PERIPHERAL IMMUNE SYSTEM AND NEURODEGENERATIVE DISEASE

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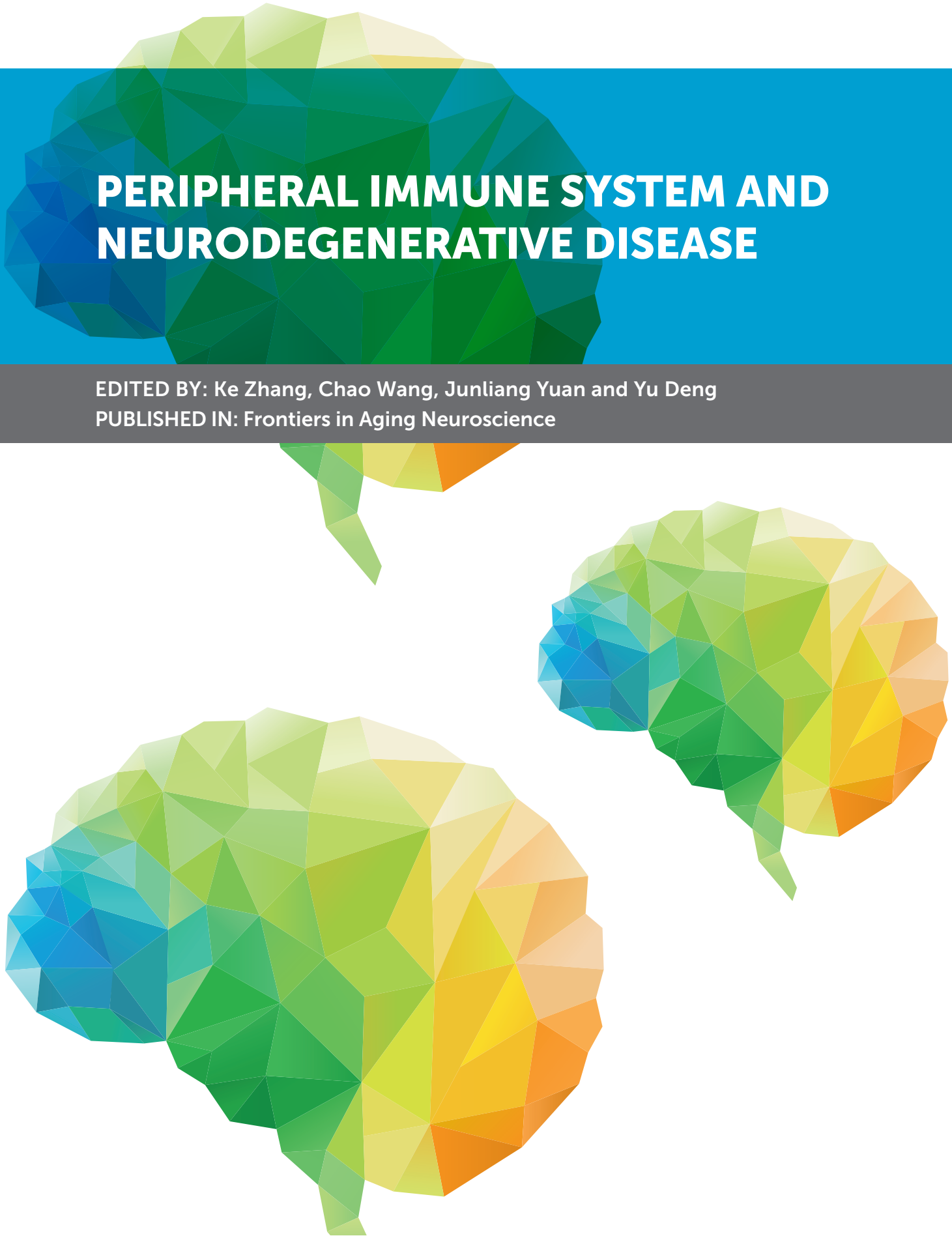
PUBLISHED IN: Frontiers in Aging Neuroscience



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ISSN 1664-8714
ISBN 978-2-88976-975-9
DOI 10.3389/978-2-88976-975-9

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PERIPHERAL IMMUNE SYSTEM AND NEURODEGENERATIVE DISEASE

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Citation: Zhang, K., Wang, C., Yuan, J., Deng, Y., eds. (2022). Peripheral Immune System and Neurodegenerative Disease. Lausanne: Frontiers Media SA.
doi: 10.3389/978-2-88976-975-9

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frontiers | Frontiers in Aging Neuroscience

TYPE Editorial
PUBLISHED 05 August 2022
DOI 10.3389/fnagi.2022.970042

Check for updates

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Neuroinflammation and Neuropathy, a section of the journal Frontiers in Aging Neuroscience

RECEIVED 15 June 2022
ACCEPTED 20 June 2022
PUBLISHED 05 August 2022

CITATION
Zhang K, Wang C, Yuan J-L and Deng Y (2022) Editorial: Peripheral immune system and neurodegenerative disease. *Front. Aging Neurosci.* 14:970042. doi: 10.3389/fnagi.2022.970042

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Editorial: Peripheral immune system and neurodegenerative disease

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KEYWORDS

neurodegenerative diseases, Alzheimer's disease, Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), peripheral immune

Editorial on the Research Topic
Peripheral immune system and neurodegenerative disease

Neurodegenerative diseases are a class of chronic and irreversible disorders characterized by progressive degeneration and loss of function of the central and/or peripheral nervous systems. The main pathological feature of neurodegenerative disease in the central nervous system (CNS) is selective neuronal loss in the brain and spinal cord, leading to cognitive and/or motor dysfunction. The immune system plays a variety of roles in the pathophysiology of neurodegenerative diseases. Current understanding of microglia from basic and clinical findings is as the main innate immune cells in the brain, which can be activated and involved in the neuroinflammation in nearly all neurodegenerative disorders. In recent years, many scientists have shifted their ground on conceptualizing neurodegenerative disease as a neuron-centric disease; rather, a close functional connection between the peripheral immune system and central nervous system has been increasingly acknowledged. An increasing number of circulating immune cells have been detected in neurodegenerative brains. In this regard, understanding how the peripheral immune system interacts with the central nervous system in terms of regulating the onset and development of neurodegenerative diseases assumes importance. Studies aim at exploring the role of the peripheral immune system in neurodegenerative diseases will help to identify new targets and improve the feasibility of therapeutic interventions. The manuscripts in this Research Topic focus on the relationship between the peripheral immune and neurodegenerative disease or neurodegenerative pathological changes. We highlight three specific themes in this topic: (1) peripheral innate/adapt immune and neurodegenerative disease;

(2) immunology-related serum or plasma or blood platelet studies on neurodegenerative disease; (3) the crosstalk between the peripheral immunity and central nervous system.

The manuscripts in this Research Topic cover three main types of neurodegenerative disease: Alzheimer's disease (AD), Parkinson's disease (PD), and Amyotrophic Lateral Sclerosis (ALS). AD, as the most common type of dementia worldwide, has already affected over 50 million people. The lack of definite diagnostic biomarkers and effective treatments is the main cause of uncontrolled AD. The progression of AD is a dynamic process, from pre-symptomatic AD, mild cognitive impairment (MCI), to AD stages. Therefore, a cost-effective, easy-to-measure biomarker to identify subjects who will develop AD, especially at the pre-symptomatic stage is urgently needed. Qin et al. investigated serum biomarkers during different AD stages and potential novel protein biomarkers of presymptomatic AD. There are thirteen proteins in the serum that were significantly different in patients with AD or MCI group. Some proteins including cathepsin D, immunoglobulin E (IgE), epidermal growth factor receptor (EGFR), matrix metalloproteinase-9 (MMP-9), von Willebrand factor (vWF), haptoglobin, and phosphorylated Tau-181 (p-Tau181) correlated with all cognitive measures. They conclude the serum level of p-Tau181 might be broadly available to identify individuals with pre-clinical AD and assess the severity of AD. Huang et al. used a meta-analysis method systematically to evaluate the association of peripheral blood cell counts and indices with AD and MCI. The changes in leukocyte, lymphocyte, neutrophil, and $CD8^+$ T cell counts, as well as the neutrophil-lymphocyte ratio and the $CD4^+/CD8^+$ ratio, are closely associated with AD, which provides us a potential diagnostic value clinical data. Besides peripheral functional immune blood cells, the complement system, an important arm of the innate immune system, is inextricably intertwined with the development of cognitive impairment. Li Z. et al. investigated and discussed the differences in complement activation pathways in cognitive impairment and type 2 diabetes mellitus (T2DM) with cognitive impairment, which provide scientific data on innate immune links between cognitive dysfunction and other diseases. In the treatment for AD, Yang et al. carried out a Randomized Controlled Trial to investigate the effects of sport stacking on the overall cognitive repairment and brain function recovery in patients with MCI and AD. It suggested that sport stacking may increase the level of neuroprotective growth factors and enhance neural plasticity. In addition, Peng and Wu reviewed a protein named Irisin, which is an exercise-stimulating cleaved product from transmembrane fibronectin type III domain-containing protein five in elderly dementia and cognitive impairment. One of the important roles of Irisin is that it can be regarded as a mediator of muscle brain cross talk to provide theoretical support for exercise therapy for patients with dementia. These findings suggested that both exercise and sport are beneficial to patients with elderly cognitive impairment, MCI, and AD.

Parkinson's Disease is the second most common neurodegenerative disease in the elderly with the fastest-growing morbidity. PD is mainly characterized by motor features, such as postural instability, bradykinesia, tremor, and rigidity, which are caused by selective loss of dopaminergic neurons in the substantia nigra pars compacta. The interaction between CNS-resident cells and peripheral immune cells in PD pathogenesis has attracted the attention of researchers. In this Research Topic, Zhang et al. systematically retrieved and evaluated the functions of natural killer cells (NK) in PD. NK cells maybe play a neuroprotective role in PD pathogenesis. Regulating the function of NK cells reveals novel targets for the management and treatment of PD. Li D. et al. comprehensively reviewed a famous factor PDGF (platelet-derived growth factors). The manuscript covers the classification, structure, biological functions, and pathogenic roles in PD of PDGF. In the course of PD, PDGF participated the pathogenesis through a variety of mechanisms such as regulating mitochondrial function, Ca^{2+} homeostasis, protein misfolding aggregation, and neuroinflammation. They also discuss the potential treatment strategy of PDGF as a target through multiple methods, especially in genetic treatment. In ALS, Yu et al. made a detailed review of crosstalk between the peripheral and central immune system from a neuroimmunological perspective which provides new insight into pathogenic mechanisms and innovative therapeutic approaches for ALS. The most noteworthy is Zang et al. comprehensively reviewed the crosstalk of central and peripheral immune systems in the three neurodegenerative diseases mentioned above. They conclude the role and molecular mechanism of the most main central immune cells (microglia and astrocytes) and peripheral immune cells (Monocytes, NK cells, T cells, Dendritic cells, and B cells) in these neurodegenerative disorders.

In summary, this Research Topic highlights the emerging role of the peripheral and central immune systems in common neurodegenerative diseases. These manuscripts provide us with the potential target in peripheral immune cells from the diagnosis to therapy. In the future, we certainly expect more studies to be added and the discussion to continue.

Author contributions

KZ and CW decided the layout, wrote the manuscript, and acted as Editors for this Research Topic. All authors contributed to the article and approved the submitted version.

Funding

This work was supported by the National Natural Science Foundation of China (81571046), a Key project of the Educational Department of Liaoning Province (LJKZ0755), and Shenyang Young and Middle-aged Innovative Talents Support Program (RC210240).

Acknowledgments

We appreciate all the editors, reviewers, and authors who contribute to this Research Topic.

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frontiers in Aging Neuroscience

ORIGINAL RESEARCH
published: 08 March 2022
doi: 10.3389/fnagi.2022.810335

Different Complement Activation Pathways Underly Cognitive Impairment and Type 2 Diabetes Mellitus Combined With Cognitive Impairment

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OPEN ACCESS

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Specialty section:
This article was submitted to Neurocognitive Aging and Behavior, a section of the journal Frontiers in Aging Neuroscience

Received: *06 November 2021*
Accepted: *31 January 2022*
Published: *08 March 2022*

Citation:
Li Z, Zhang W, Gao F, Tang Q, Kang D and Shen Y (2022) Different Complement Activation Pathways Underly Cognitive Impairment and Type 2 Diabetes Mellitus Combined With Cognitive Impairment. Front. Aging Neurosci. 14:810335. doi: 10.3389/fnagi.2022.810335

Background: The immune response and the complement system are associated with cognitive impairment and diabetes mellitus, respectively. Activation of the complement system in these diseases occurs mainly through either the classical pathway or the alternative pathway. However, the specific complement proteins involved in the development of the type 2 diabetes mellitus (T2DM) and cognitive impairment are still unclear. Here, we investigated complement proteins in serum from patients with T2DM, cognitive impairment, or both T2DM and cognitive impairment.

Objective: To investigate the levels of serum immune complement proteins in patients with T2DM, cognitive impairment, or T2DM combined with cognitive impairment and the associations between these complement proteins and risk factors for T2DM or cognitive impairment.

Methods: Clinical markers were collected from blood samples of 264 participants. Luminex multiplex assays were used to detect serum complement proteins. All statistical analyses were performed using Prism or R studio.

Results: There was a difference in serum levels of the complement proteins C1q, C3, C3b, and FH between the three different groups. Hyperglycemia was significantly correlated with elevated C3b or reduced C3, C1q, and FH. In addition, hyperlipidemia was positively correlated with elevated levels of C3, C4, C1q, and FH proteins. There was an association between C1q, C3, C4, and FH and β-pancreas cell function, whereas only FH was associated with insulin resistance. Higher serum C1q was significantly associated with an increased risk of cognitive impairment.

Conclusion: Serum levels of complement proteins were closely associated with hyperglycemia and hyperlipidemia. We found that classical complement pathway activation mainly occurred in the cognitive impairment only group, whereas the alternative pathway may reflect T2DM and T2DM with cognitive impairment.

Keywords: biomarkers, serum complement, cognitive impairment, type-2 diabetes mellitus, metabolic disorders

INTRODUCTION

Diabetes mellitus is ranked as one of the top 10 causes of death worldwide (Chatterjee et al., 2017). Type 2 diabetes mellitus (T2DM) is the most common type, accounting for 90% of all cases and is characterized by pancreatic β-cell dysfunction and insulin resistance (DeFronzo et al., 2015; Chatterjee et al., 2017). Individuals with T2DM are at a higher risk than non-diabetic individuals of developing dementia and cognitive impairment, such as Alzheimer's disease (Biessels et al., 2006, 2014; Strachan et al., 2011; McCrimmon et al., 2012; Biessels and Reagan, 2015; Biessels and Despa, 2018), and between 10 and 15% of dementia cases worldwide may be attributed to T2DM (Biessels and Reagan, 2015). This poses a leading public threat to human health and to the worldwide economy, and as such, appropriate novel biomarkers for diagnosing or predicting Alzheimer's disease are urgently needed.

A substantial body of evidence has documented that the complement system, an important arm of the innate immune system, is inextricably intertwined with the development of cognitive impairment and T2DM. A large volume of research has confirmed that the complement system is closely associated with insulin resistance (IR), β-cell function and diabetic vascular complications (Fujita et al., 2013; Ghosh et al., 2015; Flyvbjerg, 2017; Huang et al., 2018; Ajjan and Schroeder, 2019; Shim et al., 2020). A recent review reported that high complement protein C4A copy numbers and low C4B copy numbers are protective against residual β-cell function (Ajjan and Schroeder, 2019), and a clinical trial involving 95,202 participants has indicated that high baseline concentrations of complement C3 were associated with increased risk of diabetic neuropathy highlighted that complement component C3 has a role in the pathology of diabetic neuropathy (Rasmussen et al., 2018b). Complement also has a complex relationship with the CNS (Morgan and Harris, 2015; Hong et al., 2016; Coulthard et al., 2018; Morgan, 2018; Hammond et al., 2019; Lee et al., 2019; Reis et al., 2019; Dalakas et al., 2020); unbalanced or abnormal activation of complement can result in mental disorders, neurodevelopmental disorders or neurodegenerative diseases, including depression, schizophrenia, Alzheimer's disease and Parkinson's disease (Morgan and Harris, 2015; Hong et al., 2016; Sekar et al., 2016; Morgan, 2018; Hammond et al., 2019; Lee et al., 2019; Reis et al., 2019; Dalakas et al., 2020). For instance, a cohort study found that low baseline plasma levels of complement C3 were associated with a high risk of Alzheimer's disease (Rasmussen et al., 2018a). Another clinical study showed that serum levels of C1q, another complement protein, were significantly higher in major depressive disorder patients than in controls (Yao and Li, 2020).

The complement system is an enzyme cascade involving multiple proteins and three different activating pathways: classical, lectin, and alternative. Activation of these different pathways may be associated with different diseases (Botto et al., 2009; Holers, 2014). The classical pathway is initiated by the early complement components C1 complex and C4. And the lectin pathway is activated when complement associated pattern recognition molecules, including MBL, ficolins and collectins bind to carbohydrate moieties on surfaces of pathogens.

One key regulator of the alternative pathway, a loop involving the assembly of the C3 convertase C3bBb, is the complement protein FH (Botto et al., 2009; Holers, 2014). However, the specific complement system activation pathway which is associated with T2DM, cognitive impairment and associated comorbidities is not well understood.

We conducted a cross-sectional study to prospectively determine the specific complement activating pathway, which includes different complement proteins (C1q, C3, C3b, C4, FH), in the pathology of T2DM only, cognitive impairment only, and T2DM combined with cognitive impairment. In addition, we also investigated the relationship between these complement proteins and several clinical risk factors and between cognitive or diabetic functional characteristics.

MATERIALS AND METHODS

Study Participants

Our project included two separate cohorts. The first cohort was from the First Affiliated Hospital of The University of Science and Technology of China, and the second cohort was from the Provincial Sports Bureau Community, Hefei, Anhui, China. The demographics of the participants are described in **Table 1**. A total of 264 participants were enrolled in our study. We classified participants into four groups: normal (n = 70), T2DM only (n = 51), cognitive impairment only (n = 84) and T2DM combined with cognitive impairment (n = 59), predominantly according to fasting blood glucose levels (diabetes group if fasting blood glucose level $\geq$ 7 mmol/L) and scores on either Mini-Mental State Examination (MMSE) or Montreal Cognitive Assessment (MOCA) (cognitive impairment was defined as a score of $\leq$ 26 on either test). We did not consider other conditions that may fulfill the diagnostic criteria for T2DM or cognitive impairment.

Sample Preparation

Blood samples were collected from participants in the morning following an overnight fast. For each participant, blood was collected and centrifuged (2,000 g) for 10 min at room temperature (20–25°C) after allowing the blood to clot for 30 min–1 h. Following centrifugation, serum from all tubes were transferred into 200 μL aliquots, put into 1.5-mL polypropylene protein low-binding tubes which were placed immediately on dry ice and stored at -80°C. Before assays were performed, samples were thawed on ice, and aliquots of 20 μL were transferred into 1.5-mL polypropylene protein low-binding tubes and stored at -80°C. When using these samples, we placed them on ice and thawed again, such that the samples used in our assays had only two freeze cycles to avoid complement activation.

Detection of Serum Complements

Luminex multiplex assays were used to detect all five complement proteins, C1q, C3, C3b, C4, and FH. Serum samples were thawed on ice until assay application. Serum standards and backgrounds were run in duplicate. Following the manufacturer instructions for the human complement magnetic bead panel-2

TABLE 1 | Demographic and clinical characteristics of participants.

