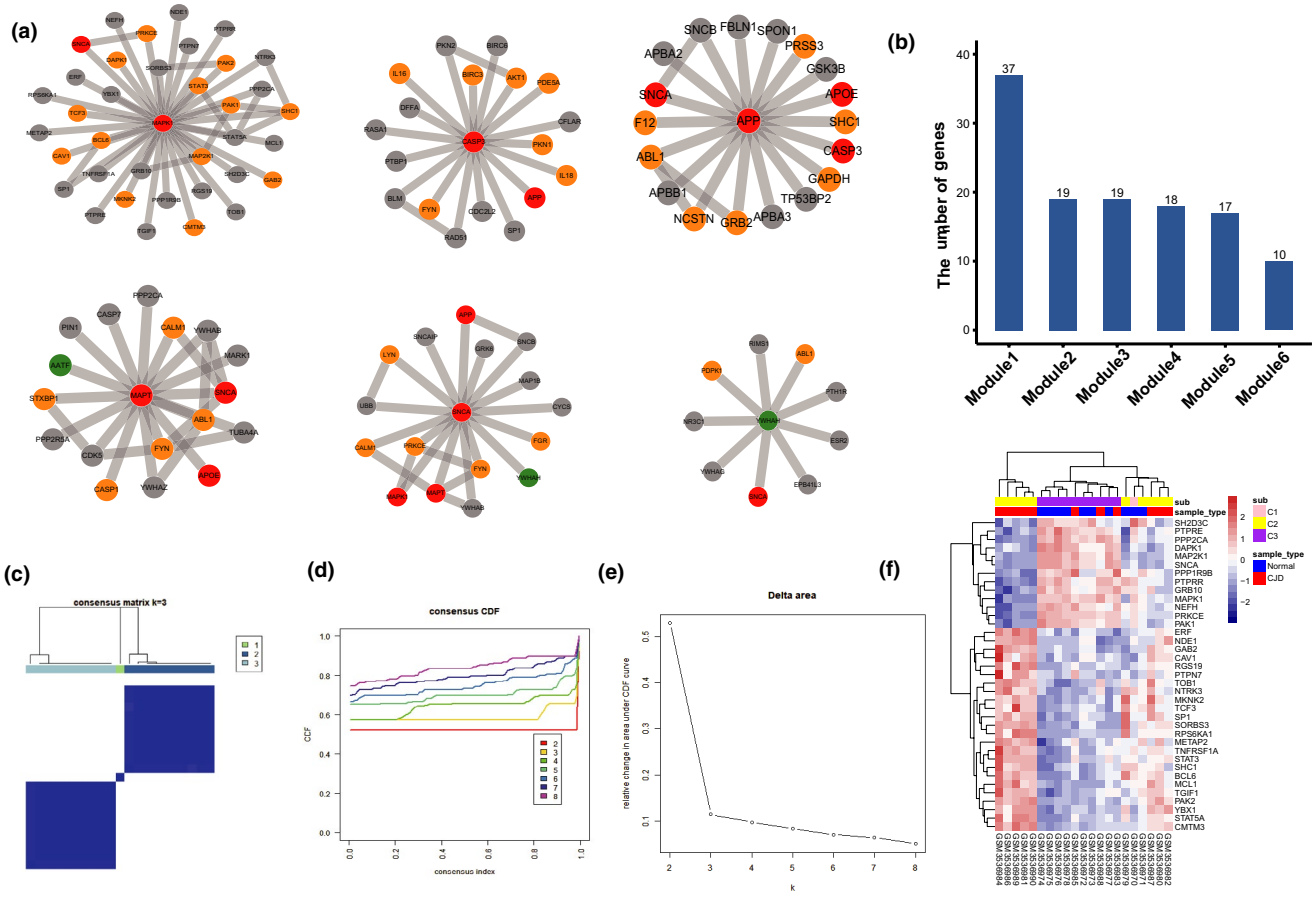

FIGURE 3 Clustering immune-associated subnetwork in Creutzfeldt–Jakob disease (CJD). (a) The critical modules extracted from the immune- or CJD-directed neighbour coexpressed network, and different types of gene were marked using different colours (green: CJD-associated genes; orange: immune-associated genes; red: immune- and CJD-associated genes; grey: other genes). (b) Distinct gene numbers of genes in different modules. (c) Cumulative distribution curves of the consensus index. (d) Relative change in area under the cumulative distribution function (CDF) curve of different group numbers. (e) Consensus cluster heatmap of samples. (f) Heatmap of gene expression. Subtype (sub) refers to the group type classified by the consensus cluster method, and sample_type refers to the disease status of the samples

