Journal of Molecular Neuroscience
https://doi.org/10.1007/s12031-021-01809-7

Peripheral Blood Biomarkers CXCL12 and TNFRSF13C Associate with Cerebrospinal Fluid Biomarkers and Infiltrating Immune Cells in Alzheimer Disease

Qianqian Wu[1] · **Wei Kong**[1] · **Shuaiqun Wang**[1]

Received: 9 October 2020 / Accepted: 2 February 2021
© The Author(s), under exclusive licence to Springer Science+Business Media, LLC part of Springer Nature 2021

Abstract
Neuroinflammation-induced neurodegeneration and immune cell infiltration are two features of Alzheimer disease (AD). This study aimed to identify potential peripheral biomarkers that interact with cerebrospinal fluid (CSF) and infiltrating immune cells in AD. Blood and CSF data were downloaded from the Alzheimer's disease Neuroimaging Initiative database. We identified differentially expressed genes (DEGs) in AD and assessed infiltrating immune cells using the Immune Cell Abundance Identifier (ImmuCellAI) algorithm. Blood-brain barrier (BBB) and immune-related genes were identified from medical databases, and common genes were used to construct a protein-protein interaction network (PPI). Potential biomarkers reflecting the clinical features of AD were screened using Pearson correlations and logistic regression analysis. We identified 210 DEGs in the AD group. ImmuCellAI indicated that blood samples from patients with AD had a higher abundance of exhausted T (Tex; 0.196 vs. 0.132) and induced regulatory T (iTreg; 0.180 vs. 0.137) cells than controls. Thirty-two genes overlapped between the BBB and immune-related genes, and 27 genes in the PPI network were associated with eight pathways, including the cytokine-cytokine receptor interaction pathway (hsa04060) and the chemokine signaling pathway (hsa04062). Pearson correlations showed that five genes were associated with the CSF biomarkers, Aβ, total, and phosphorylated tau. Logistics analysis showed that the B cell-associated genes, *CXCL12* and *TNFRSF13C*, were independent risk factors for AD diagnosis. Peripheral *CXCL12* and *TNFRSF13C* genes that correlated with immune cell infiltration in AD might serve as easily accessible biomarkers for the early diagnosis of AD.

Keywords Alzheimer disease · Cerebrospinal fluid biomarkers · Immune cell infiltration · Immune-related genes

Introduction

Alzheimer disease (AD) is a progressive and irreversible neurological degenerative disease that has a slow onset and ultimately leads to neuron death. This disease is clinically characterized by manifestations of dementia, including memory impairment, loss of recognition, impaired visual skills, and executive and behavioral dysfunction. AD affects ~30% of the population aged > 85 years and accounts for 60–70% cases of dementia in elderly populations (Sawikr et al. 2017). The prevalence of this disease increases with age, but the age of onset of AD can be quite early because the interval between onset to symptomatic presentation can be longer than 10 years (McDade et al. 2018; Mietelska-Porowska and Wojda 2017; Quiroz et al. 2018; Sawikr et al. 2017). Most patients are diagnosed at the late stage of this disease using biomarkers and imaging or cerebrospinal fluid (CSF), and only the symptoms are treated. Accordingly, AD is a leading medical concern because easily accessible and reliable biomarkers for its early detection have not been established.

The pathogenesis of AD is linked to immunological mechanisms, and neuroinflammation-induced neurodegeneration is an important feature of AD (Janssen et al. 2016; Ortiz et al. 2017; Regen et al. 2017). Increasing evidence shows that neuroinflammation is a major contributor to AD progression (Calsolaro and Edison 2016; Heneka et al. 2015; Ortiz et al. 2017; Regen et al. 2017). In light of neuroinflammation, much focus has centered on

Qianqian Wu and Wei Kong are co-first authors of this paper.

✉ Wei Kong
weikong@shmtu.edu.cn

[1] College of Information Engineering, Shanghai Maritime University, Shanghai 201306, People's Republic of China

Published online: 09 March 2021

the activation of brain glial cells in the pathogenesis of AD (Mietelska-Porowska and Wojda 2017). The activation of glial cells in the central nervous system (CNS) stimulates the deposition and accumulation of amyloid-β (Aβ) and hyperphosphorylated tau, the production of reactive oxygen species (ROS), and the production of a series of proinflammatory cytokines and molecules including interleukin-1β (IL-1β), IL-6, tumor necrosis factor-α (TNF-α), and cyclooxygenase 2 (COX-2) (Higham et al. 2019; Regen et al. 2017; Sawikr et al. 2017; Singhal et al. 2014). Aβ can directly induce neuronal cytotoxicity, death, and impairment or can do so indirectly by elevating ROS production and IL-1β released from neurons, astrocytes, and microglia (Medeiros and LaFerla 2013; Singhal et al. 2014). However, AD is mainly diagnosed clinically based on finding elevated and decreased Aβ in plaques and CSF, respectively, and excessive total-tau (T-tau) and phosphorylated tau (P-tau) in plaques and CSF. The activation of glial cells in the AD brain can only be confirmed postmortem. Biomarkers in peripheral blood that interact with those in CSF and infiltrating immune cells are urgently needed to diagnose AD.

T cells are able to target Aβ plaques, suggesting that T cells migrate from the blood to the CNS through the blood-brain barrier (BBB) or the blood-cerebrospinal fluid barrier (Fisher et al. 2010; González and Pacheco 2014; Mietelska-Porowska and Wojda 2017; Rezai-Zadeh et al. 2009). Chemokines secreted by brain cells can recruit circulating immune cells from the peripheral blood to infiltrate the CNS (Prinz and Priller 2017; Rezai-Zadeh et al. 2009). During AD pathogenesis and progression, degenerating neurons and enhanced Aβ deposition activate glial cells, astrocytes, and microglia by binding to and activating toll-like receptors (TLR) and mitogen-activated protein kinases (MAPK). These responses subsequently lead to the secretion of inflammatory cytokines and chemokines in microglial cells and the recruitment of blood-derived immune cells (Medeiros and LaFerla 2013; Singhal et al. 2014). The Immune Cell Abundance Identifier (ImmuCellAI) algorithm can reliably estimate the infiltrative levels of 24 immune cells (Miao et al. 2019). The fact that ImmuCellAI estimates are consistent with flow cytometry data suggests that ImmuCellAI could offer a promising prognostic signature for human cancers. Although suitable for application to RNA-Seq and microarray expression data derived from blood or tissue samples (Miao et al. 2019), ImmuCellAI is presently applied only to tuberculosis and human cancers (Mei et al. 2020; Shi and Qi 2020; Song et al. 2020). Furthermore, less is understood about interactions among peripheral blood, BBB damage, neuroinflammation, and immune cell infiltration during AD progression.