	Total (*N* = 264)	Normal (*N* = 70)	T2DM (*N* = 51)	CI (*N* = 84)	T2DM&CI (*N* = 59)	*P*-value
Sex						
Female	129 (48.9%)	39 (55.7%)	15 (29.4%)	51 (60.7%)	24 (40.7%)	0.001
Male	135 (51.1%)	31 (44.3%)	36 (70.6%)	33 (39.3%)	35 (59.3%)	
Age						
Mean (SD)	63.0 (± 8.0)	64.0 (± 9.5)	61.1 (± 7.8)	63.2 (± 7.6)	63.2 (± 6.7)	0.26
Complement proteins						
C1q						
Mean (SD)	93.3 (± 21.3)	93.1 (± 20.4)	85.0 (± 18.1)	101.0 (± 21.2)	89.8 (± 21.6)	0.0001
C3						
Mean (SD)	60.9 (± 25.7)	62.6 (± 29.4)	55.9 (± 21.4)	66.4 (± 24.7)	55.5 (± 24.5)	0.033
C3b						
Mean (SD)	200.8 (± 159.3)	204.4 (± 169.3)	221.2 (± 177.9)	149.0 (± 122.5)	257.0 (± 158.5)	0.0007
C4						
Mean (SD)	214.2 (± 49.9)	210.2 (± 48.3)	210.4 (± 46.8)	224.1 (± 53.2)	208.2 (± 48.6)	0.18
FH						
Mean (SD)	247.0 (± 43.9)	251.8 (± 45.1)	238.6 (± 36.8)	255.6 (± 39.5)	236.2 (± 51.0)	0.022
Clinical biochemical characteristics						
BMI						
Mean (SD)	25.0 (± 11.7)	23.3 (± 2.9)	25.1 (± 3.1)	26.1 (± 19.5)	24.9 (± 3.4)	0.61
HDL						
Mean (SD)	1.2 (± 0.4)	1.3 (± 0.5)	1.0 (± 0.4)	1.3 (± 0.4)	1.0 (± 0.3)	<0.0001
LDL						
Mean (SD)	2.8 (± 0.9)	3.0 (± 0.9)	2.6 (± 0.9)	2.9 (± 0.8)	2.5 (± 0.9)	0.003
TC						
Mean (SD)	4.7 (± 1.3)	4.9 (± 1.0)	4.8 (± 2.1)	4.8 (± 0.9)	4.2 (± 1.2)	0.012
TG						
Mean (SD)	1.7 (± 1.1)	1.4 (± 0.6)	2.3 (± 1.8)	1.5 (± 0.6)	1.6 (± 1.0)	<0.0001
FBG						
Mean (SD)	6.3 (± 2.5)	4.9 (± 0.8)	7.9 (± 2.6)	5.0 (± 0.8)	7.9 (± 3.4)	<0.0001
FINS						
Mean (SD)	10.9 (± 10.3)	8.4 (± 4.3)	13.5 (± 17.9)	9.6 (± 4.9)	12.6 (± 9.2)	0.055
GHB						
Mean (SD)	8.1 (± 2.0)	6.6 (± 1.1)	9.9 (± 2.1)	6.8 (± 0.7)	9.3 (± 1.6)	<0.0001
HbA1C						
Mean (SD)	6.9 (± 1.9)	5.6 (± 0.4)	8.4 (± 2.0)	5.7 (± 0.6)	8.2 (± 1.7)	<0.0001
Homeostasis model assessment characteristics						
HOMO-IR						
Mean (SD)	3.1 (± 3.6)	1.9 (± 1.0)	4.8 (± 6.1)	2.2 (± 1.3)	4.0 (± 3.7)	<0.0001
HOMO-IS						
Mean (SD)	0.6 (± 0.4)	0.8 (± 0.6)	0.5 (± 0.4)	0.6 (± 0.4)	0.4 (± 0.2)	0.0002
HOMO-β						
Mean (SD)	97.1 (± 248.3)	135.9 (± 80.8)	90.0 (± 146.8)	142.3 (± 85.2)	0.7 (± 470.8)	0.017
IAI						
Mean (SD)	–3.9 (± 0.8)	–3.6 (± 0.6)	–4.2 (± 1.0)	–3.7 (± 0.6)	–4.3 (± 0.6)	<0.0001
Cognitive functional characteristics						
MMSE						
Mean (SD)	27.0 (± 3.2)	28.6 (± 1.1)	28.2 (± 1.7)	25.4 (± 4.0)	24.5 (± 3.6)	<0.0001
MOCA						
Mean (SD)	23.3 (± 4.4)	27.0 (± 1.1)	26.7 (± 1.1)	19.7 (± 3.4)	21.3 (± 4.4)	<0.0001

FBG, fasting blood glucose; GHB, glycosylated hemoglobin; HbA1C, glycosylated hemoglobin A1c; HOMA-IR, homeostasis model assessment of insulin resistance; HOMA-IS, homeostasis model assessment of insulin sensitivity; HOMA-β, homeostasis model assessment of beta cell function index; IAI, insulin action index; HDL, high density lipoprotein; LDL, low density lipoprotein; TC, total cholesterol; TG, total triglyceride; HGB, hemoglobin; Ca, calcium; P, phosphate; MMSE, Mini-Mental State Examination; MOCA, Montreal Cognitive Assessment; FINS, fasting insulin; F, female; M, male. Data are presented as the median (standard deviation) where appropriate unless otherwise specified; p-values are derived from chi-square (sex) and Kruskal–Wallis tests (continuous variables).

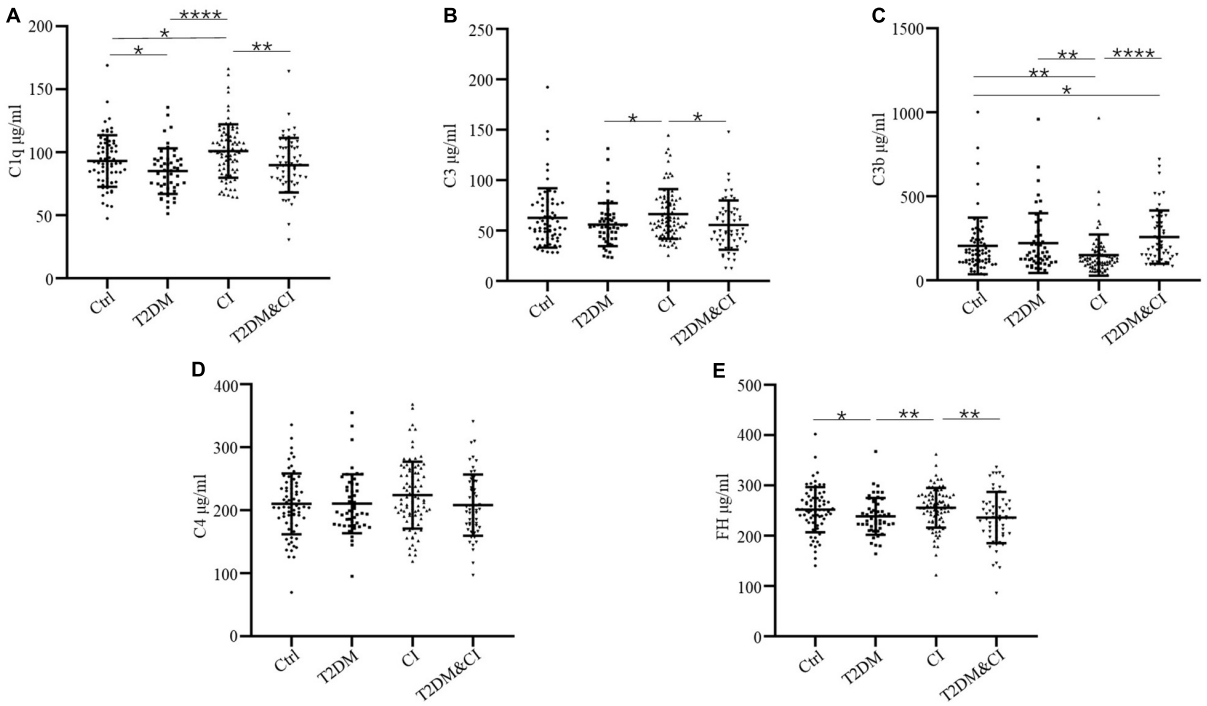

FIGURE 1 | Serum levels of complement proteins in the cross-sectional control (Ctrl), diabetes mellitus only (T2DM), cognitive impairment only (CI) and diabetes combined with cognitive impairment groups (T2DM&CI). Each point represents the value for an individual control or participant, and the horizontal line in point clusters represents the mean level and standard deviation for that group. Bar graphs indicate statistical significance of differences in values for each two-group comparison, calculated by Mann–Whitney *U*-test pairwise comparisons (*$p < 0.05$; **$p < 0.01$; ***$p < 0.005$; ****$p < 0.0001$). See **Table 1** for statistical details. **(A)** C1q, **(B)** C3, **(C)** C3b, **(D)** C4, **(E)** FH.

kit (cat. #HCMP2MAG-19K, Merck, Germany), we diluted serum at 1:8,000 with assay buffer and incubated overnight on a plate shaker with agitation (16–18 h) at 4°C after adding magnetic beads. Detection antibodies were incubated with agitation on a plate shaker for 1 h at room temperature (20–25°C), and streptavidin-phycoerythrin was then incubated for 30 min. After incubation, beads were resuspended using sheath fluid (Luminex Corporation, TX, United States), and median fluorescent intensity (MFI) values were read and analyzed using a Luminex®200™ system (Luminex Corporation, TX, United States).

Statistical Analysis

Data analyses and visualization were performed using Prism 8 (GraphPad Software Inc., La Jolla, CA) and R studio. Medians and standard deviations (SD) were calculated for demographic characteristics as described in **Table 1** and **Figure 1**. Normal data distributions were assessed using the Shapiro–Wilk test and Box-Cox transformation (log-transformed) was applied to non-normal data following the removal of any outliers. Non-parametric comparisons of complement protein concentrations between groups were performed using Mann–Whitney *U*-tests. Age was not adjusted for because there was no significant age difference between groups.

For other clinical characteristics, Kruskal–Wallis tests were used to compare continuous variables between groups, and chi-square tests (χ^2-test) were used to compare dichotomous variables. Specifically, for serum levels of complement proteins: C3 data were log-transformed; C3b data were log-transformed and five outliers were removed; six outliers were removed from C1q data, and four outliers were removed from C4 data. For HOMA index calculations, HOMA-IR values were log-transformed, and 7 outliers were then removed. Eight outliers were removed from the HOMA-β values, and then HOMA-β values were transformed to $[\text{HOMA-}\beta]^{-0.5}$. And seven outliers were removed from the insulin action index (IAI) values.

Associations between serum complement proteins and other clinical characteristics and fasting blood sugar levels were examined using linear regression models either adjusted for age and sex or not adjusted, as described in **Tables 2**, **3**. In the linear regression models, the β coefficient for the risk factors represents the cross-sectional association with the serum complement levels. In addition to examining the associations with clinical characteristics and serum complement proteins, we sought to investigate the association between complement levels and cognitive impairment using generalized linear models, either adjusted for age and sex or not adjusted (see **Table 3**). A positive odds ratio (OR) indicated that an improvement in the serum levels of complement proteins was associated with an increased risk of cognitive impairment.

Statistical significance was indicated by $p < 0.05$.

RESULTS

Comparison of Serum Complement Protein Levels Across All Groups

264 participants were enrolled in our study. Serum levels of C1q, C3, C3b, C4, and FH across each group are shown in **Table 1** and **Figure 1**. Other demographic and clinical characteristics are summarized in **Table 1**. Classification criteria for group assignment are described in the methods.

Levels of C3b in both the T2DM and CI groups were significantly different to healthy controls (mean ± SD, 257.0 ± 158.5 μg/mL for the T2DM&CI group and 204.4 ± 169.3 μg/mL for control group, $p < 0.05$). There were no other differences in protein levels between these groups.

With the exception of C4, there were significant differences in all complement protein levels measured between the T2DM and CI groups. There were significantly higher levels of C1q, C3, and FH in the CI group than in the T2DM group ($p < 0.0001$ for C1q, $p < 0.05$ for C3 and $p < 0.01$ for FH). Conversely, mean serum levels of C3b were higher in the T2DM group than in the CI group ($p < 0.01$). In the T2DM&CI group, C3b levels were significantly higher than in the CI group ($p < 0.0001$).

However, there were no differences in mean serum level of any complement proteins between the T2DM and T2DM&CI groups. In contrast to the above results, mean serum levels of C4 across all patients were indistinguishable from controls (**Figure 1**).

Association of Levels of Complement Proteins With Clinical Parameters

Consistent with the above findings, we found that clinical characteristics were also different between all groups (**Table 1**). We hypothesized that levels of complement proteins would be associated with the hyperglycemia and hyperlipemia indicators. This hypothesis was tested using single factor linear regression models. Full model statistics for all analyses are reported in **Table 2**, which shows that FBG (Fasting blood sugar), GHB (glycated hemoglobin), HbA1C (glycosylated hemoglobin A1c) were significantly associated with higher levels of C3b [β(SE), 0.0402(0.016), $p = 0.013$ for FBG; β(SE), 0.0793(0.0195), $p < 0.0001$ for GHB; β(SE), 0.0734(0.0216),$p < 0.0001$ for HbA1C], and HDL (High density lipoprotein) was significantly associated with lower levels of C3b [β(SE), ® 0.2623(0.0983), $p = 0.0082$]. Similar results were found when the data were adjusted for sex and age [β(SE), 0.046(0.0154), $p = 0.0031$ for FBG; β(SE), 0.0846(0.019), $p < 0.0001$ for GHB; β(SE), 0.0795(0.021), $p < 0.0002$ for HbA1C; β(SE), -0.2856(0.094), $p = 0.0027$ for HDL]. We observed a significant negative relationship between C3 and FBG [β(SE), -0.0212(0.01), $p = 0.034$] and a significant positive relationship between C3 and both LDL (Low density lipoprotein) and TC (Total cholesterol) [β(SE), 0.0968(0.0288), $p < 0.0009$ for LDL; β(SE), 0.0465(0.0195), $p = 0.018$ for TC] (**Table 2**).

We also found that higher levels of C1q were significantly associated with both lower levels of FBG, GHB or HbA1C and greater LDL, TC or TG [β(SE), -1.4775(0.5453), $p < 0.0072$, for FBG; β(SE), -2.6053(0.6883), $p < 0.0002$, for GHB; β(SE), -2.7452(0.7483), $p < 0.0003$, for HbA1C, β(SE), 3.7406(1.5742), $p = 0.018$, for LDL; β(SE), 1.9655(1.057), $p = 0.064$, for TC; β(SE), 2.9051(1.2432), $p = 0.02$, for TG, respectively]. These associations were also statistically significant after adjusting for age and sex, with the exception of the association between C1q and TC. Similarly, FBG, GHB and HbA1c were significantly associated with lower levels of the complement protein FH [β(SE), -2.3995 (1.0747), $p = 0.026$, for FBG; β(SE), -2.4829(1.4209), $p = 0.082$, for GHB; β(SE), -3.0892(1.5385), $p = 0.046$, for HbA1c]. Conversely, there was a significant positive association between FH with both LDL and TC and [β(SE), 11.1083(3.1002), $p < 0.0004$, for LDL; β(SE), 3.0574(2.1191), $p = 0.15$, for TC]. We found that β estimates for C4-LDL positive associations were significant whether or not the data was adjusted for age and sex ($p = 0.018$, adjusted for age and sex; $p = 1.013$, not adjusted for age and sex) (**Table 2**).

Association of Serum Complement Protein Levels With Cognitive Status and Homeostasis Model Assessment

Next, to investigate the functions of complement proteins in pathology, including cognitive impairment and insulin resistance, we derived an estimate of the association between complement proteins and insulin resistance or cognitive function.

First, to better understand the associations between complement proteins and homeostatic model assessment, including HOMA-IR, HOMA-β and IAI, we conducted linear regression analyses (full model statistics are presented in **Table 3**). We found that the complement regulator FH was significantly associated with HOMA-IR and HOMA-β. However, there was a significant negative relationship between FH and IAI when adjustments for age and sex were made [β(SE), -0.0035 (0.0013), $p < 0.0071$]. Complement proteins C1q, C3 and C4 were only significantly associated with HOMA-β, an index of islet beta cell function [β(SE), 0.0377 (0.0124), $p = 0.0026$, for C1q; β(SE), 0.0235 (0.0103), $p = 0.023$, for C3; β(SE), 0.0134 (0.0054), $p = 0.013$, for C4]. Without adjusting for age and sex, there was no association between FH and IAI [β(SE), -0.0025 (0.0013); $p = 0.053$]. In summary, we found associations between increased levels of complement proteins, particularly FH, and insulin resistance.

We further examined the relationship between the levels of complement proteins and cognitive function using general linear models. To obtain a better understanding of the relationships between complement proteins and the cognition, we stratified individuals according to the presence of cognitive impairment (i.e., impaired cognition vs. normal cognition). We found a statistically significant relationship between cognitive group and C1q. The estimated odds ratio (OR) for dementia was significantly higher in participants with elevated C1q (odds ratio (OR), 1.0155; (95% CI, 1.0036–1.0282); $P = 0.012$), even after controlling for age and sex (odds ratio (OR), 1.0146 (95% CI, 1.0024–1.0275); P = 0.021). This indicates that individuals with higher serum levels of C1q tended to have a greater likelihood of

TABLE 2 | Associations of complement protein with clinical parameters.

	C3b		C3		C1q		C4		FH	
	Unstandardized estimate (SE)	***P*-value**	**Unstandardized estimate (SE)**	***P*-value**	**Unstandardized estimate (SE)**	***P*-value**	**Unstandardized estimate (SE)**	***P*-value**	**Unstandardized estimate (SE)**	***P*-value**
Adjusted for sex and age										
BMI	0.0012 (0.0033)	0.71	0.001 (0.0022)	0.66	0.0689 (0.1173)	0.56	0.2207 (0.2752)	0.42	0.2144 (0.2324)	0.36
FBG	0.0402 (0.016)	0.013[a]	-0.0212 (0.01)	0.034[a]	-1.4775 (0.5453)	0.0072[b]	-1.5446 (1.2994)	0.24	-2.3995 (1.0747)	0.026[a]
GHB	0.0793 (0.0195)	<0.0001[c]	-0.0062 (0.0136)	0.65	-2.6053 (0.6883)	0.0002[c]	-0.7675 (1.6239)	0.64	-2.4829 (1.4209)	0.082
HbA1C	0.0734 (0.0216)	<0.0001[c]	-0.0137 (0.0147)	0.35	-2.7452 (0.7483)	0.0003[c]	-1.6784 (1.7594)	0.34	-3.0892 (1.5385)	0.046[a]
HDL	-0.2623 (0.0983)	0.0082[b]	0.002 (0.0672)	0.98	2.6561 (3.6209)	0.46	-2.4891 (8.3662)	0.77	-6.7959 (7.2062)	0.35
LDL	-0.057 (0.0443)	0.20	0.0968 (0.0288)	0.0009[c]	3.7406 (1.5742)	0.018[a]	8.6559 (3.6348)	0.018[a]	11.1083 (3.1002)	0.0004[c]
TC	-0.0133 (0.0295)	0.65	0.0465 (0.0195)	0.018[a]	1.9655 (1.057)	0.064	3.4541 (2.4484)	0.16	3.0574 (2.1191)	0.15
TG	0.0522 (0.034)	0.13	0.0362 (0.0232)	0.12	2.9051 (1.2432)	0.02[a]	1.0342 (2.9034)	0.72	4.8482 (2.4938)	0.053
Not adjusted for sex and age										
BMI	0.0006 (0.0033)	0.87	0.0018 (0.0023)	0.44	0.0875 (0.1197)	0.47	0.2636 (0.2761)	0.34	0.243 (0.2354)	0.30
FBG	0.046 (0.0154)	0.0031[b]	-0.0338 (0.01)	0.0008[c]	-1.8695 (0.5304)	0.0005[c]	-2.249 (1.252)	0.074	-3.123 (1.044)	0.0031[b]
GHB	0.0846 (0.019)	<0.0001[c]	-0.0186 (0.0137)	0.18	-3.0719 (0.6801)	<0.0001[c]	-1.599 (1.596)	0.32	-3.341 (1.398)	0.018[a]
HbA1C	0.0795 (0.021)	0.0002[c]	-0.0273 (0.0148)	0.067	-3.2713 (0.7375)	<0.0001[c]	-2.556 (1.724)	0.14	-4.029 (1.509)	0.0082[b]
HDL	-0.2856 (0.094)	0.0027[b]	0.0783 (0.0664)	0.24	6.223 (3.504)	0.077	2.663 (8.008)	0.74	0.5837 (6.9859)	0.93
LDL	-0.0581 (0.0439)	0.19	0.1043 (0.0295)	0.0005[c]	4.492 (1.576)	0.0048[b]	9.211 (3.593)	0.011[a]	12.275 (3.081)	<0.0001[c]
TC	-0.0193 (0.029)	0.51	0.0573 (0.0197)	0.004[b]	2.66 (1.049)	0.012[a]	4.193 (2.399)	0.082	4.278 (2.092)	0.042[a]
TG	0.0587 (0.0342)	0.087	0.0277 (0.0241)	0.25	2.709 (1.27)	0.034[a]	0.5836 (2.9122)	0.84	4.527 (2.528)	0.075

BMI, body mass index; FBG, fasting blood glucose; FINS, fasting serum insulin; GHB, glycosylated hemoglobin; HbA1C, glycosylated hemoglobin A1c; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TC, total cholesterol; TG, total triglycerides; HGB, hemoglobin.

The results were obtained using linear regression models with or without adjustment for age and sex. Numbers presented are unstandardized estimates (SEs) and P-values for the association of each independent variable with complement proteins. Some outliers were removed from dependent variable data or Box–Cox transformed for normalization.

[a−c]Indicates a statistically significant difference: $p < 0.05$, $p < 0.01$, $p < 0.001$.

cognitive impairment (**Table 3**). No other complement protein was associated with cognitive group.

DISCUSSION

In this study, we found that there were significant differences between patients with cognitive impairment only, T2DM, or T2DM with cognitive impairment in the levels of the complement proteins C1q, C3, C3b and the complement regulatory protein FH (**Figure 1**). These results suggest that there are different complement activation pathways underlying cognitive impairment without T2DM and cognitive impairment with T2DM.