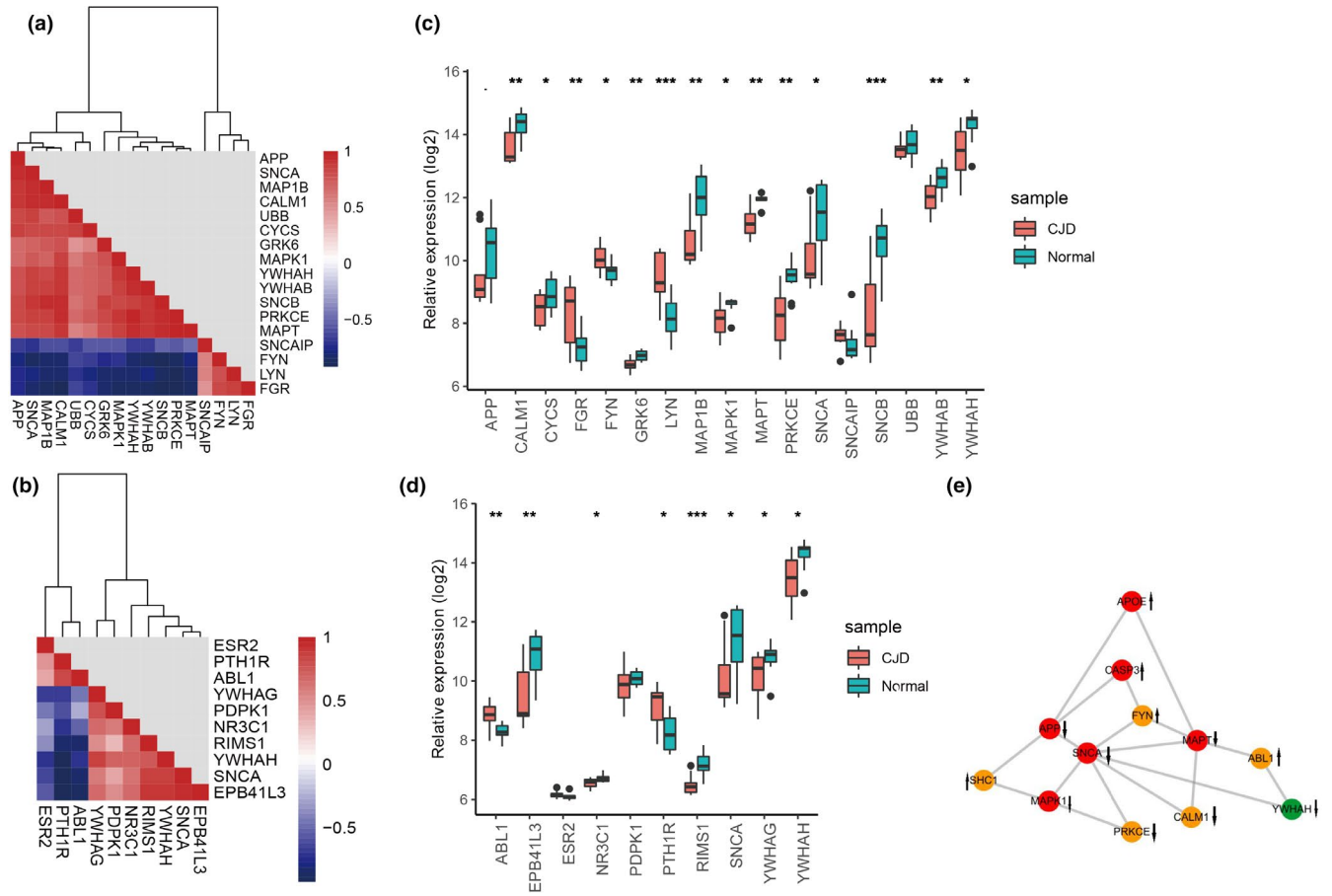

FIGURE 4 Correlation between immune- and Creutzfeldt–Jakob disease (CJD)-associated genes in clusters. (a, b) Coexpression heatmaps of genes in the SNCA and YWHAH module clusters. (c, d) The gene expression levels of the SNCA and YWHAH module between CJD and normal samples. Significantly differentially expressed genes have been labelled (*, **, and *** indicate significance level of 0.05, 0.01, and 0.001, respectively). (e) The coexpression network constructed by differentially expressed genes in a CJD-specific module