Numerous potential genetic factors associated with the pathogenesis, diagnosis, and prognosis of AD have been identified (Wang and Wang 2020; Yuen et al. 2020; Zamanian Azodi et al. 2020). These studies identified differentially expressed genes (DEGs) in patients with AD and identified potential hub genes in AD using bioinformatic methods. However, clinical verification is lacking (Yuen et al. 2020). Reduced Aβ clearance might be associated with genes that encode Fyn and epidermal growth factor receptor in AD pathogenesis (Yuen et al. 2020). However, potential peripheral blood biomarkers that might interact with biomarkers in CSF during the pathogenesis or diagnosis of AD have not been reported.

We therefore aimed to identify interactions among peripheral blood, BBB injury, neuroinflammation, and infiltration of immune cells in patients with AD. We analyzed and integrated public data about peripheral blood genes, immune cell infiltration, and CSF biomarkers for AD using bioinformatics methods. We identified common genes between DEGs in peripheral blood and genes related to immune, as well as genes associated with CSF biomarkers, including T-tau, P-tau, and Aβ. Identifying peripheral biomarkers that link interactions between the brain and blood might improve our understanding of the etiology of AD.

Materials and Methods

Data Collection

We downloaded peripheral blood, CSF, and plasma data of 304 patients with AD, early and late mild cognitive impairment, and elderly persons without cognitive impairment (controls; CN) from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (http://adni.loni.usc.edu/).

Abundance of Infiltrating Immune Cells in the Blood Samples

ImmuCellAI (http://bioinfo.life.hust.edu.cn/ImmuCellAI#!/) predicts responses of patients to immune checkpoint blockade therapy and estimates the abundance and differences in infiltration by the following 24 types of immune cells (Miao et al. 2019): B, CD4 naïve, T, CD8 naïve, CD8 T, dendritic (DC), induced regulatory T (iTreg), mucosal-associated invariant T (MAIT), natural killer, natural killer T, natural regulatory T (nTreg), cytotoxic T (Tc), central memory T (Tcm), effector memory T (Tem), exhausted T (Tex), T follicular helper (Tfh), T gamma delta (Tgd), Th1, Th17, Th2, type 1 regulatory T (Tr1) cells, neutrophil macrophages, and monocytes. We analyzed these types of immune cells in all blood samples. The immune scores of infiltrating immune

cells were calculated using ImmuneCellAI methods from a gene expression dataset including RNA-Seq and microarray data. The abundance of immune cells was re-estimated by measuring the weights of DEGs using a compensation matrix and least-squares regression.

Identification of Immune-Related Genes

The robust, public Comparative Toxicogenomics Database (CTD; http://ctdbase.org/) provides manually curated information about gene–disease relationships. We downloaded the genes associated with 24 types of immune cells in the CTD. AmiGO2 (http://amigo.geneontology.org/amigo) is an online set of tools with which to search and browse the Gene Ontology (GO) database (Balsa-Canto et al. 2016). Human immune-related genes were downloaded from AmiGO2 using the keyword, "immune". Common genes between the AmiGO2 and CTD databases were retained and further analyzed.

Identification of DEGs in AD and CN

The gene expression profiles of DEGs in the blood samples were analyzed using the Limma package (version 3.34.9, http://bioconductor.org/packages/release/bioc/html/limma.html) in R 3.4.1. Genes with $|\log_2$-fold change (AD/CN)$|>0$ were screened out. Genes with significant differences in expression between AD and CN were regarded as DEGs and selected based on a threshold of $p<0.05$. DEGs with $\log_2$ fold change (FC) >0 and $p<0.05$ were deemed significantly upregulated, and those with $\log_2$-FC <0 and $p<0.05$ were deemed significantly downregulated.

Selection of Genes Associated with Cerebral Diseases and Immunity

Genes associated with BBB damage according to published literature in PubMed, Embase, and UniProt (https://www.uniprot.org/) were screened. Genes associated with BBB damage in AD, brain inflammation, and immunity were selected. Overlapping BBB-associated genes, immune-associated genes, and DEGs were selected and regarded as candidates for selecting biomarkers for AD.

Protein-protein Interaction Network Analysis

The PPI network provides important information about interactions between gene products and indicates hub nodes. Interactions among candidates were identified using the STRING database (version 10.5, https://string-db.org). Interactions with scores >0.4 were retained and used to construct the PPI network, which was visualized using Cytoscape (version 3.6.01, http://www.cytoscape.org/).

Functional Enrichment Analysis

The database for annotation, visualization, and integrated discovery (DAVID; version 6.7, https://david.ncifcrf.gov/) is a web-accessible and expanded annotation database with novel algorithms with which to extract biological processes and pathways from large gene lists (Huang et al. 2007). Functional enrichment was analyzed using the DAVID online tool to investigate GO biological processes and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways associated with potential biomarkers of AD. The significance threshold for enrichment was $p<0.05$, and the fold discovery rate was <0.05.

Validation of Hub Genes in Microarrays

We selected microarray datasets of AD (including GSE4226, GSE4229, and GSE18309) from the National Center of Biotechnology Information Gene Expression Omnibus (https://www.ncbi.nlm.nih.gov/) using the key words, "Alzheimer disease" AND "cerebrospinal fluid" OR "peripheral blood mononuclear cells", to validate hub gene profiles. The inclusion criteria for the microarray datasets were (1) transcriptomic data from patients with and without (controls) AD; (2) data extracted from peripheral blood mononuclear cells or cerebrospinal fluid; (3) without restriction on ethnicity; and (4) >3 samples per group. The GSE4226, GSE4229, and GSE18309 datasets comprised 28 (AD and CN, $n=14$), 40 (AD, $n=18$, and CN, $n=22$), and nine (AD and CN, $n=3$ each) samples, respectively. Gene expression profiles were calculated using GEO2R (http://www.ncbi.nlm.nih.gov/geo/geo2r/).