First, serum C1q was significantly higher in individuals with cognitive impairment only than in individuals with cognitive impairment and T2DM. Previous work has revealed detrimental effects of C1q in neuronal injury and nervous system disease through the promotion of amyloid plaque accumulation, phosphorylated neurofibrillary tangles and the exacerbation of neuroinflammation (Hong et al., 2016; Morgan, 2018; Lee et al., 2019). In addition, studies have demonstrated that the classical pathway complement proteins, C1q, C3, C4 in particular, are involved in the pathology of neurological diseases, including Alzheimer's disease, through the facilitation Aβ clearance or synapse engulfment by reactive microglia (Loeffler, 2004; Propson et al., 2021). Moreover, C1q activates the complement proteins C1r and C1s by binding to Ig-G and Ig-M, resulting in the cleavage of downstream C4 and activation of the classical pathway (Sarma and Ward, 2011). However, there was no difference in serum C4 between the groups in the present study. Many recent studies have found that C4 is related to central nervous system disorders, particularly schizophrenia (Sarma and Ward, 2011). However, no study has found a link between C4 and cognitive impairment. Thus, it is likely that C4 is not involved in the pathogenesis of cognitive impairment (without T2DM).

The activation of the classical complement pathway may come from the actived astrocytes and microglia. Previous research has also shown that neuroinflammation, activation of astrocytes and microglia are hallmarks of the neurological diseases and cognitive impairment. And the reactived astrocytes and microglia secrete C3 and C1q (Propson et al., 2021). Meanwhile other pathological characteristics of the neurodegeneration, such as Aβ and hyperphosphorylated tau could also increase the levels and depositions of C1q as well (Hong et al., 2016; Schartz and Tenner, 2020). These compelling studies may help inspire thinking for the activation mechanism of the classical complement pathway.

Interestingly, serum levels of C3b, a byproduct of the alternative pathway, were lower in the cognitive impairment only group. C3b is an integral part of the C3 transformation enzyme C3Bb, which enhances the activation stage of the alternative pathway (Ghosh et al., 2015). This indicates that the alternative pathway was inhibited in the cognitive impairment only group. Studies have also shown that C3b plays an important role

TABLE 3 | Associations of insulin resistance or cognitive function with complement proteins. **(A)** Linear regression analysis assessing complement proteins associated with insulin resistance and β-cell function. **(B)** Logistic regression analysis assessing complement proteins associated with cognitive impairment.

	HOMA-IR		HOMA-β		IAI	
	Unstandardized estimate (SE)	***P*-value**	**Unstandardized estimate (SE)**	***P*-value**	**Unstandardized estimate (SE)**	***P*-value**
A						
Adjusted for sex and age						
C1q	0.0024 (0.0023)	0.29	0.0377 (0.0124)	0.0026[b]	-0.0019 (0.0026)	0.46
C3	0.0024 (0.0019)	0.19	0.0235 (0.0103)	0.023[a]	-0.0026 (0.0021)	0.22
C3b	0.0004 (0.0003)	0.22	-0.001 (0.0017)	0.57	-0.0005 (0.0004)	0.20
C4	0.0017 (0.001)	0.088	0.0134 (0.0054)	0.013[a]	-0.0017 (0.0011)	0.12
FH	0.0036 (0.0011)	0.0018[b]	0.0195 (0.0064)	0.0028[b]	-0.0035 (0.0013)	0.0071[b]
Not adjusted for sex and age						
C1q	0.0013 (0.0022)	0.57	0.0443 (0.0119)	0.0003[c]	-0.0002 (0.0025)	0.95
C3	0.0013 (0.0018)	0.46	0.03 (0.0099)	0.0027[b]	-0.0009 (0.002)	0.65
C3b	0.0005 (0.0003)	0.13	-0.0018 (0.0017)	0.30	-0.0006 (0.0004)	0.083
C4	0.0014 (0.001)	0.17	0.016 (0.0053)	0.0029[b]	-0.0013 (0.0011)	0.24
FH	0.0029 (0.0011)	0.0096[b]	0.0229 (0.0062)	0.0003[c]	-0.0025 (0.0013)	0.053

B

	Model1[d]			Model2		
	OR	**95%CI**	***P*-value**	**OR**	**95%CI**	***P*-value**
C1q	1.0146	(1.0024, 1.0275)	0.021[e]	1.0155	(1.0036, 1.0282)	0.012[e]
C3	1.0017	(0.9919, 1.0118)	0.74	1.0033	(0.9938, 1.0131)	0.50
C3b	0.9994	(0.9978, 1.0010)	0.45	0.9992	(0.9976, 1.0008)	0.32
C4	1.0027	(0.9978, 1.0077)	0.29	1.0030	(0.9981, 1.0080)	0.24
FH	1.0002	(0.9946, 1.0059)	0.94	1.0007	(0.9952, 1.0063)	0.80

HOMA-IR, homeostasis model assessment of insulin resistance; HOMA-β, homeostasis model assessment of beta cell function index; IAI, insulin action index.
The results were obtained using linear models with or without adjustment for age and sex. Numbers presented are estimates (SEs) and P-values for the association of each complement protein with homeostasis model assessment. Some dependent variables were Box–Cox transformed, and/or some observation points were removed for normalization.
[a−c]Indicates a statistically significant difference: $p < 0.05$, $p < 0.01$, $p < 0.001$.
OR, odds ratio. The results were obtained using logistic regression analysis with or without adjustment for age and sex. Numbers presented are ORs and P-values for the association of each complement protein with cognitive status.
[d]Adjusted for age and sex.
[e]Indicates a statistically significant difference: $p < 0.05$.

in the nervous system. On the one hand, it promotes brain maturation through synaptic conditioning, neuron migration and synaptic trimming to promote complete functional brain maturity (Coulthard et al., 2018; Lee et al., 2019; Reis et al., 2019) and on the other hand, C3b/iC3Bb is also deposited around the damaged brain (Lee et al., 2019; Reis et al., 2019). However, the C3 protein and other byproducts, such as C3d, are considered to be closely related to diabetes and its complications, although there is limited research on C3b and diabetes (Ajjan and Schroeder, 2019). The present study found that the serum levels of C3b were highest in the comorbid T2DM group, indicating that the activation of the alternative pathway may play a key role in T2DM-related cognitive disorder.

The main function of the component protein FH is to inhibit the formation of C3 transformase C3bBb, thereby avoiding excessive activation of the complement system (Botto et al., 2009). Clinical studies have demonstrated that FH is higher in individuals with insulin resistance, metabolic dysfunction, and obesity (Moreno-Navarrete and Fernandez-Real, 2019; Shim et al., 2020). Our current study found that serum FH was lower in the T2DM group than in the control group, which may contribute to the activation of the alternative pathway in T2DM.

Meanwhile, the alternative complement pathway is activated in obese T2DM individuals. Many studies have also demonstrated that adipose tissue is a major site of synthesis of the necessary components for alternative complement pathway (Vlaicu et al., 2016; King and Blom, 2021). Moreover, components of the alternative pathway are secreted from the activated adipocyte as well as the hepatocyte under obesogenic and hyperlipidaemic conditions in obese T2DM patients, and are especially induced by post-prandial hyperchylomicronaemia (Fujita et al., 2013). The overexpression of the complement proteins also leads to positive feedback of complement activation, expression and related pathology (King and Blom, 2021).

In our study, we found that the classical complement pathway underlies cognitive impairment and the alternative complement pathway underlies T2DM pathology when combined with cognitive impairment. The mechanisms of

the different complement activation pathways stem from different pathologic features of different types of cognitive impairment. In detail, the chronic metabolic disease T2DM and the activation of the alternative complement pathway in cognitive impaired individuals with T2DM is likely due to T2DM pathology, especially as it is closely related to the obese and activated fat cells.

In this study, we also observed that blood glucose indicators, including FBG, GHB, and HbA1c, were associated with serum levels of complement proteins. Hyperglycemia was associated with lower levels of C1q, C3, and FH but higher serum levels of C3b. This suggests that hyperglycemia may cause activation of the classical pathway and inhibit the alternative pathway. These results are consistent with some cross-sectional and longitudinal observations between plasma levels of complement protein and high blood sugar (Wlazlo et al., 2014; Borne et al., 2017). Moreover, we observed that higher LDL was correlated with higher serum levels of C1q, C3, C4 and FH. This thus suggests that hyperlipidemia may also result in activation of the classical pathway and inhibition of the alternative pathway. Contrary results suggest that hyperlipidemia and hyperglycemia may affect complement activation through different pathways (Jones, 2016), although there is a close relationship between glucose metabolism and lipid metabolism (Jones, 2016).

T2DM is a metabolic disorder characterized by chronic hyperglycemia and hyperlipidemia (DeFronzo et al., 2015; Chatterjee et al., 2017). Hyperglycemia may induce tissue damage typical of diabetic complications (Ghosh et al., 2015) and increase the hazard ratio for dementia (Crane et al., 2013). In addition, neurodegenerative diseases, especially AD, are often accompanied by symptoms of energy metabolism imbalance (Kapogiannis and Mattson, 2011). In the present study, we reveal a significant relationship between the elevated complement proteins C1q, C3, C4, and FH and enhanced β-cell function (HOMA-β) and insulin resistance (HOMA-IR). In particular, FH, which is involved in the alternative pathway, may be a vital independent risk factor for insulin resistance [β(SE), 0.0195 (0.0064), P = 0.0028]. Additionally, we found that patients with higher C1q had a higher level of cognitive dysfunction (when adjusted for sex and age; odds ratio, 1.0146 (95% CI, 1.0024–1.0275), P = 0.021), which indicates that serum C1q could be a useful novel early warning sign for cognitive impairment; other work has confirmed that C1q is associated with cognitive impairment (Stevens et al., 2007; Hong et al., 2016; Morgan, 2018; Cho, 2019; Hammond et al., 2019). These results indicate that abnormal blood glucose and lipids may be a risk factor affecting serum complement protein levels and activation of the complement pathway.

Moreover, there is a strong link between diabetes and cognitive impairment; the risk for dementia is higher in T2DM patients than in those without T2DM (Biessels et al., 2006; Biessels and Despa, 2018). The ability of some drugs, such as metformin, which is used to in the treatment of T2DM to lower blood glucose and also improve cognitive decline, also confirm the relationship between neuro-psychiatric disorders and metabolic disorders (Markowicz-Piasecka et al., 2017). However, the specific mechanism between T2DM and cognitive impairment is still not clear. Some studies have demonstrated that insulin resistance in the CNS is a mechanistic mediator of structural brain and cognitive deficits *via* inflammation, oxidative stress and direct cellular effects (Biessels and Reagan, 2015; Arnold et al., 2018). In our present study, we verified that complement activation may underlie the pathology of T2DM and cognitive impairment. This is consistent with previous evidence showing that complement proteins accumulate in the focal areas associated with T2DM or neurodegenerative disorders and consistent with several clinical experiments showing the involvement of serum complement proteins (Fujita et al., 2013; Ghosh et al., 2015; Morgan and Harris, 2015; Hong et al., 2016; Ajjan and Schroeder, 2019; Hammond et al., 2019; Lee et al., 2019; Shim et al., 2020). In addition, there is evidence that complement enrichment may cause blood–brain barrier damage and cognitive impairment *via* apoptosis of brain endothelial cells, causing infiltration of inflammatory cells and consequent opening of tight junction constructs, which modulate the generation of cytokines and chemokines (Veerhuis et al., 2011; Jacob and Alexander, 2014; Alexander, 2018; Morgan, 2018; Dalakas et al., 2020; Propson et al., 2021). We found a difference in serum levels of complement proteins between the comorbidity group and cognitive impairment group, which indicates that complement proteins cause different types of nerve injury through different activation pathways. Higher serum levels of C1q, C3 and FH in the cognitive impairment only group indicate that activation of the classical pathway and inhibition of the alternative pathway underlie the neuronal damage within this group. Conversely, activation of the alternative pathway results in T2DM-related cognitive damage.

In summary, we found different serum levels of complement proteins between patient groups and found that were risk factors for these changes. We further determined the specific complement activating pathways underlying different types of neurotoxicity in patients with cognitive impairment only and T2DM-related cognitive damage. Our findings are limited since they are based solely on mathematical models; however, they use data from a relatively large pool of individuals. Future longitudinal investigations using larger participant populations are required to validate our original findings.

LIMITATIONS

Given the nature of our study design, care needs to be taken with interpretation. First, there were no other physical diagnostic tools used as evidence of cognitive function. Neuroimaging or CSF biomarkers would permit more definitive interpretation. Second, we did not determine whether the type of cognitive impairment we measured was a diabetic complication. Long-term follow-up cohorts may verify this in the future. Third, participants in the study were primarily Anhui people and cannot adequately reflect the racial and ethnic diversity of China or the Chinese population, even the larger global population. Participants from other regions and global multi countries will need to be included

in the future. Moreover, potential confounding variables that may affect cognitive function or serum complement levels, such as unhealthy lifestyle or past disease history, were not accounted for. As such, there is much groundwork to be done before we can exploit the potential role of complement proteins as prognostic markers.

CONCLUSION

As noted above, we present new evidence that pathology relating to insulin resistance and cognitive dysfunction may be induced by different complement activating pathways in a manner dependent on abnormal blood glucose and lipid fluctuations. Given the growing morbidity of individuals with T2DM and dementia, our complement protein findings may help diagnose, predict or stratify T2DM and cognitive impairment early and accurately.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of Anhui Provincial Hospital Medical Research (approval #89). Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

ZL: acquisition, analysis, interpretation of data, drafting of the manuscript, and statistical analysis. ZL and WZ: collection of samples. FG and YS: critical revision of the manuscript for important intellectual content. YS, DK, and QT: funding acquisition and supervision. All authors have contributed to the work and agree with the presented results.

FUNDING

This work was supported by the Chinese Academy of Sciences (XDB39000000), the Foundation from Department of Science and Technology of Anhui Province (202004j07020012), and the Foundation from Anhui Provincial Health Commission (AHWJ2021b079).

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frontiers in Aging Neuroscience

REVIEW
published: 31 March 2022
doi: 10.3389/fnagi.2022.863901

Effects of the FNDC5/Irisin on Elderly Dementia and Cognitive Impairment

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Population aging is an inevitable problem nowadays, and the elderly are going through a lot of geriatric symptoms, especially cognitive impairment. Irisin, an exercise-stimulating cleaved product from transmembrane fibronectin type III domain-containing protein 5 (FNDC5), has been linked with favorable effects on many metabolic diseases. Recently, mounting studies also highlighted the neuroprotective effects of irisin on dementia. The current evidence remains uncertain, and few clinical trials have been undertaken to limit its clinical practice. Therefore, we provided an overview of current scientific knowledge focusing on the preventive mechanisms of irisin on senile cognitive decline and dementia, in terms of the possible connections between irisin and neurogenesis, neuroinflammation, oxidative stress, and dementia-related diseases. This study summarized the recent advances and ongoing studies, aiming to provide a better scope into the effectiveness of irisin on dementia progression, as well as a mediator of muscle brain cross talk to provide theoretical support for exercise therapy for patients with dementia. Whether irisin is a diagnostic or prognostic factor for dementia needs more researches.

Keywords: dementia, irisin, muscle brain cross talk, BDNF, inflammation, oxidative stress

OPEN ACCESS

Edited by:
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Specialty section:
This article was submitted to Alzheimer's Disease and Related Dementias, a section of the journal Frontiers in Aging Neuroscience

Received: *27 January 2022*
Accepted: *01 March 2022*
Published: *31 March 2022*

Citation:
Peng J and Wu J (2022) Effects of the FNDC5/Irisin on Elderly Dementia and Cognitive Impairment. Front. Aging Neurosci. 14:863901. doi: 10.3389/fnagi.2022.863901

INTRODUCTION

The world has entered an aging society. In addition to chronic diseases, the elderly is accompanied by a series of geriatric symptoms. Cognitive impairment is a classic symptom of geriatric syndrome, which occurs from mild cognitive impairment (MCI) to dementia (Sanford, 2017). MCI is an intermediate state between neurotypical cognition and neurodegenerative dementia (Petersen et al., 2018). The prevalence of MCI in the elderly population aged ≥60 years is approximately 6.4–25% and increases with age according to the American Academy of Neurology (AAN) guidelines (Cheng et al., 2017). Dementia is the most serious form of cognitive impairment; diminishes the physical and mental function of older people, quality of life, and disability; and is the fifth leading cause of death (Winblad et al., 2004).

There were some risk factors of MCI and dementia, such as cardiovascular diseases (Schumacher et al., 2013), inflammation (Huh et al., 2014), and stroke (Chen et al., 2019). Alzheimer's disease (AD) is a classic type of dementia, which is characterized at the neuropathological level by deposits of insoluble amyloid β-peptide (Aβ) in extracellular plaques and aggregated Tau proteins (Hodson, 2018). Developing evidence suggested that decreased brain-derived neurotrophic factor (BDNF) (Amidfar et al., 2020) and damaged synaptic plasticity (Skaper et al., 2017) led to dementia. However, the mechanism remains to be clarified.

Irisin, a myokine containing 112 amino acids, is secreted by skeletal muscle after exercise stimulation, which was first found in 2012 by Boström et al. (2012). It is processed from the type I membrane protein encoded by the FNDC5 gene, then secreted into the blood and circulated to several systems, and passed through the blood-brain barrier (BBB). Irisin consists of an N-terminal fibronectin III (FN III)-like domain attached to a flexible C-terminal tail and a continuous inter-subunit β-sheet dimer (Mahgoub et al., 2018). This structure is stabilized because of the hydrogen bonds and its interactions between the side chains of adjacent subunits, especially between Arg-75 and Glu-79, thus protecting the dimer ends and Trp-90/Trp-90 (Schumacher et al., 2013). Peroxisome proliferator-activated receptor γ (PPARγ) coactivator-1 α (PGC-1α) is the main regulator of FNDC5 in skeletal muscles in rodents and humans (Huh et al., 2014). Endurance exercise activates on PGC-1α to induce cleavage of FNDC5 to irisin. PGC-1α interacts with a wide range of transcription factors, and it is expressed in skeletal muscle, heart, and brain (Lin et al., 2002). It interacted with several pathways such as the p38 mitogen-activated protein kinase (MAPK) pathway stimulated by exercise (Akimoto et al., 2005), 5' adenosine monophosphate-activated protein kinase (AMPK) pathway (Chen et al., 2019), Sirtuin1 (Sirt1) pathway (Safarpour et al., 2020), and the cyclic adenosine monophosphate (cAMP) response element-binding (CREB) pathway. The cAMP-mediated PGC-1α/CREB signaling bolstered the expression of FNDC5 (Yang et al., 2018). Besides, FNDC5 and irisin expressed in many tissues, such as skeletal muscle, pancreas, brown adipose tissue (BAT), liver, and brain, especially in the hippocampus and hypothalamus, are important for memory and cognition (Dun et al., 2013; Varela-Rodríguez et al., 2016).

Irisin was associated with various metabolic diseases such as diabetes, cardiovascular disease, and obesity (Polyzos et al., 2018). It induced the expression of mitochondrial uncoupling protein 1 (UCP1) (Castillo-Quan, 2012), increasing thermogenesis and converting white adipose tissue (WAT) into BAT. Furthermore, irisin exerted favorable effects on glucose metabolism to maintain glucose homeostasis and improve insulin resistance, of which mechanisms involved β cell regeneration (Natalicchio et al., 2017), reducing gluconeogenesis and promoting glycogen synthesis (Polyzos et al., 2013; Roca-Rivada et al., 2013). Besides, irisin performed a protective function on lipid metabolism involving several pathways such as the AMPK-SREBP2 pathway (Tang et al., 2016). It was also antioxidative, anti-inflammatory, and attenuating apoptosis, functioning to alleviate mitochondrial dysfunction (Mazur-Bialy et al., 2017b; Tu et al., 2020; Zhang et al., 2020). Many studies have reported that irisin had neuroprotective functions in AD (Kim and Song, 2018; Lourenco et al., 2019; de Freitas et al., 2020). Lourenco et al. (2019) elucidated that FNDC5/irisin was decreased in AD brains and CSF and in AD experimental models, but there was no significance in plasma irisin levels. Conti et al. (2019) reported a slight increase in irisin serum levels in patients with AD. Zhang et al. (2021) suggested that serum irisin might be a biomarker of cognitive decline in vascular dementia. Bičíková et al. (2021) reported that movement was a positive modulator of aging and the PPARγ is a critical link between mental function and aging. FNDC5/irisin is stimulated by PGC-1α, indicating irisin might be the mediator of muscle and brain cross talk. Some clinical observations and mechanisms were reported.

We try to summarize the research on the relationship between irisin and cognitive impairment and to understand the mechanisms of direct neuroprotective and indirect risk reduction. This study intended to explore whether irisin is a potential serum predictor of cognitive impairment in the elderly and an underlying mediator of muscle-brain cross talk to support exercise therapy for patients with dementia.