observed that CASP3 did not have any enrichment pathways that overlapped with the other five modules. KEGG enrichment analysis suggested that some of the key pathways in the CASP3 module were associated with infection, such as "*Salmonella* infection" ($p = 5.08$ E-05), "*Yersinia* infection" ($p = 0.0001$), and "toxoplasmosis" ($p = 0.002$). In addition, some KEGG pathways related to apoptosis were abundant, including "apoptosis" ($p = 6.79$ E-06), "apoptosis–multiple species" ($p = 3.76$ E-05), and "TNF signalling pathway" ($p = 7.41$ E-05).

To gain a better understanding of how module genes drive CJD progression, we next analysed the enrichment pathways of genes in all modules by biological function enrichment analysis (Figure 5g). The top 10 results of enrichment pathways revealed by KEGG analysis included "hepatitis C" ($p = 1.22$ E-11), "apoptosis" ($p = 3.84$ E-10), "ErbB signalling pathway" ($p = 6.32$ E-09), "Alzheimer disease" ($p = 7.45$ E-09), "neurotrophin signalling pathway" ($p = 1.39$ E-08), "prolactin signalling pathway" ($p = 1.72$ E-08), "pathogenic *Escherichia coli* infection" ($p = 2.66$ E-08), "*Salmonella* infection" ($p = 6.16$ E-08), "sphingolipid signalling pathway" ($p = 1.66$ E-07), and "FcεRI signalling pathway" ($p = 2.29$ E-07). Two important immune signalling pathways were identified based on the enrichment pathway of the model (Figure 6).

Collectively, these results indicate that the pathogenesis of CJD is closely related to infection and to the immune response.

DISCUSSION

In recent years, the rapid development of molecular biotechnology has led to great progress in our understanding of CJD. Although studies on microglial immune activation in CJD have been reported, there are no studies that have described immune-associated genes as CJD biomarkers. In this study, we used a PPI network from the HPRD database, immune-associated genes from the Amigo database, and CJD-associated genes from the DisGeNET database to construct a CJD-specific coexpression network of immune- and disease-related genes. The coexpression relationship between immune- and CJD-associated genes was confirmed by layer-by-layer network screening. Using GraphWeb, we identified key disease modules and genes, and performed KEGG pathway enrichment analyses on them.

The results of the immune/CJD-related coexpression network analysis revealed that, with the exception of *YWHAB*, the other four genes with the highest connectivity were all immune-associated genes. This indicates that immune-associated genes play an