Statistical Analysis

All data were statistically analyzed using SPSS 22.0 (IBM Corp., Armonk, NY, USA). Associations between expression profiles of identified peripheral biomarkers (immune cell-specific genes) and the CSF markers (Aβ, T-tau, and P-tau) were assessed using binary Pearson correlation analyses. Values were averaged for samples with more than one duplicate, and correlations with $p<0.05$ were regarded as significant. Blood biomarkers associated with CSF biomarker concentrations were used to screen independent risk factors for AD using logistic

regression analysis with 95% confidence intervals (CI). Between-group differences in the abundance of immune cells were analyzed using Mann-Whitney *U* tests. The threshold for a significant difference was $p < 0.05$.

Results

Identification of DEGs in AD Blood and CSF Samples

Figure 1 shows a flow chart of the study. We first identified the DEGs between the AD and CN groups. After sample matching, a total of 304 overlapping blood and CSF samples were screened out, including 44 AD and 260 CN samples. Based on expression levels, we found 210 DEGs between the AD and CN groups (Table S1), including 11 and 13 that were significantly downregulated and upregulated, respectively (Table 1).

Table 1 Significantly differentially expressed genes between the elderly controls and patients with Alzheimer disease

Names	logFC	*P* value	Names	logFC	*P* value
CLDN5	−0.25	0.00	*MMP2*	0.04	0.03
BLVRB	−0.20	0.02	*ITM2B*	0.05	0.02
SLC7A5	−0.18	0.00	*VEGFA*	0.07	0.03
SLC2A1	−0.14	0.03	*CCND1*	0.07	0.00
DUX4L[a]	−0.11	0.01	*CASP1*	0.08	0.05
CSF1R	−0.10	0.02	*PSEN1*	0.10	0.02
NOXO1	−0.10	0.00	*NCF2*	0.10	0.02
ACHE	−0.09	0.04	*MAPK14*	0.12	0.04
LAMA5	−0.06	0.03	*TGFBR1*	0.14	0.01
TGFB2	−0.05	0.04	*IL1R1*	0.15	0.04
TJP1	−0.04	0.04	*FAS*	0.26	0.00
CXCL12	0.03	0.02	*MMP9*	0.28	0.04

FC fold change

[a]The gene was not related to blood-brain barrier (BBB) in literature

Abundance of Infiltrating Immune Cells

The infiltration scores of immune cells were assessed using ImmuCellAI based on the 210 DEG signatures in each sample. Figure 2a shows the proportions of the 24 types of immune cells in all individuals, and Fig. 2b shows differences between the AD and CN groups. We found that Tc, CD8 T, MAIT, and DC cells, as well as macrophages, monocytes, and neutrophils, were relatively more abundant than other types of cells in blood samples from AD. Blood samples from patients with AD contained more Tex (0.196 vs. 0.132), iTreg (0.180 vs. 0.137), and Tgd (0.146 vs. 0.107) cells and less Tcm cells (0.04 vs. 0.07) than the blood samples from the controls (Fig. 2b). These results showed that

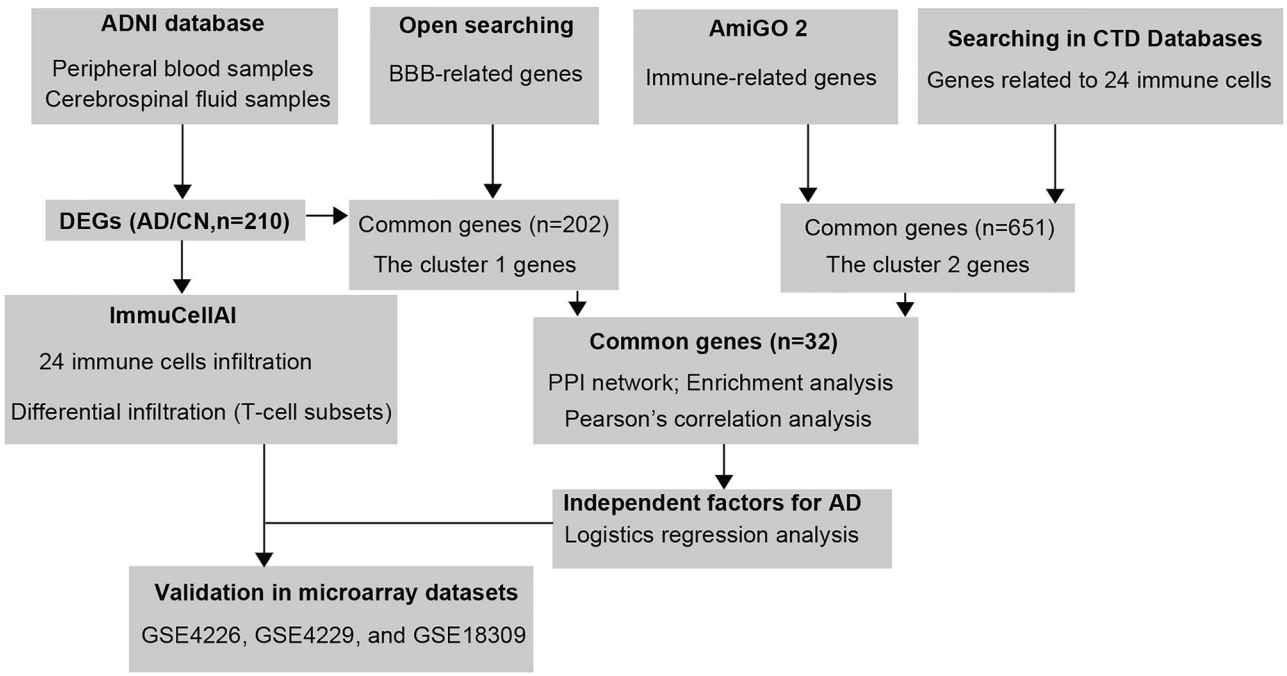

Fig. 1 The flow chart of data analysis in this paper. AD, Alzheimer disease. ADNI, Alzheimer's disease neuroimaging initiative**.** BBB, blood-brain barrier. CN, elderly controls. CTD, Comparative Toxicogenomics Database. DEGs, differentially expressed genes. ImmuCellAI, Immune Cell Abundance Identifier. PPI, protein-protein interaction