FNDC5/IRISIN IN MUSCLE-BRAIN CROSS TALK

Accumulating evidence is supporting the existence of muscle brain cross talk, a muscle-brain endocrine loop (Pedersen, 2019). Brain sensed exercise indirectly *via* adiponectin and liver-derived proteins such as fibroblast growth factor 21 (FGF21) and insulin-like growth factor 1 (IGF1), and muscle secreted myokines to regulate the brain function as a loop. The exercise was believed to decrease the risk of dementia (Santos-Lozano et al., 2016), delay the cognitive decline in patients with neurodegenerative disorders and prevent stress, anxiety, and depression (Pedersen and Saltin, 2015). The underlying mechanism might be the muscle brain cross talk. The physical activity enhanced circulating levels of myokines to enable the direct cross talk of muscle and brain, affecting neuronal proliferation and differentiation, synaptic plasticity, memory, and learning (Scisciola et al., 2021).

The exercise was tightly related to the PGC1-α/FNDC5/BDNF pathway. FNDC5 gene expression was elevated following the increased PGC-1α expression induced by exercise both in central and peripheral organs, which stimulated the expression of BDNF in the brain (Boström et al., 2012). Irisin, as a myokine dissected from FNDC5, was also mediated by PGC-1α and passed through the BBB to increase the BDNF expression and enhance learning, memory, and mood (Lourenco et al., 2019). On the one hand, periphery irisin delivered to the brain and overexpressed irisin in the brain increased BDNF. On the other hand, knockdown of FNDC5 reduced the central BDNF expression (Severinsen and Pedersen, 2020). **Figure 1** elucidated that irisin acted as a mediator of muscle brain cross talk and the effects of FNDC5/irisin on elderly cognition.

FNDC5/IRISIN ACT ON CNS

FNDC5/Irisin and Neurogenesis

Brain-derived neurotrophic factor expresses highly in the brain, and it has considerable effects on synapses (Lu et al., 2014). It, mostly released from microglia and astrocytes, acts to promote synaptic plasticity, neuronal survival, neuronal differentiation, and neuronal health (Binder and Scharfman, 2004; Zuccato and Cattaneo, 2009). It was well-related to

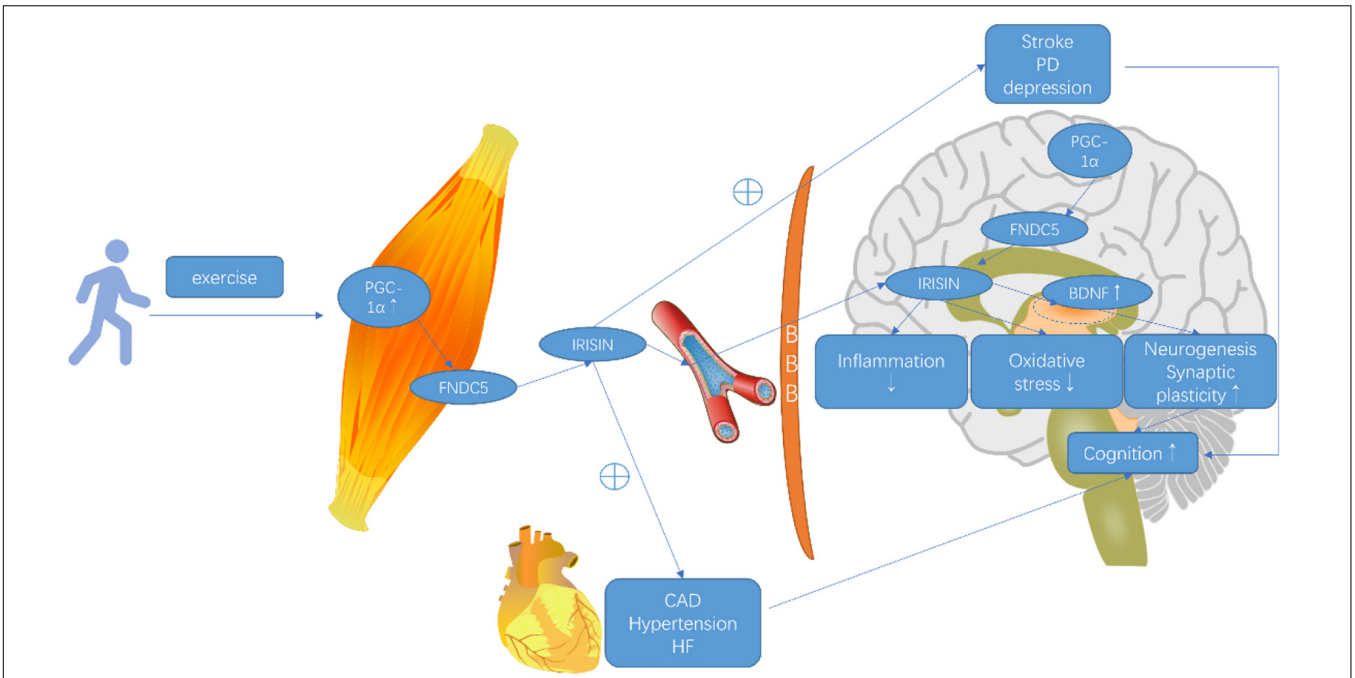

FIGURE 1 | Effects of FNDC5/irisin on elderly dementia and cognition. Exercise promoted PGC-1α, which stimulated fibronectin type III domain-containing protein 5 (FNDC5) to be dissected into irisin. Irisin was shed into a blood vessel, circulating the whole body and passing through blood–brain barrier. Irisin might increase the expression of brain-derived neurotrophic factors, which improved synaptic plasticity, neuronal survival, neuronal differentiation, and neuronal health, thus cognition. FNDC5 was also highly expressed in hippocampus and stimulated by peroxisome proliferator-activated receptor γ (PPARγ) coactivator-1 α (PGC-1α) to be cleaved into irisin. Besides, irisin acts with its anti-inflammation and anti-oxidative effects to defend cognition deficits. Furthermore, FNDC5/irisin might have positive connections to dementia-related diseases, such as coronary artery disease, hypertension, heart failure, stroke, and Parkinson's disease, and depression to protect against dementia.

neurofunction and cognition. BDNF is bound to tropomyosin-related kinase B (TrKB) receptor to exert considerable effects. Decreased BDNF/TrkB activity resulted in neurodegeneration. Downregulation of BDNF/TrkB caused neuroinflammation, increasing inflammatory cytokines such as IL-1β and IL-6. Then triggered the JAK2/STAT3 pathway, resulting in the upregulation of C/EBPβ/AEP signaling, which led to Aβ precursor protein and Tau protein cleavage, and the Aβ and Tau alterations finally caused cognitive impairment (Wang Z. H. et al., 2019). Many studies reported BDNF levels decreased in AD patients and MCI (Tanila, 2017).

Circulating and central irisin acted on the brain to exert beneficial effects. Irisin bound and modified the function of neurotransmitter receptors in the forebrain, then neurons. The receptor of irisin in the brain was integrin-αV/β5 heterodimers (Jackson et al., 2021). Recombinant irisin stimulated the cAMP/PKA/CREB pathway in human cortical slices (Lourenco et al., 2019). CREB protein is a cellular transcription factor that plays a widely confirmed role in neuronal plasticity and long-term memory formation in the brain (Sen and Stress, 2019). Irisin increased cAMP and phosphorylated CREB (pCREB) in mouse hippocampal slices, which bolstered the expression of BDNF. According to the study by Lourenco et al. (2019), irisin-induced CREB phosphorylation was mediated by PKA. Fahimi et al. (2017) reported that mice after exercises appeared appreciable increase in BDNF mRNA and protein levels, distinctively elevated synaptic load in the dentate gyrus, and increased irisin and TrkB receptor levels in the astrocytes, indicating that irisin might mediate the effects of exercise on brain function and could be a messenger of periphery and central cross talk. Zsuga et al. (2016) proposed that irisin may be a mediator between exercise and reward-related learning and motivation through the irisin-BDNF/TrKB-MEK/ERK-mTOR pathway. The TrKB linked with dopamine 3 (D3) receptor signaling such as PI3/Akt/mTOR pathway was also involved. The two pathways were under the control of BDNF and caused increased dopamine content, neuronal plasticity, and raised neuronal survival (Collo et al., 2014). Moon et al. (2013) described that irisin performed favorable effects on hippocampal neuron proliferation primarily *via* the STAT3 signaling pathway. Activation of STAT3 has been confirmed to correlate with stimulating hippocampal neurogenesis (Jung et al., 2006).

FNDC5 was highly expressed in the brain especially in the hippocampus (Wrann et al., 2013; Lourenco et al., 2019). Neuronal FNDC5 gene expression was also regulated by PGC-1α. The orphan nuclear receptor estrogen-related receptor alpha (ERRα) was a central metabolic regulator interacting with PGC-1α (Schreiber et al., 2004). Wrann et al. (2013) found that ERRα was up-regulated in the hippocampus upon exercise. Furthermore, FNDC5 regulated BDNF gene expression in a cell-autonomous manner, and BDNF decreased FNDC5 gene expression as a part of a potential feedback loop.

Elevated expression of FNDC5 strikingly up-regulated BDNF gene expression. Moreover, peripheral delivery of FNDC5 also increased BDNF expression in the hippocampus, and ERK1/2 was a critical regulator of FNDC5 expression and function on neuronal differentiation (Hosseini Farahabadi et al., 2015; Wrann, 2015). In addition to the direct regulation of FNDC5 to BDNF, irisin was also processed from FNDC5 in the hippocampus. Thus, FNDC5/irisin acted as a messenger of muscle brain cross talk, influencing the neurogenesis in cognitive impairment, in particular through the neuroprotective effects of BDNF.

FNDC5/Irisin and Inflammation

Emerging evidence suggested the importance of inflammation in the pathogenesis of AD and mild cognitive impairment (Holmes, 2013; Shen et al., 2019). According to a meta-analysis of 170 studies, patients with AD and MCI were accompanied with elevated inflammatory markers in both CSF and periphery, such as C-reactive protein (CRP), interleukin-6 (IL-6), soluble tumor necrosis factor receptor 1 (sTNFR1), soluble tumor necrosis factor receptor 2(sTNFR2), alpha1-antichymotrypsin (α1-ACT), IL-1β, soluble CD40 ligand, IL-10, monocyte chemoattractant protein-1 (MCP-1), transforming growth factor-beta 1(TGF-β1), soluble triggering receptor expressed on myeloid cells 2 (sTREM2), and so on (Shen et al., 2019).

The most common neuroinflammation is postoperative. Disruption of the BBB is the hallmark of neuroinflammation; BBB dysfunction like increased BBB permeability has been regarded as accounting for cognitive impairment (Yang et al., 2017). Surgical trauma induced the innate immune system of the brain through the nuclear factor-κB (NF-κB) pathway, leading to endothelial dysfunction and increased permeability of the BBB (Alam et al., 2018). The neuroinflammation consequences included neuronal apoptosis, damaged hippocampal neurogenesis, and impaired synaptic plasticity connections, resulting in neurodegenerative diseases (Zhang et al., 2016; Feng et al., 2017; Alam et al., 2018).

Another type of neuroinflammation is obesity-related inflammation. Obesity is related to chronic low-grade systemic inflammation (Gregor and Hotamisligil, 2011; Spencer, 2013). Inflammatory cascade was initiated by the stimulation of free fatty acid and lipopolysaccharide (LPS) receptor, toll-like receptor 4 (TLR4) on immune cells (Shu et al., 2012). The downstream factors of the TLR family signaling involve the adapter molecule MyD88, which activated NF-κB and MAPK pathways. Both of them were important for the production of cytokines and chemokines (Trinchieri and Sher, 2007; Lim and Staudt, 2013). Maric et al. (2014) suggested that the hypothalamic mRNA expression of IL-1β, IL-6, and TNF-α significantly increased in high-saturated fat (HSF)-diet rats. Qin et al. (2007) investigated that LPS-induced MAPK and STAT-3 activation, as well as the expression of IL-10, made a difference to the suppressor of cytokine signaling 3 (SOCS3) transcription and expression in macrophages and microglia, which alleviated adaptive and innate immune responses. SOCS3 activated the ERK-MAPK pathway, inhibited the NF-κB pathway, and offended cAMP-mediated signaling (Qin et al., 2007). In addition, neuroinflammation was related to microglia 1 (M1), a pro-inflammatory cell, and the anti-inflammatory microglia 2 (M2) (Sica and Mantovani, 2012). Similarly, astrocytes also have two phenotypes, pro-inflammatory astrocytes 1 (A1) and anti-inflammatory astrocytes 2 (A2) (Kwon and Koh, 2020). As a result, the neuroinflammation is under control of the polarization status of M1/M2 and A1/A2.

Irisin has already been confirmed to have anti-inflammatory effects (Pukajło et al., 2015). FNDC5 has been confirmed to attenuate adipose tissue inflammation through the AMPK pathway to induce macrophage polarization in obese mice (Xiong et al., 2018). Irisin prevented LPS-mediated liver injury by inhibiting apoptosis, nod-like receptor pyrin-3 (NLRP3) inflammasome activation, and NF-κB signaling (Li et al., 2021). Mazur-Bialy (2017) demonstrated that irisin not only promoted the activity and proliferation of macrophages and phagocytosis but also attenuated the respiratory burst of macrophages, which increased immunocompetent activity. Mazur-Bialy et al. (2017a) reported that irisin exerted its anti-inflammatory effects by downregulating the NF-κB pathway, reducing TNF-α, IL-6, and MCP-1 in adipocyte 3T3 L1 cell line, thus attenuating the obesity-related neuroinflammation. Irisin was proved to improve memory and cognition in diabetic mice by reducing the expression of IL-1β and IL-6 in the murine hippocampus (Wang K. et al., 2019). The underlying mechanism was by downregulating the P38, STAT3, and NFκB pathways, which was related to the cytokine cascade. The reactive oxygen species-NLRP3 (ROS-NLRP3) pathway was also involved in the inhibition of irisin on the neuroinflammation (Peng J. et al., 2017). Furthermore, irisin played a pivotal role in the phenotypic switch of adipose tissue macrophages from M1 to M2 to regulate neuroinflammation (Dong et al., 2016). Irisin was also involved in autophagy, which affected Tau proteins in dementia (Pesce et al., 2020). Different pathways involved in how irisin affected autophagy, such as the AMPK/SIRT1/PGC-1α pathway in pancreatic β cells in insulin resistance stage (Li Q. et al., 2019), and MAPK pathways in the hepatic I/R injury model (Bi et al., 2020). Although the mechanisms on how irisin directly influenced central autophagy were scarce, there was a consensus on the link between irisin and AMPK. The indirect effects of irisin in autophagy are reliable, and the direct pathway still needs to explore. **Table 1** summarizes the experimental studies suggesting the roles of FNDC5/irisin in inflammation.

FNDC5/Irisin and Oxidative Stress

Oxidative stress is critical in elderly cognitive impairment and AD (Chen and Zhong, 2014). The mechanisms of oxidative stress in AD included mitochondrial dysfunction, metal accumulation, hyperphosphorylated Tau protein, and inflammation. Mitochondrial dysfunction was mainly associated with ROS production resulting from Aβ (Perez Ortiz and Swerdlow, 2019). Increased Aβ1–40 and Aβ1–42 and decreased ATP synthesis and ATPase activity were reported to promote ROS generation in mitochondria (Sharma et al., 2021). Metal ions, such as Cu, Zn, and Fe, were perceived to play a pivotal

TABLE 1 | Experimental studies suggesting the roles of FNDC5/irisin in inflammation.

References	Models	Findings	Pathways
Xiong et al., 2018	HFD-induced obese mice	FNDC5 knock-down ↑inflammation and M2 to M1	Decreasing NF-κB-p65, p38, ERK, and JNK pathways
		FNDC5 overexpression ↓inflammation and ↑M1 to M2	AMPK pathway
	RAW264.7 macrophages	FNDC5↓M1 polarization	NF-κB pathway
Li et al., 2021	LPS-induced liver injury rat; LPS-challenged RAW264.7 cells	Irisin ↓inflammation and apoptosis	NLRP3 inflammasome activation and NF-κB signaling
Mazur-Bialy, 2017	RAW264.7 macrophages	Irisin ↑macrophage activity, proliferation; and phagocytosis ↓macrophage respiratory burst	Reducing ROS overproduction
Mazur-Bialy et al., 2017a	Adipocyte 3T3 L1 cell	Irisin ↓proinflammatory cytokines (TNF-α, IL-6)	NF-κB pathway
		Irisin ↑adiponectin synthesis	
Wang K. et al., 2019	Streptozotocin-induced diabetic mice	Irisin ↑memory and cognitive deficiency; ↓synaptic protein loss; ↓IL-1β and IL-6 levels in Hippocampus and CSF	Reducing the activation of P38, STAT3, and NF-κB pathways
Peng J. et al., 2017	OGD-induced PC12 cell line	Irisin ↓oxidative stress, inflammation, and apoptosis; ↓IL-1β and IL-18; ↓ROS and MDA	NLRP3 inflammatory signaling
Dong et al., 2016	HFD-fed mice	Irisin ↓inflammation; ↑M1 to M2	AMPK and Akt pathway
Li Q. et al., 2019	INS-1 cells	Irisin ↓autophagy; ↑INS-1 cell function and survival	AMPK/SIRT1/PGC-1α pathway
Bi et al., 2020	Hepatic IR old rats	Irisin ↓inflammation	MAPK pathways

Abbreviations: HFD, high fat diet; LPS, lipopolysaccharide; ROS, reactive oxygen species; NLRP3, NOD-like receptor pyrin 3; OGD, oxygen-glucose deprivation; MDA, malondialdehyde; HepG2, human hepatocellular carcinoma cells; IR, ischemia-reperfusion.

role in AD (Faller and Hureau, 2012). Metal ions accumulation was also associated with Aβ for its metal binding sites for Zn2+, Cu2+, and Fe3+. Theoretically, Aβ binds to Cu2+ or Fe3+ resulting in reduced Cu+ and Fe2+, respectively. The binding was accompanied by the production of hydrogen peroxide (H2O2), which reacted with Fe2+ to generate Fe3+ and hydroxyl radicals (OH) (Gaeta and Hider, 2005; Chen and Zhong, 2014). Metal mal-metabolism increased the oxidative stress. Violet et al. (2014) suggested the Tau protein alterations contributed to the impaired safeguarding function of DNA and RNA, promoting the aggregation of nucleic acid oxidative damage in the AD brain. Finally, as mentioned before, the inflammation arose the generation of ROS.

FNDC5/irisin has been confirmed the anti-oxidative effects in many studies. Zhang et al. (2020) suggested that FNDC5 decreased ROS production, MDA level, and NADPH oxidase activity *via* its subunit p67phox and increased SOD1 and SOD2 expression in doxorubicin-treated hearts. Besides, FNDC5/irisin exerted the anti-oxidative effects *via* the AKT/GSK3β/FYN/Nrf2 signaling in an mTOR-independent manner. Wang et al. (2020) reported that irisin attenuated oxidative stress *via* 8-OHdG and reversed Sirt3 and UCP-1 pathways to promote mitochondrial membrane potential (MMP), ATP production, and the catalase to alleviate reactive oxygen radical generation, mitochondrial fusion and fission in the osteoarthritis model. Irisin targeted mitochondria to promote SOD-2 activity and prevented the loss of MMP, decreased the ROS activity, and finally relieved the oxidative stress in the ischemia/reperfusion (I/R) heart (Wang et al., 2018). Besides, in an ischemia/reperfusion (I/R) liver model, irisin was shown to reduce oxidative stress *via* improving UCP-2 expression, which led to reduced ROS production, restrained mitochondrial fission, and increased mitochondrial DNA copy to improve mitochondrial biogenesis (Bi et al., 2019). The Nrf2/HO-1/HMGB1 signaling participated in the anti-oxidative performance of irisin, increasing the expression of anti-oxidative factors such as SOD-1, glutathione peroxidase (GPx), and catalase-9 (Cat-9) (Mazur-Bialy and Pocheć, 2021). Activation of the AMPK-Sirt1-PGC-1α pathway and Akt/ERK1/2 pathway were involved in the irisin's anti-oxidative effect (Li et al., 2017; Wu et al., 2020). **Table 2** summarizes the experimental studies suggesting the roles of FNDC5/irisin in oxidative stress.

FNDC5/IRISIN ACT ON DEMENTIA-RELATED DISEASE

FNDC5/Irisin and Coronary Artery Disease

Coronary artery disease (CAD) was associated with dementia as they shared common risk factors such as aging, obesity, type 2 diabetes (T2DM), and hypercholesterolemia. The prevalence of both dementia and CAD increases with age, with the prevalence of dementia in those with acute myocardial infarction (AMI) increasing from 1.2% in those aged 65–69 years to 14.8% in those aged above 85 years (Fowkes et al., 2016).

Various studies suggested serum irisin levels were decreased in patients with CAD, indicating the positive effects of irisin on CAD (Khorasani et al., 2019; Wang S. et al., 2019; Guo et al., 2020). In a myocardial infarction (MI) mouse model, irisin appeared to suppress cardiomyocyte apoptosis and fibrosis and promote angiogenesis *via* the ERK signaling, which collectively improved the cardiac function and reduced the infarct size of the post-MI model (Liao et al., 2019). Zhao et al. (2016) found that

TABLE 2 | Experimental studies suggesting the roles of FNDC5/irisin in oxidative stress.