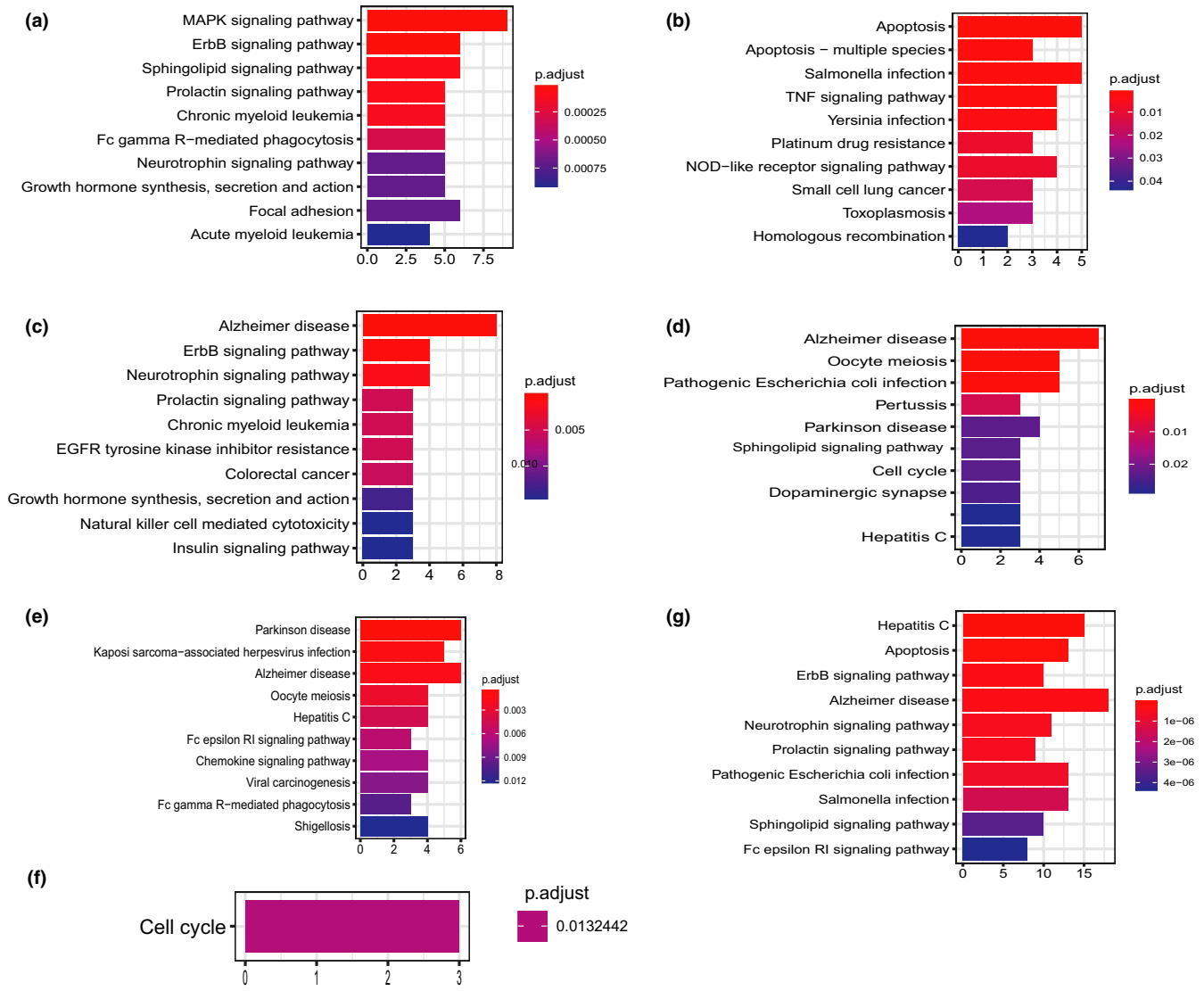

FIGURE 5 Biological function analysis of the significant immune-associated clusters in Creutzfeldt–Jakob disease (CJD). (a–f) Bar chart of KEGG pathway enrichment for the MAPK1, CASP3, APP, MAPT, SNCA, and YWHAH module genes, respectively. (g) The KEGG enrichment results for all module genes. KEGG, Kyoto Encyclopedia of Genes and Genomes

important role in CJD. Further analysis of 20 CJD-associated genes extracted from a CJD-directed neighbour coexpression network showed that the level of coexpression between CJD- and immune-associated genes was high, especially with regard to *NEFL*, *VIM*, *PKN1*, *SPTAN1*, and other immune-associated genes, which were expressed differently in CJD and normal samples.

Six modules (MAPK1, CASP3, APP, MAPT, SNCA, and YWHAH) were identified using GraphWeb to determine which genes were more closely related to CJD. It is worth noting that the five modules other than YWHAH included immune- and CJD-associated genes. In particular, the MAPK1 module had the largest number of immune-associated genes (14). Of the six modules, SNCA and APP contained the highest proportion of immune genes (52.9% and 52.6%, respectively). In particular, *SNCA* directly interacts with immune-associated genes such as *APP*, *MAPT*, *MAPK1*, *FYN*, *CALM1*, and *LYN*. Of these, *APP*, *MAPT*, and *MAPK1* are immune- and CJD-associated genes and are among the key nodes of the six modules. *FYN*, *CALM1*, and *LYN* were the immune-associated genes with the highest expression levels. We therefore speculate that the SNCA module plays an important role in the occurrence and development of CJD.