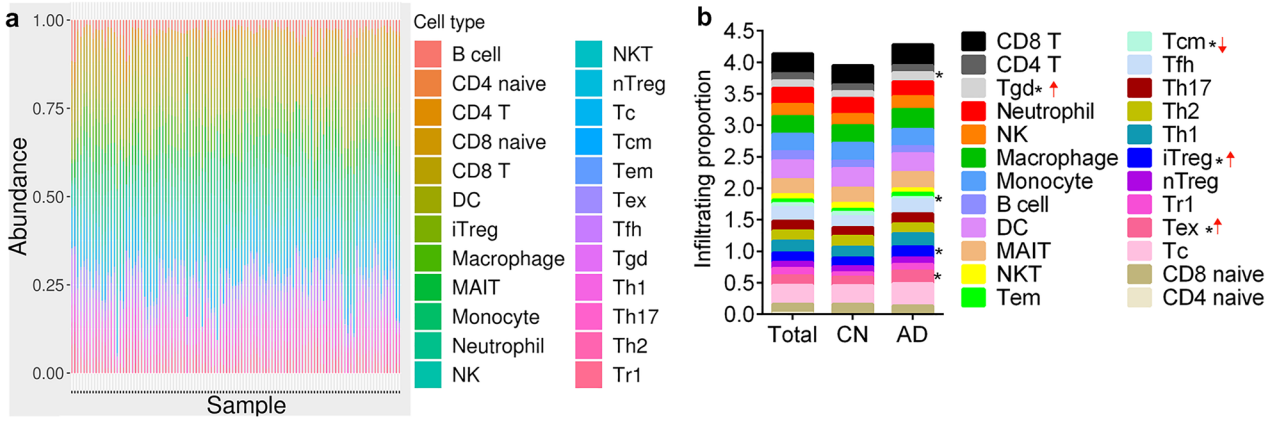

Fig. 2 Abundance of 24 immune cells in blood samples. **a** The abundance of 24 immune type cells in 109 individuals. **b** The abundance of immune cells in groups and the whole cohort. CN, elderly controls. AD, Alzheimer disease. There is a significant difference between the two groups in the abundance of corresponding immune cells ($p < 0.05$, by t test). Tc, cytotoxic T cells. Treg, regulatory T cells. iTreg, induced Treg cells. nTreg, natural Treg cells. Tgd, T gamma delta cells. Tfh, T follicular helper cells. Tex, exhausted T cells. DC, dendritic cells. NK: natural killer cells. Tem, effector memory T cells. MAIT, mucosal-associated invariant T cells. Tr1, type 1 regulatory T cells. NKT, natural killer T cells. Tcm, central memory T cells

the levels of T cell infiltration differed between the patients with AD and the controls.

Screening Genes Related to Immune Cells

We mined the literature and identified 560 genes, including 134 receptor genes that are associated with the BBB (Table S2) and 202 DEGs between AD and CN, including 23 that were significant (Table 1). The 202 DEGs were named cluster 1.

Based on the screening of the AmiGO2 and CTD databases, we identified 3324 and 2022 genes that were associated with immune cells and the 24 immune cell types, respectively. We identified 651 common genes and named them cluster 2 (Table S2).

We finally identified 32 genes that were common to clusters 1 and 2 (Table 2). Among them, 15, seven, six, six, and five genes were associated with macrophages, neutrophils, T cells, B cells, and monocytes, respectively.

Network and Enrichment Analysis of Immune-Related DEGs in AD

Figure 3 shows that the PPI network derived from the 32 genes comprised 27 DEGs, including upregulated neutrophil cytosolic factor 2 (*NCF2*), matrix metalloproteinase 9 (*MMP9*), and chemokine stroma-derived factor 1 (*CXCL12*) and downregulated transforming growth factor (TGF)-β 2 (*TGFB2*) and colony-stimulating factor 1 receptor (*CSF1R*). The degrees of *TNF*, C–C chemokine ligand (*CCL5*), *CXCL2*, *CXCL12*, *CCL3*, *CXCL1*, and *MMP9* gene interactions were high in this network (range, 11–21).

Functional enrichment analysis showed that these 27 DEGs were associated with 18 biological processes, including inflammatory response (GO: 0006954), immune response (GO: 0006955), neutrophil chemotaxis (GO: 0030593), and chemokine-mediated signaling pathway (GO: 0070098), and eight KEGG pathways including cytokine-cytokine receptor interaction (hsa04060), chemokine signaling pathway (hsa04062), TNF signaling pathway (hsa04668), and rheumatoid arthritis (hsa05323) (Table S3).

Correlations Between Blood Gene Expression and CSF Biomarkers

Associations between the 27 DEG profiles and CSF biomarkers (Aβ, T-tau, and P-tau) were assessed using Pearson correlation analyses. The results showed that expression of five genes, including *TNFRSF13C*, *CXCL3*, *CXCL12*, *CCL4L1*, and *CCL1*, were significantly correlated with levels of Aβ, T-tau, and/or P-tau in CSF (Table 3). For instance, *CCL1* and *CCL4L1* expression in blood samples correlated with Aβ contents in CSF ($r = 0.142$ and 0.140, $p < 0.05$, respectively), whereas *CXCL12* expression correlated negatively with Aβ content ($r = -0.158$, $p < 0.05$) and positively with T-tau content in CSF samples ($r = 0.156$, $p < 0.05$).