References	Models	Findings	Pathways
Zhang et al., 2020	DOX-induced Mice; DOX-induced H9C2 cells	FNDC5 ↓cardiac oxidative damage	AKT/GSK3β/FYN/Nrf2 signaling
		FNDC5 ↓cardiomyocyte apoptosis	AKT/mTOR signaling
Wang et al., 2020	DMM-induced OA mice	Irisin ↓autophagy and apoptosis;	PGC-1α; UCP-1; Sirt3
Wang et al., 2018	Myocardial I/R mice; A/R injury H9c2 cells	Irisin ↓apoptosis; ↓MMP loss; protects against I/R-injured myocardium	SOD2 targeting to mitochondria
Bi et al., 2019	Hepatic I/R Mice; H/R injury HL-7702 cell	Serum irisin increased after ischemia and 4 h after reperfusion then decreased.	PGC-1α; UCP 2; Fis-1;Drp-1
		Irisin ↓organ injury and apoptosis; ↓inflammation; ↓excessive mitochondrial fission; ↑mitochondrial biogenesis; ↓oxidative stress (↓liver MDA level)	
Mazur-Bialy and Pocheć, 2021	LPS-induced RAW264.7 macrophages	Irisin ↓respiratory burst and apoptosis; ↑Nrf2, HO-1 SOD1, SOD2, GPx, Cat-9; ↓HMGB1	Nrf2/HO-1/HMGB1 pathway
Wu et al., 2020	alcat1 knockout MI Mice; NRK cells treated with H2O2	Irisin ↓oxidative stress and apoptosis in NRK cells	AMPK-Sirt1-PGC-1α pathway
Li et al., 2017	MCAO Mice; PC12 neuronal cells with OGD	Plasma irisin levels are negatively associated with brain infarct volume, neurological deficit and inflammation.	Akt and ERK1/2 pathways
		Irisin ↓ inflammation and oxidative stress	

Abbreviations: DOX, doxorubicin; DMM, destabilized medial meniscus; OA, osteoarthritis; I/R, ischemia/reperfusion; A/R, anoxia/reoxygenation; SOD, superoxide dismutase; H/R, hypoxia/reoxygenation; UCP, uncoupling proteins; Drp-1, dynamin related protein 1; Fis-1, fission 1; GPx, glutathione peroxidase; Cat-9, catalase-9; HMGB1, high-mobility group box 1; Nrf2, nuclear factor erythroid 2-related factor 2; HO-1, heme oxygenase-1; NRK, normal rat kidney; ALCAT1, acyltransferase1; MCAO, middle cerebral artery occlusion; OGD, oxygen and glucose deprivation.

in histone deacetylases (HDAC)-over-expressed H9c2 cardio-myoblasts that went through hypoxia/reoxygenation-induced injury, irisin treatment increased cardio-myoblast survival and decreased the LDH release to alleviate cytotoxicity. Besides, irisin repressed the cell apoptosis *via* reducing active-caspase 3 and annexin V signals, mitigating the loss of MMP to protect mitochondrial damage. Furthermore, irisin held back the opening of mitochondrial permeability transition pore, which was critical for myocardial injury.

FNDC5/Irisin and Hypertension

Hypertension is associated with an increased incidence of vascular dementia (Sharp et al., 2011). Midlife systolic blood pressure (SBP) was suggested to be a significant predictor of cognition that deficits later in life (Launer et al., 1995). In the elderly, dysfunction of cerebral autoregulation led to vulnerable cerebral hemodynamics. Autoregulation protected the brain from hypertension but increased the risk of cerebral hypotension. Inappropriate antihypertensive therapy might further increase the risk of chronic cerebral hypoperfusion and subsequent dementia (Feldstein, 2012). Higher diastolic blood pressure (DBP) and lower SBP were correlated with impaired cognition (Nilsson et al., 2007; Tsivgoulis et al., 2009).

Irisin made a difference in regulating blood pressure through central and peripheral pathways; central irisin increased cardiac output and blood pressure by activating hypothalamic paraventricular nucleus of the hypothalamus (PVN) neurons, while peripheral irisin secreted from skeletal muscle reduced blood pressure *via* Adenosine triphosphate-sensitive potassium (KATP) channels to dilate vessels (Zhang et al., 2015). Besides, Irisin improved hypertension by protecting endothelial function *via* the AMPK-Akt-eNOS-NO and Nrf2 signaling pathway, the Nrf2 signaling pathway also participated in alleviating oxidative stress in the hypothalamus (Fu et al., 2016; Huo et al., 2020). Huang et al. (2022) proposed that irisin inhibited the NF-κB signaling pathway to lower blood pressure, along with reduced angiotensin II type 1 receptor (AT1R) expression and function.

FNDC5/Irisin and Heart Failure

A considerable number of patients with heart failure (HF) have cognitive problems (Cannon et al., 2017). Vascular dysfunction and loss of cardiac perfusion pump function can trigger the typical AD feature such as Aβ accumulation and hyperphosphorylated Tau tangles, as HF and AD shares common risk factors like inflammation and oxidative stress (Daniele et al., 2020).

Irisin exerted positive influences on mitochondrial dysfunction, oxidative stress, metabolic imbalance, and energy expenditure in HF (Ho and Wang, 2021). Cohort and experimental studies were conducted to elucidate the correlation between irisin and HF. Several cohorts showed increased serum irisin levels in patients with HF (Shen et al., 2017; Kalkan et al., 2018; Abd El-Mottaleb et al., 2019). Peng Q. et al. (2017) suggested that irisin ameliorated H2O2-induced apoptosis in H9c2 cardio-myoblasts and improved cell viability *via* miR-19b/PTEN/AKT/mTOR pathway. Li R. et al. (2019) found that irisin-induced protective autophagy and alleviated apoptosis signaling attenuated the myocardial hypertrophy and cardiomyocytes apoptosis. The AMPK-ULK1 pathway might be involved in the underlying mechanisms (Li et al., 2018).

FNDC5/Irisin and Stroke

Stroke is a pronounced disease related to cognition impairment and contributes to damaged life quality (Obaid et al., 2020).

Stroke is divided into the ischemic and hemorrhagic stroke, the former makes up 85% (Amarenco et al., 2009; Beal, 2010). A total of 23.9% of older stroke survivors developed dementia (Allan et al., 2011). Taking ischemic stroke as an example, brain injury secondary to the stroke was a result of the post-stroke excitotoxicity, oxidative and nitrative stress, inflammation, and apoptosis (Khoshnam et al., 2017). Besides, Goulay et al. (2020) have reported that stroke exacerbated the deposition of Aβ.

Irisin has been reported to perform neuroprotective effects on stroke (Liu et al., 2020). Irisin mitigated brain injury after stroke *via* inhibiting inflammation and oxidative stress and preventing BBB dysfunction (Peng J. et al., 2017; Guo et al., 2019). Jin et al. (2019) suggested that irisin attenuated the brain injury after the cerebral ischemia/reperfusion (I/R) injury especially in the hippocampus region through the Notch signaling pathway. Irisin promoted the Notch1, Notch intracellular domain (NICD), and Hes1 expression, which were reported to exert effects in AD and other neurodegenerative diseases. Irisin alleviated neuronal apoptosis, accompanied by decreasing the caspase-3 expression, a critical apoptotic effector. Besides, irisin reduced the inflammation, decreasing the TNF-α and IL-1β levels (Berezovska et al., 1998; Alberi et al., 2013). Yu et al. (2020) reported that irisin protected the neurological function in a middle cerebral artery occlusion (MCAO) I/R injury model *via* suppressing the TLR4 and NF-κB pathways. Others also elucidated the neuroprotective effects of irisin in mice with MCAO and OGD neuronal cells *via* Akt and ERK1/2 signaling pathways (Li et al., 2017). Irisin relieved the post-ischemic inflammation by downregulating TNF-α and IL-6 expression, suppressed the microglial infiltration, and decreased the MPO-1+ cell numbers, as well as reduced the post-ischemic oxidative stress by decreasing the levels of 4-HNE and MDA. Furthermore, mitochondrial dynamics were involved in the ischemic stroke, and mitochondrial defects are critical for AD (Yan et al., 2013; Anzell et al., 2018). Irisin improved mitochondrial function *via* AMPK pathway as the AMPK was a guardian of mitochondrial homeostasis (Tang et al., 2016; Herzig and Shaw, 2018; Siteneski et al., 2018; Xin et al., 2020). In summary, irisin exerted neuroprotective effects after stroke to prevent cognitive impairment primarily through its anti-inflammatory and anti-oxidative effects, as well as the beneficial effects on mitochondria.

FNDC5/Irisin and Parkinson's Disease

Parkinson's disease is the second most frequent senile neurodegenerative disease (Mhyre et al., 2012). Patients with PD often developed cognitive deficits and dementia, especially in elderly patients (Aarsland et al., 2017). PD-dementia is a classic type of dementia.

Irisin played a protective role in PD. In a mouse model of PD, irisin treatment prevented dopaminergic neurons from apoptosis and degeneration (Zarbakhsh et al., 2019). Mahalakshmi et al. (2020) elucidated the benefits of exercise on PD, and irisin was a mediator of exercise-induced BDNF. Raefsky and Mattson (2017) suggested that irisin might protect neuronal mitochondria function in PD *via* antioxidation, autophagy, and DNA repair regulations.

FNDC5/Irisin and Depression

Depression and dementia often occur at the same time in the elderly (Bennett and Thomas, 2014). Depression is both the risk factor and prodrome of dementia (Gutzmann and Qazi, 2015). The interreaction of depression and dementia is complex.

Irisin improved depressive neuropathology by regulating mitochondria function *via* PGC-1α signaling and modifying synaptic plasticity *via* BDNF signaling (Jo and Song, 2021). Hou et al. (2020) proposed that irisin attenuated the postoperative depressive-like behavior and reduced neuron death and cytokines release from astrocytes through inhibiting the surface expression of epidermal growth factor receptors (EGFR) in the mice model. Siteneski et al. (2018) also suggested that central irisin administration manifested antidepression effect, associated with the adjustment of gene expression of PGC-1α, FNDC5, and BDNF in the hippocampus and prefrontal cortex of mice.

FUTURE DIRECTION

There is a long way to intervene and delay the progression of elderly cognitive impairment. Based on the irisin secretion and function to optimize the exercise protocol such as the amount of exercise, the form of exercise, and the duration of exercise, further research is needed. Factors affecting exercise, such as age, frailty, sarcopenia, and fracture, also need to be considered. Although many studies have been reported to support the favorable effects of FNDC5/irisin, there are some limitations. Many studies are based on experimental studies, and direct studies of irisin on central autophagy are scarce. Besides, the difference of plasma irisin levels alterations in patients with dementia was not significant and has not reached a consensus. Interfering factors such as age, gender, race, and disease duration differences cannot be ignored. There are also some controversial results and views. On the one hand, Raschke et al. (2013) argued that the beneficial effect of irisin observed in mice can be translated to humans. Although there are many registered clinical trials to clarify the effects of irisin on the human body, large-scale clinical research and long-term follow-up are required to study the relationship between FNDC5/irisin and cognition. Besides, to carry out the animal experiments and clinical research simultaneously and to conduct comparative analysis are very necessary to elucidate the difference in FNDC5/irisin effects in the mice and human body. On the other hand, the current irisin detection method is still insufficient. ELISA has been widely used in the examination of irisin levels in serum or other specimens in humans and animals. However, Albrecht et al. (2015) argued that ELISA kit for irisin may not be accurate. Besides, ELISA can be influenced by a series of factors such as preservation conditions, temperature, antibody, and operational contingency. As a result of the conflicting opinions, conducting comparative studies on the sensitivity and specificity of current ELISA kits is a research direction. A high-quality meta-analysis or systematic review of the efficacy of ELISA kits for irisin also can be considered. Most of the ELISA kits for irisin were for laboratory research only, not for drug, diagnostic, or other use. Exploring new methods with high sensitivity and specificity as well as diagnostic value in

clinical conditions is also the direction of future efforts, such as the application of sensors or nanotechnology.

CONCLUSION

Cognitive impairment is a worldwide public health problem, which seriously affects the quality of life of the elderly and increases the burden of care. Clarifying the pathological mechanism of dementia and exploring drugs to prevent, treat, and delay the course of dementia have always been the direction of efforts. Physical exercise and lifestyle are believed to defend against cognitive decline in the elderly. Irisin might be a mediator of muscle and brain cross talk mainly through the PGC-1α/FNDC5/BDNF pathway. More information is needed to optimize exercise protocols based on irisin for patients with dementia. Our review discussed the favorable effects of irisin on cognitive impairment, such as the positive effect irisin on neurogenesis and synapse; anti-inflammatory and anti-oxidative effects; and possible connections of irisin on dementia-related diseases such as CAD, hypertension, HF, stroke, PD, and depression. The serum irisin level alterations in dementia have not reached a consensus. Large-scale clinical research and long-term follow-up are required to explore whether serum irisin is a diagnostic or prognostic factor for dementia. The current detection method for irisin is still limited to ELISA. It is also an exploratory direction to find more sensitive, specific, and simple detection methods.

AUTHOR CONTRIBUTIONS

JP participated in literature collection, preparation, and wrote the draft. JW supervised the whole project. Both authors participated in the conception and study design, contributed to the manuscript revision, and approved the submitted version.

FUNDING

This work was supported by China National Key R&D Program (Nos. 2018YFC2002100 and 2018YFC2002103) to JW.

ACKNOWLEDGMENTS

We thank the participants.

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REVIEW
published: 25 April 2022
doi: 10.3389/fnagi.2022.872134

The Emerging Role of Central and Peripheral Immune Systems in Neurodegenerative Diseases

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For decades, it has been widely believed that the blood–brain barrier (BBB) provides an immune privileged environment in the central nervous system (CNS) by blocking peripheral immune cells and humoral immune factors. This view has been revised in recent years, with increasing evidence revealing that the peripheral immune system plays a critical role in regulating CNS homeostasis and disease. Neurodegenerative diseases are characterized by progressive dysfunction and the loss of neurons in the CNS. An increasing number of studies have focused on the role of the connection between the peripheral immune system and the CNS in neurodegenerative diseases. On the one hand, peripherally released cytokines can cross the BBB, cause direct neurotoxicity and contribute to the activation of microglia and astrocytes. On the other hand, peripheral immune cells can also infiltrate the brain and participate in the progression of neuroinflammatory and neurodegenerative diseases. Neurodegenerative diseases have a high morbidity and disability rate, yet there are no effective therapies to stop or reverse their progression. In recent years, neuroinflammation has received much attention as a therapeutic target for many neurodegenerative diseases. In this review, we highlight the emerging role of the peripheral and central immune systems in neurodegenerative diseases, as well as their interactions. A better understanding of the emerging role of the immune systems may improve therapeutic strategies for neurodegenerative diseases.

Keywords: peripheral immune system, central nervous system, neurodegenerative diseases, Amyotrophic lateral sclerosis, Alzheimer's disease, Parkinson's disease

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Specialty section:
This article was submitted to Neuroinflammation and Neuropathy, a section of the journal Frontiers in Aging Neuroscience

Received: *09 February 2022*
Accepted: *25 March 2022*
Published: *25 April 2022*

Citation:
Zang X, Chen S, Zhu J, Ma J and Zhai Y (2022) The Emerging Role of Central and Peripheral Immune Systems in Neurodegenerative Diseases. Front. Aging Neurosci. 14:872134. doi: 10.3389/fnagi.2022.872134

INTRODUCTION

Neurodegenerative diseases are characterized by progressive dysfunction and the loss of neurons in the CNS. In recent years, the incidence of neurodegenerative diseases associated with aging, especially Alzheimer's disease (AD), has increased exponentially with the aging of the global population (Schwartz and Deczkowska, 2016). However, there are no effective therapies to stop or reverse the progression of neurodegenerative diseases. Studies have shown that the aggregation and deposition of misfolded proteins play key roles in neurodegenerative diseases (Hartl, 2017; Abdel-Nour et al., 2019). In the last decade, research on the role of the immune system in neurodegenerative diseases has progressed. Both the innate and adaptive immune systems have been shown to be involved in the inflammatory mechanisms associated with the accumulation of misfolded proteins in the brain (Ciccocioppo et al., 2020).

The central immune system is composed of neurons, glial cells as well as other immune cells. Traditionally, studies have considered the peripheral and central immune systems to be separate processes because the BBB blocks peripheral immune cells and humoral immune factors (Jeon et al., 2021). However, there is increasing evidence that peripheral immune system plays an important role in neuropathology. Peripheral immune cells can participate in the progression of neuroinflammatory and neurodegenerative diseases by infiltrating the brain (Greenhalgh et al., 2020). In addition, peripherally released cytokines can cross the BBB to cause direct neurotoxicity and contribute to the activation of glial cells (Fani Maleki and Rivest, 2019). Activated glial cells lead to further secretion of pro-inflammatory chemokines and cytokines, thereby recruiting more immune cells to the CNS (Prinz and Priller, 2017; Vainchtein and Molofsky, 2020). Although neurodegenerative diseases have different etiologies and pathogeneses, they all share the characteristic of neuroinflammation. In recent years, neuroinflammation has received significant interest as a potential therapeutic target for many neurodegenerative diseases. In this review, we summarize the emerging role of the peripheral and central immune systems in neurodegenerative diseases, as well as their interactions, which may have important implications for understanding the pathogenesis and progression and provide new ideas for therapeutic strategies to treat neurodegenerative diseases.

THE CENTRAL NERVOUS SYSTEM

Microglia

Microglia are long-lived resident macrophages in the CNS. As part of CNS homeostasis, microglia remain quiescent under physiological conditions and perform extremely strong immune surveillance through highly mobile processes (Nimmerjahn et al., 2005). This continuous state of movement enables microglia to respond rapidly to neuropathological changes. On the one hand, these cells play a phagocytic role, engulfing pathogens and cell debris that invade the brain. On the other hand, microglia are also able to transform into an activated phenotype under certain stimuli, accompanied by transcriptional changes to perform inflammatory functions (Amor et al., 2022).

Abbreviations: AD, Alzheimer's disease; AB, *Achyranthes bidentata* Blume; ALS, Amyotrophic lateral sclerosis; APCs, antigen-presenting cells; AST-IV, Astragaloside IV; Aβ, β-amyloid; BBB, blood–brain barrier; CNS, central nervous system; CTLs, cytotoxic T lymphocytes; CX3CL1, CX3C chemokine ligand 1; DN, dopaminergic neuron; DAMPs, danger-associated molecular patterns; DCs, dendritic cells; eQTL, expressed quantitative trait loci; HD, Huntington's disease; HFD, Hua-Feng-Dan; LRRK2, Leucine-rich repeat kinase 2; MDMs, monocyte-derived macrophages; MHC-, major histocompatibility complex-; mHTT, mutant HTT; MMPs, matrix metalloproteinases; MN, motor neuron; moDCs, monocyte-derived dendritic cells; MS, multiple sclerosis; NETs, neutrophil extracellular traps; NF- κ B, nuclear factor-kappa B; NFTs, neurofibrillary tangles; NLRP3, NOD-like receptor thermal protein domain associated protein 3; NMJ, neuromuscular junction; NO, nitric oxide; NVU, neurovascular unit; OLs, oligodendrocytes; OPCs, Oligodendrocyte precursor cells; OPN, Osteopontin; PD, Parkinson's disease; ROS, reactive oxygen species; SC, spinal cord; SNpc, substantia nigra pars compact; TCM, traditional Chinese herbal medicine; Teffs, effector T cells; TGF-β1, transforming growth factor-β1; TLR, Toll-like receptors; Tregs, regulatory T, lymphocytes; VEGFA, vascular endothelial growth factor A; α-syn, alpha-synuclein.

In general, the activation of microglia can be simplified into two states, which have been traditionally divided into M1 (classic activation) and M2 (alternative activation) (**Figure 1**). Sustained activation of the M1 phenotype results in the secretion of excessive amounts of pro-inflammatory cytokines and neurotoxic molecules, which in turn damage the organism. In contrast, M2 microglia promote tissue repair and regeneration through the production of anti-inflammatory cytokines and neurotrophic factors to exert neuroprotective effects. In recent years, however, it has been shown that activated microglia express canonical gene products associated with the M1 and M2 phenotypes (Rahimian et al., 2021). In neuropathological conditions, microglial activation falls on a continuum, and these cells exhibit a mixed phenotype mediated by a complex cascade of surrounding signals. Classifying microglia based on M1 and M2 polarization states is not sufficient to describe the multiple states of microglial activation (Ransohoff, 2016).

Recent studies have shown that microglia can make direct connections with different regions of neurons, which is a more precise modulation that regulates neuronal responses and cell fate (Cserép et al., 2020, 2021). Microglia can sense ATP produced by neuronal activation and break ATP down into adenosine, which inhibits adenosine receptors on the surface of active neurons, thereby inhibiting excessive neuronal activation and inducing negative feedback control of neuronal activity (Badimon et al., 2020). In some neurodegenerative conditions, however, microglia lose this ability to sense ATP molecules and produce adenosine. Therefore, in-depth research on the mechanism of microglia-neuronal communication may provide new ideas for the treatment of certain neurodegenerative diseases.