SNCA (synuclein alpha) encodes an α-synaptic soluble nuclear protein that is expressed in the presynaptic space and around the nucleus in CNS neurons, where it regulates the transport of synaptic vesicles and controls the release of neurotransmitters. A recent study found that the amino acid residues at positions 67–78 in α-synuclein form a tilted peptide, which plays a key role in the formation of amyloid fibres and toxicity in nerve cells, and is closely associated with neurodegenerative diseases [19]. Studies have determined that abnormal protein deposition with neurotoxicity is caused by excessive increases in cell excitability or destruction of axon transport and microtubule function through the tyrosine protein kinase Fyn signalling pathway [20]. CJD has a similar pathogenesis to neurodegenerative diseases, such as the formation and deposition of abnormal protein conformations, synaptic dysfunction, abnormal and missing

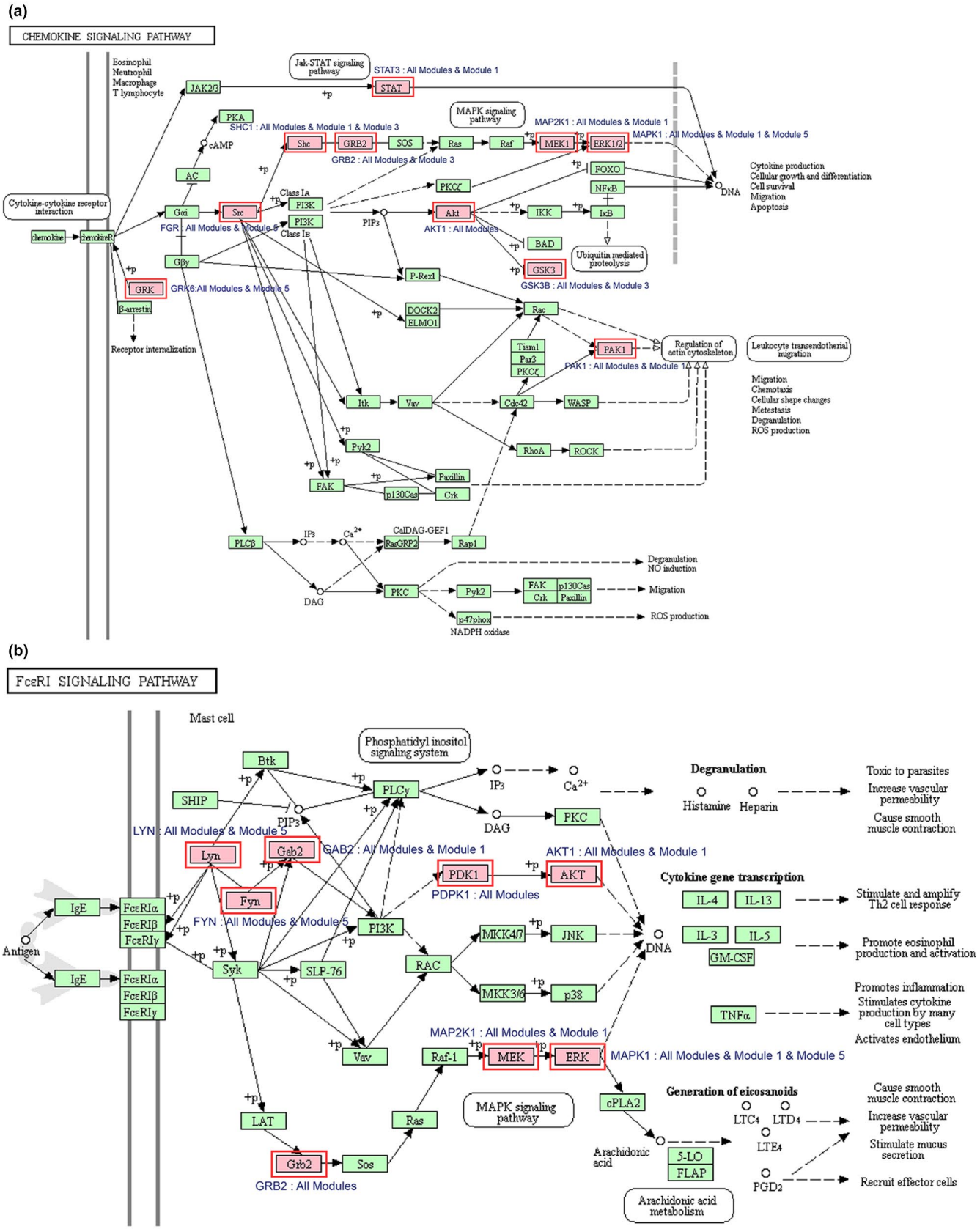

FIGURE 6 KEGG pathway enrichment analysis in signalling pathways. (a) The map of the chemokine signalling pathway. (b) The map of the FcεR1 signalling pathway. KEGG, Encyclopedia of Genes and Genomes