Potential Blood Biomarkers for AD

Based on the correlations between the DEGs and CSF biomarkers, we speculated that their expression might be a risk factor for the diagnosis of AD. Logistics analysis indicated that two genes, namely *TNFRSF13C* (odds ratio [OR], −1.574; 95%

Table 2 The list of the 32 genes that were common to clusters 1 and 2

Gene	Description/annotation	Sig. Down/up
TNFRSF13C	B cell-activating factor receptor	NA
TNF	TNF, macrophage/monocyte-derived	NA
TGFB2	Glioblastoma-derived T cell suppressor factor	Down
RELA	Nuclear factor of kappa light polypeptide gene enhancer in B cells 3	NA
PTGS2	Macrophage activation-associated marker protein P71/73	NA
PPBP	Macrophage-derived growth factor/neutrophil activating peptide-2	NA
NCF2	Neutrophil cytosolic factor 2	Up
MMP9	Macrophage gelatinase	Up
IL3RA	Colony-stimulating factor 2 receptor, alpha, low affinity (granulocyte macrophage)	NA
IL27RA	T cell cytokine receptor	NA
CYBB	Neutrophil cytochrome b 91 kDa polypeptide	NA
CXCL6	Alveolar macrophage chemotactic factor	NA
CXCL5	Epithelial-derived neutrophil-activating protein 78	NA
CXCL3	Macrophage inflammatory protein 2-alpha/beta; dendritic cell inflammatory protein 1; cytokine-induced neutrophil chemoattractant 2	NA
CXCL2	Macrophage inflammatory protein 2; cytokine-induced neutrophil chemoattractant 3	NA
CXCL14	B cell and monocyte-activating chemokine	NA
CXCL13	B cell-attracting chemokine 1	NA
CXCL12	pre-B cell growth-stimulating factor	Up
CXCL1	Cytokine-induced neutrophil chemoattractant 1	NA
CSF2RB	Colony stimulating factor 2 receptor, beta 1, low-affinity (granulocyte-macrophage)	NA
CSF1R	LOW QUALITY PROTEIN: macrophage colony-stimulating factor 1 receptor	Down
CSF1	Colony stimulating factor 1 (macrophage)	NA
CD109	Activated T cell marker CD109	NA
CCL5	Regulated upon activation normal T-cell expressed and secreted	NA
CCL4L2	Macrophage inflammatory protein-1b2; monocyte adherence-induced protein 5-alpha	NA
CCL4L1	Macrophage inflammatory protein-1b2; monocyte adherence-induced protein 5-alpha	NA
CCL3	Macrophage inflammatory protein 1 alpha	NA
CCL27	Cutaneous T cell attracting chemokine	NA
CCL26	Macrophage inflammatory protein 4-alpha	NA
CCL2	Monocyte chemoattractant protein 1	NA
CCL1	T cell activation protein 3	NA
BCL2	B cell CLL/lymphoma 2	NA

Sig significant ($p < 0.05$)

NA not applicable

CI 0.045–0.951, $p = 0.043$) and *CXCL12* (OR, 101.63, 95% CI 2.002–5158.642, $p = 0.021$, were independent risk factors for AD diagnosis (Table 4). Both genes were associated with B cells (Table 2).

Validation of Genes Associated with B and T Cells in Microarray Datasets

Based on the above results, we found that the levels of T cell subset infiltration differed in AD and that the *TNFRSF13C* and *CXCL12* genes associated with B cells were independent risk factors for a diagnosis of AD. The profiles of T cell-related genes (*IL27RA*, *TGFB2*, *CD109*, *CCL5*, *CCL2*, and *CCL1*) and B cell-related genes (*CXCL13*, *RELA*, *CXCL14*, *BCL2*, *TNFRSF13C*, and *CXCL12*) among the 27 DEGs in the PPI network were validated in AD samples. Three datasets (GSE4226, GSE4229, and GSE18309) were downloaded, and the expression profiles of the 12 genes were determined (Fig. 4). *CXCL13*, *CXCL14*, *BCL2*, *IL27RA*, *CCL2*, and *CCL1* were upregulated in AD samples from one to three datasets, and *CXCL12* and *TGFB2* were downregulated in GSE18309. However, most of these genes were not significantly deregulated in AD.

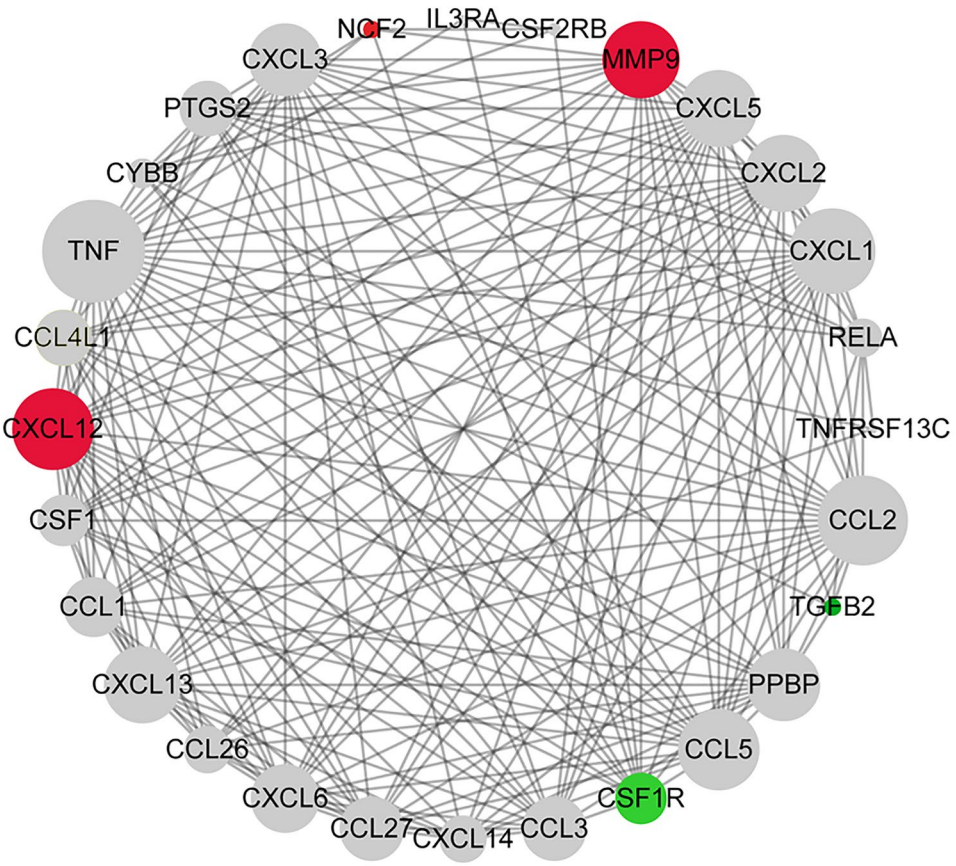

Fig. 3 The protein-protein interaction network of the 32 common genes in Alzheimer disease. This network consists of 27 nodes, including three upregulated genes (red) and two downregulated genes (green). Gray color indicates genes with insignificant differential expression between patients and controls. Node size corresponds to interaction degree in the network

Discussion

Screening easily accessible and reliable biomarkers is of major significance for the early diagnosis and timely treatment of AD. Blood neutrophils, brain-derived neurotrophic factors, and miRNA are associated with and might be used as biomarkers for AD (Balietti et al. 2018; Dong et al. 2019; Swarbrick et al. 2019). We found higher levels of circulating T-cell subsets, including Tex, iTreg, and Tgd cells in patients with AD than in the controls.