In fact, it is widely believed that microglia are able to not only interact with the immune components of the CNS but also crosstalk with peripheral immune components that infiltrate the CNS (Liu et al., 2020). Moreover, microglia have many important but not yet fully understood roles in protecting the brain from disease, and this offers the possibility of developing targeted molecular therapies. However, under pathological conditions, activated microglia can exert deleterious effects, and microglia can mediate the onset and development of neuro-inflammatory responses through a range of transcription factors and multiple cellular signaling pathways (Cai et al., 2018). Therefore, targeting the microglial inflammatory signaling pathway may be a potential approach to treat neurodegenerative diseases.

Astrocytes

Astrocytes provide nutritional support for neurons, regulate the metabolic balance of the nervous system, and play an important role in promoting the formation and function of synapses and maintaining the structure of the brain and the BBB (Alvarez et al., 2011; Jeon et al., 2021). In addition, an increasing number of studies have proven that astrocytes are a double-edged sword. Similar to the M1 and M2 polarization states of macrophages, murine reactive astrocytes are defined as A1 and A2 (Liddelow et al., 2017). The A1 astrocytes are pro-inflammatory and neurotoxic and are associated with the progression of neurodegenerative diseases. In contrast, A2

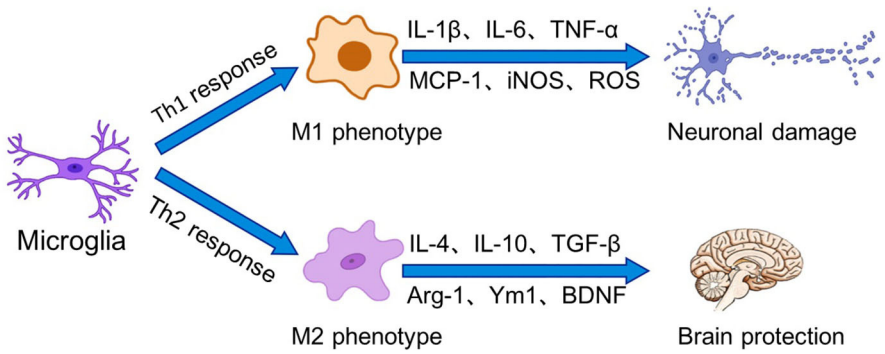

FIGURE 1 | Microglia perform surveillance functions of the extracellular environment of the brain in the resting state. In the presence of stressors, microglia can be activated into two states, which have been traditionally divided into M1 (classical activation) and M2 (alternative activation). Binding of receptors on microglia to Th1 cytokines drives microglia polarization to the M1 phenotype, leading to increased inflammation and oxidative stress as well as BBB dysfunction. In contrast, Th2 cytokines prompt microglia to polarize to the M2 phenotype. M2 phenotype microglia promote tissue repair and regeneration through the production of anti-inflammatory cytokines and neurotrophic factors for to exert neuroprotective effects.

astrocytes have a neuroprotective function. In a mouse cell model, microglia express interleukin (IL)-1α, tumor necrosis factor (TNF), and complement component 1q (C1q) in response to lipopolysaccharide (LPS) stimulation, and the combined effects of these cytokines are critical for the activation of A1-type astrocytes (Liddelow et al., 2017; Ridler, 2017). After activation, A1 astrocytes are involved in neuroinflammation-mediated neurotoxicity in various ways, inducing neuronal and mature differentiated oligodendrocyte death and participating in the progression of neurodegenerative diseases (Liddelow et al., 2017) (**Figure 2**).

Furthermore, neurodegenerative diseases are distinguished by idiopathic neuronal loss in different parts of the CNS, and these damaged neurons are not compensated by tissue regeneration. Instead, these cells are gradually replaced by extracellular matrix components, which are mainly produced by endothelial cells, activated fibroblasts and astrocytes (D'Ambrosi and Apolloni, 2020). This fibroglial response has dual roles in tissue protection and repair inhibition. Scar-forming astrocytes are usually adjacent to the lesion and prevent the spread of proinflammatory cytokines and cellular debris to some extent (Becerra-Calixto and Cardona-Gómez, 2017). However, a recent study demonstrated that this fibrotic response exacerbates the progression of degenerative diseases. Therefore, it has been suggested that converting the fibrosis-supporting matrix deposition state of astrocytes and myofibroblasts to a fibrosis-supporting regressive or reversible matrix degradation state and reducing scar formation may help to improve the pathological processes of amyotrophic lateral sclerosis (ALS) and AD (D'Ambrosi and Apolloni, 2020).

Oligodendrocytes

Mature oligodendrocytes (OLs) are found throughout the gray and white matter of the CNS (Boulanger and Messier, 2014). OLs are myelin-forming cells in the CNS and provide metabolic support to neurons by forming myelin sheaths around axons (Saab et al., 2016). In recent years, studies have demonstrated that oligodendrocyte loss and demyelination are characteristic of neurodegenerative diseases. Oligodendrocyte precursor cells (OPCs) are the progenitors of terminally differentiated OLs. As a repair mechanism, when demyelination occurs, the proliferation and differentiation rate of OPCs increases to generate new OLs (Snaidero et al., 2020; Neely et al., 2022). This may be crucial for the pathological recovery process in such diseases. Recent studies have revealed that OLs may also have immune-related functions, with a variety of immunomodulatory factors expressed in OLs, such as cytokines/chemokines and their receptors (Zeis et al., 2016; Raffaele et al., 2021). In multiple sclerosis, OLs and OPCs are not passive targets, but modulators of active immunity (Falcão et al., 2018).

Endothelial Cells

The neurovascular unit (NVU) establishes close structural and functional connections between neurons, glial cells (astrocytes, oligodendrocytes and microglia) and vascular cells (endothelial cells and pericytes) (Castellani and Schwartz, 2020; Mészáros et al., 2020). NVU contributes to the development and maintenance of the BBB, ion balance, and nutrient transport (Vedam-Mai, 2021). Endothelial cells are an important cellular component of the NVU. Compared to non-neural tissue endothelial cells, brain endothelial cells have high expression of connexins at intercellular junctions, conferring barrier restriction properties for paracellular permeability (Procter et al., 2021). During the inflammatory process, peripheral cytokine act on endothelial cells, leading to impaired barrier function. Meanwhile, inflamed endothelial cells upregulate the expression of adhesion molecules that facilitate the recruitment of circulating peripheral immune cells and antibodies across the barrier (Varatharaj and Galea, 2017; Marogianni et al., 2020). A recent study identified a new pathway for microvascular endothelial cells to degrade myelin debris via the autophagy-lysosome system, which promotes the progression of demyelinating diseases by promoting inflammation, angiogenesis and fibrotic scar formation (Zhou et al., 2019).

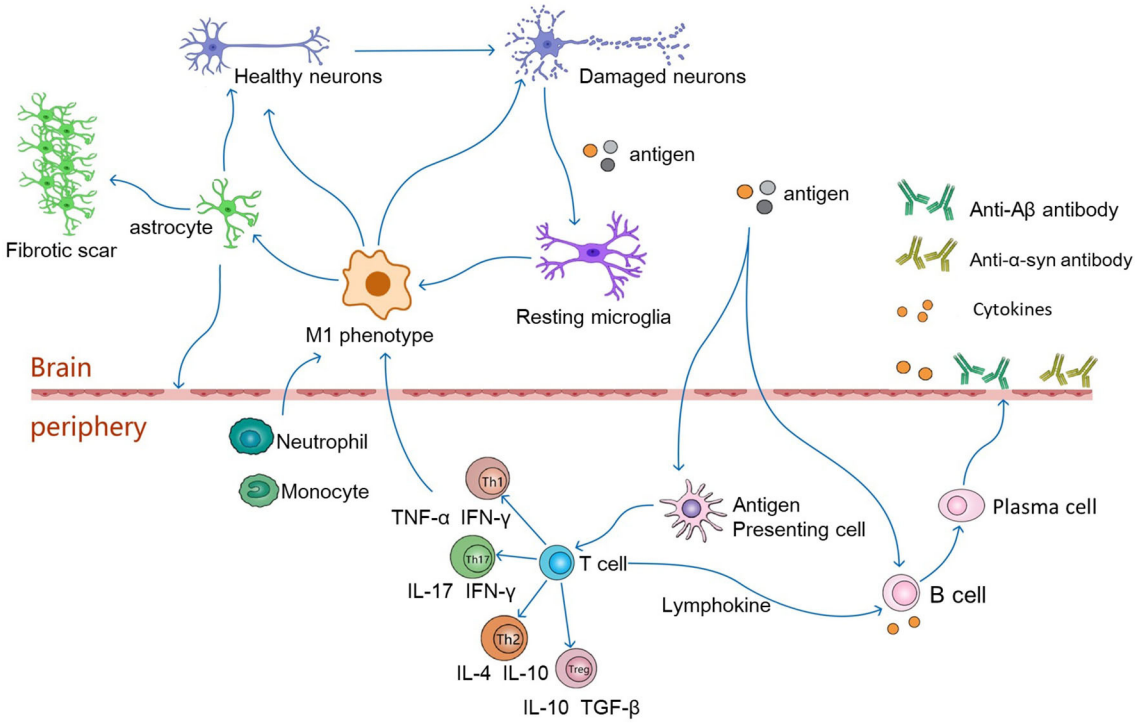

FIGURE 2 | In pathological conditions, damaged neurons release autoantigens to activate resting microglia, which differentiate into the pro-inflammatory phenotype of M1. The M1 phenotype microglia secrete pro-inflammatory factors(IL-6, TNF-α, IFN-γ) to activate astrocytes, which induce the activation of A1 phenotype astrocytes. Reactive astrocytes contribute to the formation of glial scarring. It also leads to destruction of the BBB, which in turn leads to infiltration of CNS by peripheral immune cells. Antigens may enter peripheral lymphoid tissue, where they are presented by antigen-presenting cells to naive T cells, which differentiate into antigen-specific T effector cells (Th1, Th2, Th17 or Tregs). These cells then secrete anti-inflammatory or pro-inflammatory factors to regulate neuronal survival. Th1 and Th17 cells cross the BBB, produce neurotoxic and pro-inflammatory factors that interact with glial cells, leading directly to neuroinflammation and damage to motor neurons. Th2 and Tregs migrate from the periphery to the central nervous system and interact with glial cells to function as motor neuron protectors. In addition, antigens can directly stimulate B cells, which are activated to produce pro-inflammatory factors that travel along blood vessels to the brain and participate in neurodegeneration. Activated T cells secrete lymphokines to activate B cells, which proliferate and differentiate into plasma cells. Plasma cells can further produce cytokines and antibodies, such as anti-Aβ or anti-α-synuclein antibodies, which cross the blood-brain barrier into the brain to alleviate neuronal degeneration.

Pericytes

Pericytes are microvascular wall cells that are embedded in the basement membrane and surrounding microvasculature. As mentioned previously, pericytes are important cellular components of the neurovascular unit (Uemura et al., 2020). Pericytes interact with endothelial cells, neuronal cells, glial cells and perivascular macrophages, and this interaction is the basis for performing these functions (Ding et al., 2018; Uemura et al., 2020). In recent years, pericytes have been shown to be involved in neuro-inflammatory as well as neurodegenerative diseases (Nyúl-Tóth et al., 2017). Pericyte-derived pleiotrophin is thought to be a neurotrophic factor required for neuronal survival. The loss of pericytes leads to the breakdown of BBB, blood flow and loss of neurons in the mouse brain (Nikolakopoulou et al., 2019).

THE PERIPHERAL IMMUNE SYSTEM

Neutrophils

During infection, neutrophils are recruited to the area of infection to engulf invading microorganisms through phagocytosis. Under physiological conditions, there are almost no neutrophils present in the CNS due to the BBB (Guo et al., 2021). However, under different pathological conditions, such as infection, trauma or neurodegeneration, a large amount of neutrophil infiltration occurs in the CNS.

Studies have shown that neutrophils disrupt the BBB by releasing free radicals, proteolytic enzymes and matrix metalloproteinases (MMPs) (Strecker et al., 2017; Manda-Handzlik and Demkow, 2019). Neutrophil extracellular traps (NETs) are reticulated ultrastructures that are released into the extracellular space after neutrophil activation and damage the BBB (Naegele et al., 2012). Zenaro et al. observed neutrophil-microglia crosstalk in AD and the presence of NETs within blood vessels and the brain parenchyma, suggesting that NETs affect the BBB in Alzheimer's disease and contribute to neuronal damage (Zenaro et al., 2015). In addition, the authors showed that the migration of neutrophils produces IL-17 in the cortex and hippocampus, which not only has a direct toxic effect on neurons and is involved in the disruption of the BBB but recruits additional neutrophils (Kebir et al., 2007; Zenaro et al., 2015). Disruption of the BBB has become one of the mechanisms of peripheral-central nervous system crosstalk and is an early

marker of neurodegenerative diseases (Giannoni et al., 2020; Ishii and Iadecola, 2020).

Monocytes

Monocytes are the largest white blood cells in peripheral blood and can differentiate into monocyte-derived macrophages (MDMs) or monocyte-derived dendritic cells (moDCs). Similar to neutrophils, monocytes are barely detectable in the CNS under physiological conditions (Gomez Perdiguero et al., 2015; Mrdjen et al., 2018; Croese et al., 2021). When brain homeostasis is disrupted, MDMs can invade the brain through an intact or compromised BBB (Engelhardt et al., 2017; Greenhalgh et al., 2020). Infiltrating blood-derived monocytes play a major role in controlling neuropathic events in the CNS, including scar degradation, anti-inflammatory and neurotrophic factor production (Shechter et al., 2013; Schwartz, 2017). Greenhalgh et al. (2018) found evidence of direct communication between MDMs and microglia and differential regulation of each other's functions. MDMs inhibit the critical functions of microglia and counteract harmful acute and long-term microglia-mediated inflammation.

NK Cells

NK cells are bone marrow (BM)-derived haematopoietic cells that are widely distributed in peripheral lymphoid organs and the circulatory system (Hazenberg and Spits, 2014). NK cells can secrete cytokines and chemokines that affect the host immune response or release cytoplasmic granules containing perforin and granzyme to induce apoptosis in target cells (Voskoboinik et al., 2015; Zhou et al., 2020). In addition, some NK cells can kill target cells through the death receptor pathway via Fas ligand and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) (Guillerey et al., 2016; Yang et al., 2018). Under pathological conditions, NK cells are recruited to the CNS by chemokines. For example, during neuroinflammation, neuronal production of CX3C chemokine ligand 1 (CX3CL1) is necessary to direct CX3CR1-expressing NK cells into the brain (Huang et al., 2006; Hamann et al., 2011).

NK cells have different immunomodulatory functions in different neurological diseases. For example, NK cells interact with glial cells to regulate the neuroinflammatory response in neurodegenerative diseases (Huang et al., 2006; Hertwig et al., 2016). NK cells regulate Treg recruitment and microglial phenotype by interacting with infiltrating Treg cells and resident microglia. In addition, NK cells affect CNS physiology by killing glial cells and secreting IFN-γ (Saikali et al., 2007; Moodley et al., 2011).

Dendritic Cells (DCs)

Dendritic cells (DCs) are the predominant antigen-presenting cells (APCs). In the steady state, DCs are present in the CNS, expressing major histocompatibility complex class II (MHCII) and the leukocyte integrin CD11c (Santos et al., 2020). The expression of MHCII and CD11c is generally used to identify the existence and position of DCs in the brain (Bulloch et al., 2008; Colton, 2013). Through the identification of cell morphology and surface markers, multiple studies have shown that under physiological conditions, DCs are located in the meninges, choroid plexus, cerebrospinal fluid, and perivascular spaces in the CNS (McMenamin et al., 2003; Schain et al., 2018). These sites are all highly vascularized regions, supporting the idea that cerebral DCs are of vascular origin rather than from within the brain. Anandasabapathy et al. (2011) demonstrated that endogenous dendritic cells in the meninges and choroidal plexus of the steady-state brain are most likely derived from local precursor dendritic cells that entered the perivascular region of the brain early in life, and this conclusion suggests that such precursor dendritic cells are derived from bone marrow rather than monocytes.

The release of chemokines and adhesion molecules allows peripheral DCs to migrate to the meninges or choroid plexus, recognize antigens and present antigens to T cells (Sabahi et al., 2021). DCs can provide the co-stimulatory signals required for T cell activation, promoting the activation and proliferation of CD8+ cytotoxic T cells (CTLs) and CD4+ helper T cells with diverse proinflammatory cytokine profiles (Colton, 2013). These proinflammatory effects may cause tissue damage under certain conditions. However, a recent study showed that the bone marrow-derived dendritic cells induce neuroprotective Tregs in a PD model, providing neuroprotective effects through modulation of adaptive immunity (Schutt et al., 2018). In conclusion, these studies demonstrate the role of DC in the regulation of inflammatory and neurodegenerative diseases.

T Cells

T cells are key immune cells of the adaptive immune system. Several studies have shown that the connection between the innate and adaptive immune systems is also implicated in the progression of neurodegenerative diseases. T cells within the CNS are often considered pathogenic, especially in the context of neuroinflammatory diseases, and excessive inflammatory responses are thought to be modulators of the pathogenesis of neurodegenerative diseases (Ellwardt et al., 2016). Abnormal T cells promote neuroinflammation through direct crosstalk with glial cells in the brain and the secretion of pro-inflammatory mediators (Dai and Shen, 2021). However, recent studies have shown that T cells play an active role in limiting inflammation and CNS damage, infection and neurodegeneration (Ellwardt et al., 2016). The balance between T cells, which can play an injurious or protective role in CNS, remains to be further investigated.

CD4+ T cells are the main regulators of the immune response by secreting a variety of cytokines, recruiting immune cells to the site of infection, and initiating the differentiation of CD4+ T cell subsets with different effector functions (Morgan et al., 2021). Naïve CD4+ T cells (Th0) differentiate into antigen-specific T effector cells, including T helper 1 (Th1), T helper 2 (Th2), and T helper 17 (Th17) cells and regulatory T cells (Tregs) (**Figure 2**). CD8+ T cells mainly mediate cellular immune responses and are known as cytotoxic T cells (CTLs).

Th1 and Th17 cells are generally regarded to be producers of pro-inflammatory cytokines and directly promote neuroinflammation by secreting inflammatory mediators such as IL-1, IL-6, IL-17, TNF-α and IFN-γ (Dardalhon et al.,

2008). Furthermore, these cells enhance microglia-mediated neurotoxicity by upregulating the release of reactive oxygen species (ROS) and nitric oxide (NO) (Liu et al., 2020). In contrast, Th2 cells are thought to be anti-inflammatory and produce anti-inflammatory cytokines (IL-4). Th2 cells and Tregs enhance microglia-mediated neuroprotection (Gendelman and Appel, 2011; Mayne et al., 2020). Th1 and Th2 cells are essential for maintaining a healthy CNS environment, the ratio of Th1 to Th2 cytokines (Th1/Th2) can be used to reflect the pattern of the immune response, and alterations in this ratio are thought to be a trigger for neurodegenerative diseases (Burgaletto et al., 2020).

The critical role of regulatory T lymphocytes (Tregs) in immune tolerance and the control of inflammatory responses makes them potential therapeutic targets for many diseases (Sheean et al., 2018). Tregs exert neuroprotective effects by regulating microglial activation. In the early stages of ALS, this regulation manifests as the upregulation of Tregs and elevated levels of M2-type neuroprotective microglia (Beers et al., 2011). However, as the disease progresses, Treg levels decrease, pro-inflammatory cytokine levels increase, and microglia shift to a neurotoxic M1 phenotype (Chen et al., 2014; Sheean et al., 2018). Studies have shown that the number of Tregs is negatively correlated with the progression of ALS (Beers et al., 2011). In SOD1-mutant mice, passive transfer of mSOD1 Tregs to ALS mice lacking functional T lymphocytes induced M2 microglia in the spinal cord and prolonged survival (Banerjee et al., 2008; Beers et al., 2011).

In humans, gamma/delta (γδ) T cells are a relatively small subset of T lymphocytes (Xu et al., 2020). γδ T cells function as innate immune cells, and they have recently been shown to share many key features of adaptive immunity (Davey et al., 2017, 2018). Previous studies have demonstrated that γδ T cells can regulate immune responses associated with inflammation and have a pro-inflammatory role in the CNS. γδ T cells can promote local amplification of the immune response in the CNS, altering the interstitial microenvironment of the inflamed brain and ultimately leading to BBB disorders (Schirmer et al., 2013; Benakis et al., 2016; Wo et al., 2020). However, Ponomarev et al. showed that γδ T cells could regulate CNS inflammation and disease recovery through Fas/Fas ligand-induced brain-native T cell apoptosis (Ponomarev and Dittel, 2005). The different functions depend on the time and location of γδ T cells, as well as on the different subtypes. However, more evidence on different subtypes of γδ T cells is needed to determine their various roles in CNS inflammation (Wo et al., 2020).