autophagy, and inflammation [21]. Our biofunctional analysis also confirmed that the pathways enriched in CJD are closely related to neurodegenerative diseases. The KEGG enrichment analysis of the SNCA module revealed several important immune-related pathways, including FcεRI signalling, chemokine signalling, and FcγR-mediated phagocytosis. The FcεRI receptor belongs to a protein family that includes multiple immune recognition receptors, and the cross-linking of FcεRs stimulates the factors *Lyn* and *Sky* to activate downstream MAPK signalling pathways [22]

MAPK (mitogen-activated protein kinase) is widely expressed in the CNS. Stimulated by various extracellular factors, *MAPK* participates in the pathological process of neurodegenerative diseases by regulating cell proliferation, differentiation, growth, and apoptosis. The MAPK pathway has been shown to be closely involved in neuronal apoptosis in neurodegenerative diseases [22]. In vitro studies have reported that activated MAPK induces hyperphosphorylation of the tau protein, initiates apoptosis and other mechanisms, and participates in the development of Alzheimer disease [23]. The neuronal microtubule-associated protein tau (encoded by *MAPT*) is abnormally expressed in the CSF of patients with CJD, and the determination of total and phosphorylated tau protein in CSF are valuable indicators in the diagnosis and differential diagnosis of CJD [24–26].

Our biological function analyses showed that immune/CJD-related genes were enriched in pathways associated with infection and the immune response. In recent years, an increasing number of epidemiological and experimental studies have shown that persistent infection in the brain can lead to protein misfolding and aggregation, resulting in oxidative stress injury, abnormal autophagy, apoptosis, and programmed necrosis, ultimately leading to neuronal damage and promoting the development of neurodegenerative diseases [27–33]. Continuous infection and abnormal protein deposition may lead to severe microglial activation. Activated microglia trigger chemotaxis and phagocytosis through mer receptor tyrosine kinases and release cytokines, proteases, and superoxides to remove harmful substances and protect neurons, thereby triggering neuroinflammation [34,35]. Previous studies have confirmed an increase in expression of pro- and anti-inflammatory cytokines and immune mediators in the CSF and brains of sCJD patients [8,36]. The sustained and excessive activation of microglia prevents nerve repair and leads to synaptic and oxidative damage, and mitochondrial dysfunction [37], which may play a role in inducing and promoting the neurodegeneration of CJD.

CONCLUSION

The analyses from our study shown that immune- and CJD-associated genes are strongly correlated not only at a network level, but also in their patterns of expression. The functional enrichment analysis of each module showed that they may be closely related to the pathogenesis, infection, diagnosis, and treatment of CJD. KEGG analysis of the module genes also identified key nodes of the six CJD-specific modules, which may have potential as clinical biomarkers and therapeutic targets, and are worth exploring in future basic research and clinical studies.

There were a few limitations to our study. Due to the low incidence of CJD, this study was based on clinical data from a very small number of CJD samples. Owing to the difficulty in obtaining CJD samples, the study of its aetiology is currently limited to bioinformatic methods. Further experiments are needed in the future to experimentally confirm the results of this study.

CONFLICT OF INTEREST

The authors declare no conflict of interest. AmiGo16, DisGeNET, HPRD, and GEO are public databases. All patient data in these databases were collected by informed consent and are anonymized. As our study is based on this open-source data, no ethics approval was required.

AUTHOR CONTRIBUTIONS

Xiaoou Hai: Data curation (lead), formal analysis (lead), investigation (lead), methodology (lead), project administration (lead), resources (lead), software (lead), supervision (lead), validation (lead), visualization (lead), writing–original draft (lead). **Jiaming Zhou:** Writing–original draft (equal), writing–review & editing (equal). **Guangyan Liu:** Conceptualization (lead), funding acquisition (lead), project administration (lead).

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are included in this published article and its supplementary information file. The original data are available upon reasonable request to the corresponding author.

ORCID

Guangyan Liu https://orcid.org/0000-0002-5891-1830

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

Fig S1

Fig S2

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