Table 3 Correlation of the differentially expressed genes with cerebrospinal fluid biomarkers

Genes	Cerebrospinal fluid biomarker		
	Aβ	T-tau	P-tau
TNFRSF13C	0.163*	−0.019	−0.060
CXCL3	−0.021	0.163*	0.122
CXCL12	−0.158*	0.156*	0.100
CCL4L1	0.140*	−0.094	−0.102
CCL1	0.142*	−0.020	−0.094

T-tau total Tau, *P-tau* phosphorylated tau

*$p < 0.05$, respectively

Both *TNFRSF13C* and *CXCL12* were identified as potential blood markers of AD, as their high and low expression levels were independent risk factors for AD, respectively.

Circulating lymphocytes and macrophages infiltrate the brains of patients with progressive neurodegenerative diseases of the CNS such as AD, multiple sclerosis (MS), and Parkinson disease (Rosenberg 2012; Sawikr et al. 2017). The Aβ-mediated release of microglial inflammatory cytokines, including TNF-α, can promote the transendothelial migration of T lymphocytes (Mietelska-Porowska and Wojda 2017; Schaerli and Moser 2005; Yang et al. 2013). In response to inflammation, cells at sites of damage secrete high levels of inflammatory cytokines and chemokines, which subsequently recruit immune cells from peripheral blood (Marsh et al. 2016). During the onset and development of AD, Aβ deposition or responses to cell damage activate glial cells, microglia, and TLR and MAPK signaling to recruit circulating immune cells (Ho et al. 2005; Medeiros and LaFerla 2013; Singhal et al. 2014). We showed here that the expression of the genes encoding circulating proteins, including NCF2, MMP9, and CXCL12, were upregulated in patients with AD compared with the controls. The upregulation of *CXCL12*, a pre-B cell growth-stimulating factor, was associated negatively with the levels

Table 4 Correlations of the differentially expressed genes with Alzheimer disease diagnosis

Genes	Univariate			Multivariate		
	β	OR (95% CI)	*P*	*β*	OR (95% CI)	*P*
TNFRSF13C	−1.550	0.212 (0.046–0.976)	0.046	−1.574	0.207 (0.045–0.951)	0.043
CXCL3	0.923	2.528 (0.150–42.349)	0.521			
CXCL12	4.483	88.531 (1.831–4281.430)	0.023	4.621	101.630 (2.002–5158.642)	0.021
CCL4L1	−0.345	0.708 (0.340–1.477)	0.358			
CCL1	−0.756	0.469 (0.066–3.336)	0.450			

OR odds ratio, *CI* confidence interval

of Aβ, and positively with increased T-tau and P-tau in CSF samples. These results showed that *CXCL12* interacts with blood and CSF in AD.

Levels of 24 types of infiltrative immune cells and immune-related prognostic/diagnostic signatures in human diseases can now be determined using ImmuCellAI (Miao et al. 2019; Song et al. 2020; Sun et al. 2020). The ImmuCellAI results are consistent with flow cytometry data and might serve as prognostic signatures for human cancers (Mei et al. 2020; Song et al. 2020). For instance, the infiltration of Th1 cells and M1 macrophages correlates with a favorable prognosis, and the higher expression levels of the M1 macrophage markers, *CD68* and *CSF1R*, are also associated with a good prognosis among patients with osteosarcoma (Song et al. 2020). The present study used ImmuCellAI to estimate the abundance of 24 types of immune cells based on the expression of 210 DEGs in blood samples. We found that patients with AD had a higher abundance of Tex, iTreg, and Tgd cells, and a lower proportion of Tcm cells. These T cell subsets have distinct functions in the immune system (Prinz and Priller 2017), but none of the markers of these cells was associated with the diagnosis of AD.

Most T cells in CSF are Tem cells that express the receptors CD62L and CCR7, and have a homing capacity for guiding theirs recruitment to target tissues (Pepper and Jenkins 2011; Prinz and Priller 2017). These cells play important roles in antitumor and anti-disease immunity with long-term memory (Liu et al. 2015; Klebanoff et al. 2005; Pepper and Jenkins 2011; Sallusto et al. 2004). Within a few hours post-stimulation, Tem cells secrete IFN-γ, IL-4, and IL-5 and are therefore characterized by a rapid effector function (Pepper and Jenkins 2011). The expression of CXCL12 prevents Tc cells from infiltrating tumor tissues in tumor models in vivo (Garg et al. 2018), and when expressed in the bone marrow, CXCL12 promotes the migration of Treg cells from peripheral blood to bone marrow (Zou et al. 2004). Here, we confirmed that *CXCL13* and *CXCL14*, which are associated with B cells, and *IL27RA*, *CCL5*, and *CCL2*, which are associated with T cells, were upregulated in AD compared with the controls. The upregulated expression of *IL27RA*, *CCL5*, and *CCL2* in the blood was consistent with the increased infiltration of iTreg, Tgd, and Tex cells.

However, only *CXCL12* and *TNFRSF13C* were independent risk factors for AD. Notably, *TNFRSF13C* encodes a B cell-activating factor receptor (BAFFR) during the ontogeny, terminal differentiation, maturation, and survival of B cells (Mihalcik et al. 2010; Ntellas et al. 2020). This gene is undetectable in B cell precursors, but is overexpressed in B cell neoplasms such as B cell acute lymphoblastic leukemia (Turazzi et al. 2018; Tussiwand et al. 2012). The enhanced BAFFR-signaling induced by the *H159Y* (rs61756766) polymorphism in *TNFRSF13C* correlates with MS (Ntellas et al. 2020). We showed here that *TNFRSF13C* downregulation and CXCL12 overexpression contribute to a diagnosis of AD. The present findings suggest that the abundance of B cells might play a crucial role in the etiology of AD.