CD8+ T cells have been shown to be involved in the pathophysiology of diseases associated with neurodegeneration. CD8+ T cells drive CNS axonal degeneration in normal senescent mice in a T cell receptor- and granzyme B-dependent manner, and this deleterious effect is further enhanced in the presence of inflammation (Groh et al., 2021). In addition, Coque et al. (2019) found that autoreactive CD8+ T cells directly interacted with motor neurons and triggered death. Recently, however, there has been an increase in interest in CD8+ T cells, as CD8+ T cells have been shown to have other functions than neurotoxicity. Studies have shown that peripheral infection induces a type of CNS tissue-resident memory CD8+ T cells in the brain. These memory CD8+ T cells contribute to the control of CNS infection, showing rapid activation, enhanced cytokine production and mediated protection after brain infection (Griffin and Metcalf, 2011; Urban et al., 2020).

B Cells

B cells are important regulators of CNS homeostasis and disease states and play an important role in the pathogenesis of various CNS diseases by acting peripherally or compartmentalizing within the CNS. In individuals with CNS inflammation, the number of B cells in the cerebrospinal fluid increased several-fold, and in the CNS parenchyma and perivascular space, these cells increased at least several orders of magnitude (Machado-Santos et al., 2018).

In some PD patients, decreased levels of B lymphocyte subsets in the peripheral blood were detected, which may be related to altered B cell-related gene expression (Kobo et al., 2016). Although no B cells have been identified in the post-mortem brain tissue of PD patients, studies have shown IgG deposition on dopaminergic neurons in the substantia nigra and Lewy bodies in the CNS (Orr et al., 2005). Another study showed increased levels of alpha-synuclein-specific autoantibodies in the blood and cerebrospinal fluid of PD patients (Shalash et al., 2017). This finding reflects the role of B cell antibodies in PD. Despite the mounting evidence of the involvement of B cells in neurodegenerative diseases, whether the changes in adaptive immune are causal or secondary to CNS injury still needs further study (Sabatino et al., 2019).

REGULATION OF PERIPHERAL AND CENTRAL IMMUNITY IN NEURODEGENERATIVE DISEASES

Amyotrophic Lateral Sclerosis (ALS)

Amyotrophic lateral sclerosis (ALS) is a severe degenerative disease of the CNS. ALS is characterized by the progressive degeneration of upper motor neurons in the motor cortex and lower motor neurons in the brainstem and spinal cord, resulting in severely impaired motor function in patients (Brown and Al-Chalabi, 2017). The main symptoms of ALS are related to motor dysfunction, and patients experience progressive muscle weakness. As the disease progresses, patients gradually lose all muscle control, eventually leading to death, with an average life expectancy of only 2-5 years after onset (Tunca et al., 2018). An increasing number of studies have shown that patients with ALS can develop non-motor symptoms, such as cognitive and behavioral impairments during the course of the disease in 50% of patients and concomitant behavioral variant frontotemporal dementia (FTD) in 13% of patients (Elamin et al., 2013; Hardiman et al., 2017). Most ALS cases are sporadic (SALS) with no clear genetic origin. Only approximately 10% of cases are familial ALS (FALS) caused by genetic mutations, of which Cu^{2+}/Zn^{2+} superoxide dismutase (SOD1) mutations are considered to be the most prominent and earliest identified genetic cause of ALS. Other disease-specific gene mutations

include the C9orf72, TDP43, and FUS mutations (Sreedharan et al., 2008; Vance et al., 2009; Renton et al., 2011).

Neuroinflammation is thought to be involved in the heterogeneity of ALS. The crosstalk between activated microglia and astrocytes and pro-inflammatory peripheral immune cells in the CNS, together with the immune molecules these cells release, is significantly associated with disease progression and survival in ALS patients. We summarized the emerging role of the peripheral and central immune responses in ALS with the aim of providing clear insights into potential new treatments for ALS.

Microglia are known to play dual roles in the pathogenesis of ALS. It has been shown that more than 90% of ALS patients have accumulated cytoplasmic TDP-43 aggregates in the post-mortem spinal cord (SC) (Spiller et al., 2016). TDP-43 increases BBB permeability and impairs the release of neurovascular unit components, leaving the brain vulnerable to systemic immune responses during inflammation (Zamudio et al., 2020; de Boer et al., 2021). The novel inducible mouse rNLS8 model of ALS was used to show that activated microglia selectively cleared neuronal hTDP-43, exerting an important neuroprotective effect (Spiller et al., 2018). Furthermore, Liao et al. observed increased mRNA levels of the M2 phenotype markers Ym1, CD163 and BDNF in murine mSOD1 microglia during the early stages of ALS, suggesting that microglia express the M2 anti-inflammatory phenotype during the early stages of ALS (Liao et al., 2012). However, during ALS disease progression, mSOD1-expressing microglia have dual phenotype and functional profiles. As the disease progresses, mSOD1 microglia in the spinal cords of ALS mice have increased levels of the M1 marker NOX2 and secrete reactive oxygen species (ROS) and pro-inflammatory cytokines (Frakes et al., 2014; Zhao et al., 2015). Frakes et al. (2014) demonstrated that NF-κB was activated in the G93A mouse model as ALS progressed and regulated the conversion of ALS microglia to a pro-inflammatory, neurotoxic state.

Studies of human cases and animal models of ALS have shown that astrocytes are a key factor in the progression of ALS. On the one hand, astrocytes play a role in the specific degeneration of spinal cord motor neurons in ALS. On the other hand, astrocytes can regulate BBB permeability and can crosstalk with CNS immune components and infiltrating peripheral immune components (Novellino et al., 2020). A recent study showed that transforming growth factor-β1 (TGF-β1) expression was upregulated in astrocytes in murine and human ALS (Endo et al., 2015). TGF-β1 is thought to be a negative regulator of the neuroprotective inflammatory response, forming an IFN-γ-dominated environment in infiltrating T cells. The overproduction of TGF-β1 hastens disease progression by disrupting the protective effects of microglia and T cells (Endo et al., 2015). In addition, previous studies have demonstrated that astrocytes can amplify the dual effects of microglia during the pre-symptomatic and symptomatic phases. In the $SOD1^{G93A}$ ALS model, IKK2/NF-κB activation in astrocytes drives Wnt-dependent microglial proliferation (Ouali Alami et al., 2018). This response prolongs the pre-symptomatic phase of ALS, delays muscle denervation, and reduces disease burden. However, during the symptomatic phase, this response enhances pro-inflammatory microglial activation, leading to accelerated ALS progression and shortened survival (Ouali Alami et al., 2018). Therefore, enhancing pre-symptomatic immune responses may be a viable therapeutic option for pre-symptomatic patients.

Mast cells are associated with myofibers and motor endplates and form NETs by interacting with neutrophils (Trias et al., 2018). Mast cells, phagocytic neutrophils, and NETs were abundant around the neuromuscular junction (NMJ) and in degenerating motor axons, which that suggested a role for immune effector cell interactions in driving ALS progression (Trias et al., 2017, 2018).

The topic of whether monocytes infiltrate the CNS and their impact on ALS progression is currently controversial. A parabiosis experiment found that CNS microglia are a closed system with no evidence of recruitment from the circulation (Ajami et al., 2007). However, another study showed that in the glial cells of ALS patients, there is increased expression of the chemokine MCP-1, which attracts monocytes and myeloid dendritic cells, which in turn recruit monocytes and moDCs (Henkel et al., 2004). Furthermore, Zondler et al. (2016) demonstrated that in ALS, circulating monocytes are dysregulated in terms of both subtype composition and function and that peripheral monocytes invasion of the CNS is increased. To some extent, peripheral monocytes have a protective role during the early stages of ALS. In a recent study, Komiya et al. assessed whether CCR2+ monocytes infiltrated the CNS of ALS mice by examining the distribution of the CCR2 protein in a CCR2-reporter mouse model (Komiya et al., 2020). This study demonstrated that during ALS disease progression, CCR2 expression expanded from CNS-infiltrating monocytes to centrally resident microglia and neurons, resulting in the toxic transformation of microglia and neurons, leading to accelerated ALS pathology.

As mentioned above, despite the controversy, a growing number of studies have shown infiltration of peripheral monocytes into the CNS. However, it is controversial whether infiltrating monocytes have a beneficial or detrimental effect on ALS progression. Therefore, future studies on monocytes are needed to provide new insights into the pathogenesis of ALS.

In ALS, NK cells determine the onset and progression of motor neuron degeneration. Garofalo et al. (2020) found that in $hSOD1^{G93A}$ mice, NK cells can directly kill spinal cord motor neurons. In addition, NK cells can also interact with resident microglia and infiltrating Treg cells, contributing to the motor impairment. A recent study demonstrated that NK cells promote ALS progression in a gender- and age-specific manner, which suggests that we should consider gender and age variables when designing immunotherapy for ALS (Murdock et al., 2021).

In addition to the innate immune response, the adaptive immune system is involved in the pathological process of ALS. In mSOD1 transgenic mice, CD4+ T cells provided supportive neuroprotection by modulating the trophic and cytotoxic balance of glial cells (Beers et al., 2008). Among them, Tregs act as suppressors of the excessive immune responses. In mice with a mutant form of SOD1, secondary transfer of Tregs induced the M2 phenotype in microglia in the spinal cord and delayed the onset of clinical symptoms, demonstrating the

neuroprotective effect of Tregs by modulating the activation of microglia (Banerjee et al., 2008; Sheean et al., 2018). Tregs tend to increase in the early stages of ALS, suppressing microglial activation by secreting IL-4 (Beers et al., 2017). As the disease progresses, the increased levels of proinflammatory cytokines lead to the transformation of microglia into a neurotoxic M1 phenotype, which may be associated with a decreasing tendency of Tregs due to the absence of FoxP3 expression (Beers et al., 2017). During the rapid developmental stage of ALS, not only do Tregs levels decrease, but patients show an increase in Th1 and Th17 proinflammatory T cell subsets and a decrease in Th2 cells in peripheral blood, and the immune phenotype is skewed toward a Th1/Th17 cell-mediated proinflammatory phenotype that correlates with the severity and progression of the disease (Jin et al., 2020).

In contrast, CTL infiltration in the CNS of ALS patients and mSOD1 mice is usually considered detrimental to motor neurons. MHCI is a key molecule associated with the interaction of monocytes with CD8+ T lymphocytes. Coque et al. showed that $SOD1^{G93A}$-expressing CD8+ T cells selectively triggered motor neuron death in an MHCI-dependent manner via the granzyme and Fas death pathways (Coque et al., 2019). In addition, Nardo et al. showed that CD8+ T cells interacted with microglia expressing MHCI, accelerated motor neuron death and reduced survival in $SOD1^{G93A}$ mice (Nardo et al., 2018). However, CD8+ T cells were shown to be more than just neurotoxic. Sustained expression of MHCI in motor neurons protected mSOD1 mice from ALS astrocyte-induced toxic effects (Song et al., 2016). Nardo et al. (2018) showed that the activation of MHCI in the peripheral nervous system of ALS mice was considered an early protective response. In addition, damaged MNs in SOD1-associated ALS actively recruit immune cells. Infiltration of CD8+ T cells and macrophages promotes myelin regeneration, delays muscle denervation and prolongs survival time (Nardo et al., 2016).

In summary, the role of the immune system is often complex and multifaceted. The same cell groups will have a positive or negative effect at different stages of the disease or with different stimuli. Future studies could focus on these different stages and the modulation of stimuli to promote more neuroprotective effects of immune cells.

Alzheimer's Disease (AD)

Alzheimer's disease (AD) is the most common neurodegenerative disease and the most common cause of cognitive decline in the elderly population. Characteristic pathological changes in AD include the deposition of β-amyloid (Aβ) in the brain to form senile plaques and the hyper-phosphorylation of tau protein to cause neurofibrillary tangles (NFTs) (Chen and Mobley, 2019). Despite tremendous efforts to determine the etiology of AD, the exact pathogenesis of AD is not fully understood, and there are no effective drugs or therapies to stop or reverse the progression of AD. Numerous studies have demonstrated that neuroinflammation plays a key role in the pathogenesis of AD and that crosstalk between peripheral immune cells and the CNS is involved in the onset and progression of AD. To date, the role of peripheral immunity in AD is still not well understood, although a large number of studies have demonstrated its involvement in all phases of AD.

Microglia may have dual roles in the pathogenesis of AD (Li et al., 2020). Activated microglia have the capacity to remove excess Aβ plaques and cellular debris (Ries and Sastre, 2016). Loss-of-function mutations in genes associated with microglial transmembrane proteins, such as TREM2 and CD33, are associated with reduced microglial phagocytosis and are considered genetic risk factors for AD (Hansen et al., 2018; Cao et al., 2021). As the disease progresses, the function of microglia changes. On the one hand, continuous stimulation with high cytokine concentrations leads to the transformation of microglia into a dysfunctional senescent state, with diminished phagocytosis leading to Aβ accumulation and the loss of neural support functions (Hickman et al., 2008; Salani et al., 2019). One the other hand, the danger-associated molecular patterns (DAMPs), including Aβ and phosphorylated tau, are the activators of microglia. These persistent stimuli drive chronic microglial activation, making them neurotoxic (Thadathil et al., 2021). Astrocytes can take up and internalize Aβ from the extracellular environment and participate in its degradation. A recent study demonstrated that IL-3 derived from astrocytes in the mouse brain was protective in a mouse model of AD and served as a key mediator of astrocyte-microglia crosstalk, which may be a strategy for therapeutic intervention in AD (McAlpine et al., 2021). However, the pathological role of reactive astrocytes in AD has been demonstrated. Chun et al. (2020) found that heavily reactive astrocytes could cause neurodegeneration in AD and act as a new hallmark of AD. This damage may be due to hydrogen peroxide production by heavily reactive astrocytes leading to neuronal death, brain atrophy, and cognitive impairment. Aβ plaques and tau aggregates can stimulate NLRP3 inflammasome within astrocytes and microglia cells, thereby causing activation of caspase-1 and the release of inflammatory cytokines that trigger pathophysiological changes in AD (Haseeb et al., 2022). The inhibition of NLRP3 inflammasome reduces pathological features such as Aβ deposition and Tau phosphorylation.

In addition, pericytes of the CNS are involved in AD pathology. In a mouse model of AD, pericytes are involved in Aβ-induced capillary constriction in the brain (Nortley et al., 2019). The loss of pericytes accelerates amyloid angiopathy and brain amyloidosis by reducing the clearance of Aβ from the interstitial fluid of the brain (Sagare et al., 2013).

Monocytes appear to have dual roles in AD pathophysiology. Murine monocytes were reorganized into two major subpopulations based on their chemokine receptor and Ly6C expression levels: a pro-inflammatory subset and an anti-inflammatory subset. On the one hand, the $Ly6C^{low}$ monocyte subpopulation patrolled the vascular lumen and enhanced tissue repair, phagocytosed toxic elements, including Aβ, and alleviated neurodegenerative processes (Naert and Rivest, 2013). On the other hand, $Ly6C^{high}$ monocytes could infiltrate the brain parenchyma and produce pro-inflammatory cytokines that promoted microglial activation (Fani Maleki and Rivest, 2019).

A recent study demonstrated that the inflammatory properties of circulating neutrophils change with increasing age and that

neutrophil phenotype may correlate with the rate of cognitive decline in AD patients. Thus, an altered neutrophil phenotype may serve as a prognostic blood biomarker for AD disease progression (Dong et al., 2018). In addition, the results of animal studies of AD suggest that neutrophils may be associated with the destruction of the BBB (Baik et al., 2014). Zenaro et al. (2015) observed in transgenic model mice with AD that the integrin LFA-1 controls neutrophil extravasation into the CNS and parenchyma and is present in areas with Aβ deposits. Neutrophils in these areas are directly toxic to neurons and the BBB by releasing NETs and IL-17 and may recruit more neutrophils into the CNS (Kolaczkowska and Kubes, 2013). Neutrophil depletion or integrin LFA-1 blockade reduced AD severity in animal models, suggesting that neutrophil-directed therapy may benefit AD patients (Zenaro et al., 2015).

Infiltration of the brain by peripheral NK cells and the resulting neuroinflammatory changes have been observed in human AD and 3xTg-AD mice (Zhang et al., 2020; Lu et al., 2021). It has been suggested that NK cells are biomarkers of the early stages of AD (Le Page et al., 2015, 2018). A study showed that the accumulation of NK cells in the aging brain impairs neurogenesis, and NK cell depletion reduces neurogenesis and neuroinflammation in the aging brain and AD patients (Jin et al., 2021). Another study demonstrated the critical role of NK cells in promoting neuroinflammation and AD-related cognitive decline. Depletion of NK cells with an anti-NK1.1 antibody significantly improved cognitive function in 3xTg-AD mice, and microglia from NK cell-depleted 3xTg-AD mice exhibited homeostatic-like morphology and decreased expression of pro-inflammatory cytokines (Zhang et al., 2020). In summary, targeting NK cells and neuroinflammation may provide new pathways for the treatment of AD. NK cell killing and degranulation remain unchanged during healthy aging and AD development, although numerous changes in NK phenotype and function occur (Le Page et al., 2015). Further studies on the altered phenotype and function of NK cells may help to provide insight into the relationship between NK cells and aging or with neurodegenerative diseases.

In contrast, the role of adaptive immunity in AD has not been adequately explored. Increased Aβ-specific CD4+ T cell and B cell responses were found in the blood samples of AD patients, suggesting that Aβ can antigenically induce adaptive immune responses (Monsonego et al., 2003). In a Rag-5×fAD mouse model of T-, B- and NK cell-deficient mice, the Aβ plaque load was significantly increased, the neuroinflammatory phenotype of microglia was exacerbated, and phagocytosis was reduced (Marsh et al., 2016). This finding suggests that the adaptive immune system plays an important role in limiting AD amyloid pathology. However, Kim et al. observed the progression of AD required B cells (Kim et al., 2021). At the onset of the disease, therapeutic depletion of B cells significantly delayed the progression of AD in mice. Meanwhile, in another study, decreased brain Aβ levels and increased microglial proliferation were observed in an aged PSAPP mouse model of functional T and B cell ablation (Späni et al., 2015). These conflicting findings suggest that the role of the adaptive immune system in the development of AD remains controversial.

The role of Tregs in AD pathophysiology has been controversial in recent years. The transplantation of Treg cells into 3xTg-AD transgenic mice led to the observation of improved AD pathology in mice, as well as the observation of reduced brain Aβ load and reduced production of inflammatory cytokines (Baek et al., 2016). However another study found that a transient depletion of Foxp3(+) Tregs may contribute to AD disease remission (Baruch et al., 2015). Such conflicting results imply a complex role for Tregs in AD pathology. It is worth noting that these two conflicting results may be due to the different disease stages. In the early stages of the disease, Tregs may promote beneficial activation of microglia and inhibit deleterious pro-inflammatory glial proliferation (Dansokho et al., 2016). However, in later stages of the disease, systemic Foxp3+ Treg plays a negative role in the pathology of AD by altering the function of the choroid plexus and thereby reducing leukocyte recruitment to the CNS (Baruch et al., 2015). Future studies are needed to evaluate the therapeutic potential of Treg-based immunomodulatory approaches in AD.

Due to the altered permeability of the BBB in AD patients, immune cells can travel to and from the brain. Recent studies have shown that crosstalk between microglia and astrocytes is critical for T cell recruitment to the CNS (Burgaletto et al., 2020). Previous studies have demonstrated an increased percentage of lymphocytes in the brain parenchyma of AD patients. A recent study demonstrated the presence of clonally expanded CD8+ TEMRA cells in the cerebrospinal fluid of AD patients and that clonally expanded CD8+ T cells patrolled the cerebrospinal fluid during age-related neurodegeneration (Gate et al., 2020). Laurent et al. demonstrated the activation of microglia and astrocytes in a THY-Tau22 mouse model of tau pathology and cognitive dysfunction (Laurent et al., 2017). Furthermore, the infiltration of CD8+ T cells associated with early chemokine responses, particularly those involving CCL3, was observed in the hippocampus in mice. This finding demonstrates the important role of adaptive immunity in AD pathophysiology.

Parkinson's Disease (PD)

Parkinson's disease (PD), which is also known as tremor palsy, is the second most common neurodegenerative disease after AD. PD is characterized by a prominent loss of dopaminergic neurons in the substantia nigra (SN) and pathological intraneuronal aggregation of alpha-synuclein (α-syn) in Lewy vesicles (Shahnawaz et al., 2020). PD pathogenesis is still unclear, and the increased incidence is related not only to aging but to environmental factors and genetic defects that can lead to the degeneration of dopaminergic (DA) neurons in the brain. Leucine-rich repeat kinase 2 (LRRK2) is the most commonly mutated gene in familial PD (Deniston et al., 2020). Over the past decade, an increasing number of studies have focused on the role of the immune system in AD, and pro-inflammatory immune-mediated mechanisms are believed to play important roles in disease progression. However, the extent to which changes in peripheral immunity affect the CNS remains a matter of debate.