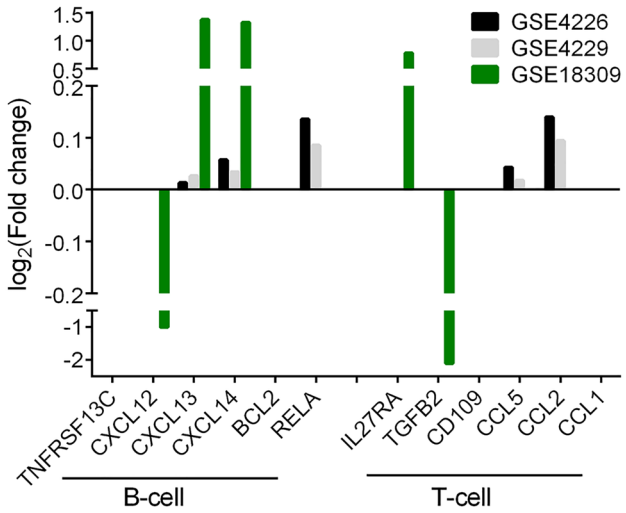

Fig. 4 The profiles of B cell- and T cell-related genes in microarray datasets. GSE4226, GSE4229, and GSE18309 were downloaded from the Gene Expression Omnibus (https://www.ncbi.nlm.nih.gov/). Gene expression profiles were calculated using GEO2R (http://www.ncbi.nlm.nih.gov/geo/geo2r/). FC, fold change

Conclusions

The abundance of Tex, iTreg, and Tgd cells in peripheral blood was higher in patients with AD than in the controls. The expression of the B cell-related genes *TNFRSF13C* and *CXCL12* was elevated in the peripheral blood and correlated with T-tau and Aβ levels in the CSF of patients with AD. Therefore, *TNFRSF13C* and *CXCL12* gene expression in the peripheral blood might serve as an easily accessibly biomarker of AD. The interaction between B cell activity and T cell infiltration in AD appears significant. However, a limitation of this paper is that the above results are not verified. The exact mechanism remains unclear and requires investigation.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s12031-021-01809-7.

Author Contribution Conception and design of the research: Qianqian Wu and Wei Kong. Acquisition, analysis, and interpretation of data: Qianqian Wu, Shuaiqun Wang, and Wei Kong. Statistical analysis: Qianqian Wu. Drafting the manuscript: Qianqian Wu. Manuscript revision for important intellectual content: Wei Kong. All authors have read and approved the manuscript.

Funding This work was supported by Natural Science Foundation of Shanghai (No.18ZR1417200, obtained by Wei Kong) and National Natural Science Foundation of China (No.61803257; obtained by Shuaiqun Wang).

Data Availability The original microarray datasets of Alzheimer disease (including GSE4226, GSE4229, and GSE18309) are available from the National Center of Biotechnology Information Gene Expression Omnibus (https://www.ncbi.nlm.nih.gov/)。Peripheral blood, CSF, and plasma data of patients with Alzheimer disease and controls were downloaded from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (http://adni.loni.usc.edu/).

Declarations

Ethics Approval and Consent to Participate This article does not contain any studies with human participants or animals performed by any of the authors; therefore, the ethical approval and consent to participate are not applicable.

Competing Interests The authors declare that they have no competing interests.

References

Balietti M, Giuli C, Conti F (2018) Peripheral blood brain-derived neurotrophic factor as a biomarker of Alzheimer's disease: are there methodological biases? Mol Neurobiol 55:6661–6672. https://doi.org/10.1007/s12035-017-0866-y

Balsa-Canto E, Henriques D, Gábor A, Banga JR (2016) AMIGO2, a toolbox for dynamic modeling, optimization and control in systems biology. Bioinformatics 32:3357–3359

Calsolaro V, Edison P (2016) Neuroinflammation in Alzheimer's disease: current evidence and future directions. Alzheimers Dement 12:719–732

Dong X, Nao J, Shi J, Zheng D (2019) Predictive value of routine peripheral blood biomarkers in Alzheimer's disease. Front Aging Neurosci 11. https://doi.org/10.3389/fnagi.2019.003322019.00332

Fisher Y, Nemirovsky A, Baron R, Monsonego A (2010) T cells specifically targeted to amyloid plaques enhance plaque clearance in a mouse model of Alzheimer's disease. PLoS One 5:e10830

Garg B et al (2018) NFκB in pancreatic stellate cells reduces infiltration of tumors by cytotoxic T cells and killing of cancer cells, via up-regulation of CXCL12. Gastroenterology 155:880–891.e888. https://doi.org/10.1053/j.gastro.2018.05.0512018.05.051

González H, Pacheco R (2014) T-cell-mediated regulation of neuroinflammation involved in neurodegenerative diseases. J Neuroinflammation 11:201

Heneka MT et al (2015) Neuroinflammation in Alzheimer's disease. Lancet Neurol 14:388–405. https://doi.org/10.1016/S1474-4422(15)70016-5

Higham JP, Malik BR, Buhl E, Dawson JM, Ogier AS, Lunnon K, Hodge JJL (2019) Alzheimer's disease associated genes ankyrin and tau cause shortened lifespan and memory loss in drosophila. Front Cell Neurosci 13:260–260. https://doi.org/10.3389/fncel.2019.00260

Ho GJ, Drego R, Hakimian E, Masliah E (2005) Mechanisms of cell signaling and inflammation in Alzheimer's disease. Curr Drug Targets: Inflammation Allergy 4:247–256

Huang DW et al (2007) DAVID Bioinformatics Resources: expanded annotation database and novel algorithms to better extract biology from large gene lists. Nucleic Acids Res 35:W169–W175

Janssen B et al (2016) Imaging of neuroinflammation in Alzheimer's disease, multiple sclerosis and stroke: Recent developments in positron emission tomography. Biochim Biophys Acta (BBA) - Mol Basis Dis 1862:425–441. https://doi.org/10.1016/j.bbadis.2015.11.011

Klebanoff CA et al (2005) Central memory self/tumor-reactive CD8+ T cells confer superior antitumor immunity compared with effector memory T cells. Proc Natl Acad Sci 102:9571–9576

Liu HL et al (2015) Clinical significance of preoperative CD8+ central memory T cells for operable pancreatic adenocarcinoma. Dig Surg 32 (6):433-438

Marsh SE et al (2016) The adaptive immune system restrains Alzheimer's disease pathogenesis by modulating microglial function. Proc Natl Acad Sci USA 12:P463–P464

McDade E et al (2018) Longitudinal cognitive and biomarker changes in dominantly inherited Alzheimer disease. Neurology 91:e1295–e1306

Medeiros R, LaFerla FM (2013) Astrocytes: conductors of the Alzheimer disease neuroinflammatory symphony. Exp Neurol 239:133–138

Mei J, Xu R, Xia D, Yang X, Wang H, Liu C (2020) Profiles and clinical significance of immune cell infiltration in pancreatic adenocarcinoma. bioRxiv. https://doi.org/10.1101/2020.1103.1130.017327

Miao YR, Zhang Q, Lei Q, Luo M, Xie GY, Wang H, Guo AY (2019) ImmuCellAI: a unique method for comprehensive T-cell subsets abundance prediction and its application in cancer immunotherapy bioRxiv:872184

Mietelska-Porowska A, Wojda U (2017) T lymphocytes and inflammatory mediators in the interplay between brain and blood in Alzheimer's disease: potential pools of new biomarkers. J Immunol Res 2017:4626540. https://doi.org/10.1155/2017/4626540

Mihalcik SA, Huddleston PM, Wu X, Jelinek DF (2010) The structure of the TNFRSF13C promoter enables differential expression of BAFF-R during B cell ontogeny and terminal differentiation. J Immunol 185:1045–1054

Ntellas P et al (2020) TNFRSF13C/BAFFR P21R and H159Y polymorphisms in multiple sclerosis. Mult Scler Relat Disord 37:101410–101422. https://doi.org/10.1016/j.msard.102019.101422

Ortiz G et al (2017) Oxidative stress: Love and hate history in central nervous system. Adv Protein Chem Struct Biol 108:1-31. https://doi.org/10.1016/bs.apcsb.2017.01.003

Pepper M, Jenkins MK (2011) Origins of CD4(+) effector and central memory T cells. Nat Immunol 12:467–471. https://doi.org/10.1038/ni.2038

Prinz M, Priller J (2017) The role of peripheral immune cells in the CNS in steady state and disease. Nat Neurosci 20:136–144. https://doi.org/10.1038/nn.4475

Quiroz YT et al (2018) Association between amyloid and tau accumulation in young adults with autosomal dominant Alzheimer disease. JAMA Neurol 75:548–556

Regen F, Hellmann-Regen J, Costantini E, Reale M (2017) Neuroinflammation and Alzheimer's disease: Implications for microglial activation. Curr Alzheimer Res 14:1140-1148. https://doi.org/10.2174/1567205014666170203141717

Rezai-Zadeh K, Gate D, Town T (2009) CNS infiltration of peripheral immune cells: D-Day for neurodegenerative disease? J Neuroimmune Pharmacol 4:462–475

Rosenberg GA (2012) Neurological diseases in relation to the blood-brain barrier. J Cereb Blood Flow Metab 32:1139–1151

Sallusto F, Geginat J, Lanzavecchia A (2004) Central memory and effector memory T cell subsets: function generation, and maintenance. Annu Rev Immunol 22:745–763. https://doi.org/10.1146/annurev.immunol.22.012703.104702

Sawikr Y, Yarla NS, Peluso I, Kamal MA, Aliev G, Bishayee A (2017) Neuroinflammation in Alzheimer's disease: the preventive and therapeutic potential of polyphenolic nutraceuticals. Adv Protein Chem Struct Biol 108:33-57. https://doi.org/10.1016/bs.apcsb.2017.02.001

Schaerli P, Moser B (2005) Chemokines: control of primary and memory T-cell traffic. Immunol Res 31:57–74

Shi B, Qi J (2020) The pattern and prognostic relevance of immune activity scores and tumor-infiltrating immune cells in metastatic clear cell renal cell carcinoma: evidence from multiple datasets. Int Immunopharmacol 85:106651

Singhal G, Jaehne EJ, Corrigan F, Toben C, Baune BT (2014) Inflammasomes in neuroinflammation and changes in brain function: a focused review. Front Neurosci 8:315

Song YJ et al (2020) Immune landscape of the tumor microenvironment identifies prognostic gene signature CD4/CD68/CSF1R in Osteosarcoma. Front Oncol 10:1198. https://doi.org/10.3389/fonc.2020.01198

Sun S et al (2020) Development and validation of an immune-related prognostic signature in lung adenocarcinoma. Cancer Med 9:5960–5975

Swarbrick S, Wragg N, Ghosh S, Stolzing A (2019) Systematic review of miRNA as biomarkers in Alzheimer's disease. Mol Neurobiol 56:6156–6167

Turazzi N et al (2018) Engineered T cells towards TNFRSF13C (BAFFR): a novel strategy to efficiently target B-cell acute lymphoblastic leukaemia. Br J Haematol 182:939–943. https://doi.org/10.1111/bjh.14899

Tussiwand R, Rauch M, Flück LA, Rolink AG (2012) BAFF-R expression correlates with positive selection of immature B cells. Eur J Immunol 42:206–216

Wang X, Wang L (2020) Screening and identification of potential peripheral blood biomarkers for Alzheimer's disease based on bioinformatics analysis. Med Sci Monit 26:e924263. https://doi.org/10.12659/MSM.924263

Yang YM, Shang DS, Zhao WD, Fang WG, Chen YH (2013) Microglial TNF-α-dependent elevation of MHC class I expression on brain endothelium induced by amyloid-beta promotes T cell transendothelial migration. Neurochem Res 38:2295–2304

Yuen SC, Zhu H, Leung SW (2020) A Systematic Bioinformatics Workflow With Meta-Analytics Identified Potential Pathogenic Factors of Alzheimer's Disease. Front Neurosci 14:209

Zamanian Azodi M, Rezaei-Tavirani M, Rezaei Tavirani M (2020) Investigating the effects of ibuprofen on the gene expression profile in Hippocampus of mice model of Alzheimer's disease through bioinformatics analysis. Iranian Journal of Pharmaceutical Research 19:352–359

Zou L et al (2004) Bone marrow is a reservoir for CD4+ CD25+ regulatory T cells that traffic through CXCL12/CXCR4 signals. Can Res 64:8451–8455

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.