Since the discovery of activated microglia in the substantia nigra pars compact (SNpc) of the midbrain in PD patients

by McGeer et al. (1988), microglial activation and subsequent neuroinflammation have been shown to play multiple roles in the degeneration of dopaminergic neurons in AD patients. Microglia contribute to the clearance of misfolded α-syn aggregates in PD (Brück et al., 2016). However, α-syn can in turn activate NLRP3 inflammasome in microglia through interaction with Toll-like receptors (TLR). This leads to translocation of NF-κB, which induces increased expression of pro-inflammatory cytokines as well as impaired mitochondria, thereby damaging dopaminergic neurons (Gustot et al., 2015). Targeting the α-syn/TLRs/NF-κB/NLRP3 inflammasome axis may have some potential application in the treatment of PD (Li et al., 2021). However, at present, modulators of inflammasome are limited by clinical effectiveness as well as safety factors, making it difficult to achieve translation to the clinic.

In addition, LRRK2 mutations associated with PD can drive microglial activation, leading to increased microglial phagocytosis and increased production of inflammatory factors, as well as reactive oxygen species (ROS) (Subramaniam and Federoff, 2017; Kim et al., 2018). Microglia, which were originally neuroprotective, become toxic to dopaminergic neurons due to the overproduction of cytokines and ROS. In addition to their recognized role in neuroinflammation, glial cells are involved in the intercellular transmission of α-syn through exosome release. Guo et al. (2020) observed that exosomes released from microglia could induce nigrostriatal degeneration and play a key role in the pathogenesis of PD.

Immunoreactive astrocytes with elevated density and phenotypic changes have been identified in post-mortem PD brains; however, the specific function of astrocytes in PD pathology remains unclear (Braak et al., 2007). On the one hand, astrocytes can play a protective role in disease progression by effectively isolating and degrading pro-inflammatory extracellular α-syn. On the other hand, high concentrations of extracellular α-syn induce astrocyte inflammatory responses in a TLR4-dependent manner, which may exacerbate stressful conditions in brains with synucleinopathy (Rannikko et al., 2015). A recent study by Sonninen et al. (2020) suggested that LRRK2- and GBA-mutant astrocytes contributed to the development of PD. In addition, the activation of microglia by classic inflammatory mediators converts astrocytes to the neurotoxic A1 phenotype. Yun et al. (2018) found that NLY01 was a potent GLP1R agonist with good neuroprotective effects by directly blocking the microglia-mediated conversion of astrocytes to the A1 neurotoxic phenotype. Astrocytes are involved in the disruption of the BBB in PD patients. Lan et al. (2022) found that α-syn oligomers lead to the activation of astrocytes, which increased the production and release of vascular endothelial growth factor A (VEGFA) and nitric oxide (NO), both of which can lead to the degradation of BBB integrity. It is worth noting that recent studies have demonstrated that PD is also associated with OLs (Bryois et al., 2020). Agarwal et al. (2020) described the human single-nuclei transcriptomic atlas for the SN and found a significant association between the risk of PD and oligodendrocyte-specific gene expression, which also reveals a possible role of OLs in the etiology of PD.

Monocytes may be an essential element in the pathogenesis of PD, and the overexpression of expressed quantitative trait loci (eQTL), which is specific to monocytes, has been shown to be associated with PD (Raj et al., 2014). The disease-specific gene expression profile of peripheral blood mononuclear cells in early AD correlates with the severity of the disease (Schlachetzki et al., 2018). Grozdanov et al. (2014) demonstrated the dysregulation of peripheral blood monocytes in PD patients, in which an increased proportion of pro-inflammatory monocytes was accompanied by activation of the CCR2-CCL2 axis in PD. The authors suggest that this increase in classic monocytes and elevated CCL2 serum levels may be related to the secretion of inflammatory mediators by microglia. The FAS/FASLG system, which regulates monocyte subpopulations, may be a potential target for PD therapy. A recent study showed that pathological α-syn activates LRRK2 expression and kinase activity in monocytes, promoting the recruitment of pro-inflammatory monocytes to the brain, which in turn drives the neuroinflammatory response in PD. Thus, LRRK2 kinase inhibitors may attenuate pro-inflammatory monocyte responses in the brain (Xu et al., 2022).

The role of NK cells in PD pathogenesis is still unclear. However, a recent study showed that NK cells have a protective effect on Lewy body (LB)-related neurodegenerative diseases. On the one hand, human NK cells can efficiently internalize and degrade α-syn aggregates via the endosomal/lysosomal pathway. On the other hand, NK cells are involved in resolving extracellular α-syn load by producing IFN-γ and activating or differentiating antigen-presenting cells, including microglia. In a preclinical mouse model of PD, systemic depletion of NK cells led to worsened motor symptoms and nuclear protein pathology (Earls et al., 2020). Recent evidence indicated that the misfolded a-syn may be retrogradely transported from the enteric nervous system to the CNS along the vagus nerve (Hill et al., 2021). Therefore, whether immune cells affect α-syn pathology in the periphery, especially in the intestine, may be an important research direction for future studies.

There is increasing consensus that the adaptive immune system is also involved in the pathogenesis of PD (Tansey and Romero-Ramos, 2019). CD4+ T cells and CD8+ T cells have been found in the SN region in postmortem specimens from PD patients and MPTP-induced mice, and this immune response promotes dopaminergic neuron (DN) degeneration through the Fas/FasL cytotoxic pathway (Brochard et al., 2009). Sommer et al. found that a large amount of Th17 is present in the substantia nigra of PD patients and that IL-17 secreted by T lymphocytes is essential for neuronal death (Sommer et al., 2018). Additionally, in an *in vitro* model constructed using patient-induced multifunctional stem cells, antagonism of both IL-17 and its receptor was able to prevent neuronal death. In addition, the adaptive immune system interacts with immune cells in the CNS. It is suggested that glial cells may be involved in the Th17-mediated cell death of PD neurons described above (Muffat et al., 2016; Sommer et al., 2018). Activated microglia secrete inflammatory mediators that mediate antigen presentation to CD4+ T cells via the MHC-II pathway, resulting in cell proliferation, slow degeneration and dopaminergic neuronal death (Marogianni et al., 2020).

However, Tregs are a subtype of CD4+ T cells. Tregs exert neuroprotective effects on animal models of PD by inhibiting immune activation and microglial attack of α-syn and preventing the loss of dopaminergic neurons in the substantia nigra (Reynolds et al., 2007). Recent studies have shown that the pathogenesis of PD involves two stages of CTL-mediated immune responses. The first stage is early robust CTL infiltration, which causes slight neuronal loss and α-syn aggregation, but no dopaminergic neuronal death was found at this stage. More modest CD8+ T cell infiltration was observed in the next stage, amplifying α-synuclein pathology and neurodegeneration. The authors suggested that CD8+ T cells promote substantia nigra dopaminergic neuron dysfunction and death in PD prior to the appearance of overt Lewy bodies (Galiano-Landeira et al., 2020). This pathogenicity of CD8+ T cells should be confirmed by appropriate models. Further studies on the number and phenotype of infiltrating CTL at different stages of the disease could help in the development of immunotherapies targeting these T cells.

In summary, we have presented the emerging role of the central and peripheral nervous systems in ALS, AD and PD. Meanwhile, the immune system also plays a crucial role in other neurodegenerative diseases, such as multiple sclerosis or Huntington's disease.

Multiple sclerosis (MS) is traditionally defined as a chronic immune-mediated demyelinating disease of the CNS. The activation of glial cells plays a key role in the process of demyelination, neuronal and axonal damage (Baaklini et al., 2019). Similar to the pathogenesis of AD, neuroinflammation in MS is also characterized by the activation of microglia and astrocytes in the CNS. This leads to the secretion of additional pro-inflammatory cytokines and chemokines that further exacerbate neuroinflammation and BBB destruction, thereby recruiting more peripheral immune cells (Linnerbauer et al., 2020; Vainchtein and Molofsky, 2020). In addition, such interactions between glial cells have an effect on OPC/OLs, thus affecting the demyelination remyelination process (Chu et al., 2021).

Approximately 85% of patients present with a relapsing-remitting phenotype, which is dominated by peripheral immune responses. Peripheral immune cells disrupt BBB infiltration into the brain parenchyma, producing focal areas of primary demyelination (Lassmann, 2018). In contrast, the primary and secondary progressive forms of MS are dominated by neurodegeneration and enhanced innate immune responses, resulting in severe axonal damage, neuronal death and synaptic loss (Faissner et al., 2019). In contrast to other neurodegenerative diseases, immunotherapies have had great success in targeting relapsing-remitting MS. These therapies primarily target the peripheral immune system and therefore have limited effectiveness in the treatment of progressive MS (Healy et al., 2022). Further studies on the activation state, pathogenic role and interaction of glial cells with peripheral immune cells may help to identify the new potential therapeutic opportunities.

Huntington's disease (HD) is a rare genetic neurodegenerative disorder caused by the amplification of CAG repeats in the Huntington (HTT) gene and the accumulation of mutant proteins (mHTT) (Bates et al., 2015). The pathophysiology of HD remains unclear, but previous studies have highlighted the role of the immune system and neuroinflammation in HD pathology. mHTT is highly expressed in microglia and peripheral immune cells. As an inflammatory stimulus for these cells, mHTT may promote inflammatory responses through direct toxic effects, impaired glutamatergic homeostasis, or mitochondrial dysfunction (Taherzadeh-Fard et al., 2011; Weiss et al., 2012). Previous studies have demonstrated that increased microglia activation and dysregulation of astrocytic neuroinflammatory signaling pathways are associated with the progression of HD (Hsiao et al., 2013). However, peripheral adaptive immune cells rarely infiltrate into the CNS. At present, HD remains incurable, and immunotherapy and anti-inflammatory drug treatments are not effective. Many molecular targets as well as gene therapies are currently under clinical investigation (Devadiga and Bharate, 2022).

THERAPEUTIC STRATEGIES FOR NEURODEGENERATIVE DISEASES

Previous studies have demonstrated the effectiveness of strategies harnessing peripheral blood innate immune cells in clearing Aβ from brain parenchyma and blood vessels, thereby slowing the progression of AD in mouse models (Koronyo et al., 2015; Rentsendorj et al., 2018; Koronyo-Hamaoui et al., 2020). Koronyo et al. (2015) indicated that the cerebral infiltration of monocytes was beneficial to disease outcome, due to the effects on restriction of astrogliosis, cellular uptake and enzymatic degradation of Aβ. Rentsendorj et al. shown that Osteopontin (OPN), which is highly expressed in bone marrow monocytes, is an important regulator of macrophage polarization toward an anti-inflammatory immunophenotype and clearance of pathogenic Aβ (Rentsendorj et al., 2018). However there is still controversy as to whether peripheral monocytes can enter the AD brain and whether they can be used as a treatment for neurodegenerative diseases (Reed-Geaghan et al., 2020).

Adaptive immunity is also essential in the pathogenesis of neurodegenerative diseases, and the understanding of the adaptive system has a positive effect on facilitating immune-mediated treatment of neurodegenerative diseases. For example, Tregs exert neuroprotective effects by regulating microglia, effector T cells (Teffs), motor neurons (MN). Although the passive transfer of mSOD1 Tregs to ALS mice lacking functional T lymphocytes has made great progress in prolonging survival, there is still a long way to go to achieve successful clinical translation (Banerjee et al., 2008; Beers et al., 2011). A first-in-human phase 1 trial has shown that autologous Tregs infusion can slow disease progression in ALS patients. However, autologous Tregs are inefficient in targeting the CNS and require frequent and large infusions to induce therapeutic effects (Thonhoff et al., 2018).

As shown above, the pathological amyloid aggregation of α-Syn protein is the main pathological hallmark of PD,

TABLE 1 | The role of central and peripheral immune cells on the pathogenesis of neurodegenerative diseases.

	Amyotrophic lateral sclerosis (ALS)	Alzheimer's disease (AD)	Parkinson's disease (PD)	References
Microglia	• Exerted an neuroprotective effect in the early stages of ALS. • Converted to a proinflammatory, neurotoxic state as ALS progressed.	• Activated microglia have been shown to clear excess Aβ plaques and cellular debris. • Neurotoxic and diminished phagocytosis and as AD progresses.	• Microglia contribute to the clearance of misfolded α-syn aggregates in PD. • Involved in the degeneration of dopaminergic neurons.	(Liao et al., 2012; Frakes et al., 2014; Zhao et al., 2015; Brück et al., 2016; Ries and Sastre, 2016; Hansen et al., 2018; Kim et al., 2018; Spiller et al., 2018)
Astrocytes	• Astrocytes are active participants in neuronal damage in ALS by producing neurotoxicmediators. • Regulated BBB permeability and can crosstalk with CNS immune components and infiltrating peripheral immune components.	• Reactive astrocytes are involved in AD pathology. • Astrocytes can also take up and internalize Aβ from the extracellular environment and participate in its degradation.	• Played a protective role in PD progression by isolating and degrading proinflammatory extracellular α-syn. • LRRK2- and GBA-mutant astrocytes contribute to the development of PD. • Involved in the disruption of the BBB.	(Rannikko et al., 2015; De Biase et al., 2017; Chun et al., 2020; Linnerbauer et al., 2020; Novellino et al., 2020; Sonninen et al., 2020; Lan et al., 2022)
Monocyte	• Exerted protective effects in the early stages of ALS. • Accelerated the progression of ALS during disease progression.	• Monocytes appear to have dual roles in AD pathophysiology.	• Peripheral blood mononuclear cells are dysregulated, with an increased proportion of proinflammatory monocytes. • Pathological α-syn promotes recruitment of proinflammatory monocytes to the brain.	(Naert and Rivest, 2013; Grozdanov et al., 2014; Zondler et al., 2016; Fani Maleki and Rivest, 2019; Komiya et al., 2020; Xu et al., 2022)
NK cells	• NK cells promote ALS progression in a gender- and age-specific manner.	• Infiltration of the brain by peripheral NK cells and the resulting neuroinflammatory changes have been observed in human AD and animol model.	• Internalize and degrade α-syn aggregates through the endosome/lysosome pathway. • Produce cytotoxicity against hyperactive microglia.	(Earls and Lee, 2020; Earls et al., 2020; Jin et al., 2021; Lu et al., 2021; Murdock et al., 2021)
Dendritic cells	• The role of DCs in ALS pathogenesis is still unclear.	• Dendritic cell-based immunotherapy against AD can be used as potential therapeutic approach.	• Tolerogenic bone marrow-derived DCs (BMDCs) induced Tregs.	(Brezovakova et al., 2018; Schutt et al., 2018)
T cells	• The circulating CD4+ T cells are involved in ALS progression through multiple mechanisms. In animal model, CD4+ T cells provided supportive neuroprotection.	• The role of the T cells in the development of AD remains controversial.	• Tregs exert neuroprotective effects through the interaction of the peripheral and central immune systems.	(Reynolds et al., 2007; Beers et al., 2008)
B cells	• The role of B cells in ALS pathogenesis is still unclear.	• Played an essential role on cerebral Aβ pathology. • The role of the B cells in the development of AD remains controversial.	• No B cells were identified in the post-mortem brain tissue of PD patients. • IgG deposition was found on dopaminergic neurons in the substantia nigra and Lewy bodies.	(Orr et al., 2005; Späni et al., 2015; Kim et al., 2021)

and neuroinflammation also plays an important role in the progression of PD. Modulating the activation state of glial cells by directly targeting cellular neuroinflammation, inhibiting harmful pro-inflammatory neurotoxicity, and enhancing their anti-inflammatory protective function is also a new approach to PD treatment (Subramaniam and Federoff, 2017). For example, NK cells can internalize and degrade α-syn aggregates through the endosome/lysosome pathway (Earls et al., 2020). NK cells can also interact with microglia, thereby producing cytotoxicity against hyperactive microglia (Earls and Lee, 2020). In addition, immunotherapy is also a very promising protocol for the treatment of PD, and the research on active and passive immunity against α-syn has been a novel starting point for the therapy of PD. Immunotherapy improves disease progression in patients with early-stage PD by reducing extracellular α-Syn load (Mandler et al., 2015). In clinical trials, active immunization mainly includes two humanized immunogens, PD01A and PD03A. PD passive immunization mainly includes PRX002, BIIB054 humanized antibodies, and two other anti-α-syn monoclonal antibodies MEDI1341 and BAN0805 are

in the early stages of development. The active and passive immunity of α-syn has been described in detail in other literature (Zella M. A. S. et al., 2019; Zella S. M. A. et al., 2019). Recent studies demonstrated that vaccination based on DCs can provide a bridge between innate and adaptive immune responses (Brezovakova et al., 2018). By using dendritic cells as natural adjuvants, the host immune system is enhanced and the production of specific antibodies helps to clear pathological aggregation of intracellular proteins (Brezovakova et al., 2018; Sabahi et al., 2021). The vaccination based on DCs maintains the balance of the immune response and may have advantages over traditional protein-based vaccination (Sabahi et al., 2021). Since the first attempts at immunotherapy for neurodegenerative diseases, promising advances have been made, however, further studies are required to confirm its efficacy in neurodegenerative diseases.

Traditional Chinese herbal medicine (TCM) has been used for thousands of years as one of the therapies for neurodegenerative diseases, including dementia. In recent years, with the development of modern pharmacological research techniques, bioactive components isolated from TCM beneficial to patients with neurodegenerative diseases have been identified and purified, and their mechanisms of action have been extensively studied.

Previous studies have demonstrated that herbal monomers and extracts modulate AD by reducing β-amyloid production and regulating autophagy, oxidative stress, microglia polarization, and mitochondrial function (Chen S. Y. et al., 2020). For example, *Achyranthes bidentata* Blume (AB), a traditional Chinese medicine, is widely used in the treatment of dementia. ABS inhibits amyloid deposition and reduces the activation of microglia and astrocytes. It also modulates ERK and NF-κB pathways, decreases levels of proinflammatory cytokines in the brain and reduces neuroinflammation (Lin et al., 2019). Astragaloside IV (AST-IV) can exert anti-inflammatory effects on microglia by inhibiting the TLR4/NF-κB signaling pathway and promote the transformation of microglia to a neuroprotective M2 phenotype (Yu et al., 2019). In addition, QMAD, a dichloromethane soluble fraction of the Chinese herb QuMai, induces Tregs by altering intracellular signaling that restricts AKT phosphorylation (Reid-Adam et al., 2013), which may be a new strategy for the treatment of ALS.

A growing number of studies have demonstrated the crosstalk between the gut microbiota, the peripheral immune system and the CNS. Meanwhile, the dysregulation of the gut microbiota was shown to be necessary for the infiltration of peripheral immune cells into the brain. Also, studies on microglia-gut connections have suggested the important role of brain-gut microbiota in neurodegenerative diseases (Perez-Pardo et al., 2018; Wang et al., 2018). This also provides new ideas on the possible mechanisms of TCM for the treatment of neurodegenerative diseases. For example, Hua-Feng-Dan (HFD) is used to treat neurological dysfunction such as PD. The cinnabar and realgar in HFD were effective in restoring LPS and rotenone induced alterations in intestinal flora. This effect on intestinal microbes has also been shown to be associated with neuroprotective effects. HFD produces a protective effect against LPS and rotenone-induced DA neurotoxicity by delaying DA neuron loss, increasing TH protein expression, and reducing microglia activation (Chen C. et al., 2020). GV-971, prepared from marine brown algae extract, could remodel the composition of intestinal flora in AD mice, thereby inhibiting Th1 cell differentiation and M1-type microglia activation (Wang et al., 2019). These studies demonstrate that herbal monomers and compound herbs may suppress inflammatory responses in neurodegenerative diseases by regulating intestinal flora.

CONCLUSION

In this review, we highlight the emerging role of the peripheral and central immune systems in neurodegenerative diseases, as well as their interactions (**Table 1**). In recent years, the prevalence of neurodegenerative diseases has been gradually increasing, imposing a huge economic and emotional burden on society and patients. However, there are no effective therapies to stop or reverse the progression of neurodegenerative diseases. Despite the recent progress, clinical trials so far have been disappointing. We propose in this review that for immunotherapy of neurodegenerative diseases, it is important to focus on not only CNS immunity or peripheral immunity but also their interactions. And the limitations of the current understanding of interactions between CNS and peripheral immunity are challenges that need to be urgently overcome. A detailed understanding of the key steps in the process of immune cell infiltration from the peripheral circulation to the CNS and aggressive interventions in these steps may lead to the development of more effective therapies to manage these intractable neurodegenerative diseases. In addition, the natural active ingredients in TCM have multifaceted pharmacological effects, which provide new ideas for the multi-targeted treatment of neurodegenerative diseases. It has been reported that TCM is involved in the pathogenesis of neurodegenerative diseases through multiple pathways, and future studies will be based more on the mechanism of action of TCM on the peripheral and central immune systems. A portion of TCM drugs have been applied in clinical trials, providing novel treatment strategies for neurodegenerative diseases.

AUTHOR CONTRIBUTIONS

YZ contributed to the conception and design of this review and finally approved the version to be submitted. XZ, SC, JZ, and JM designed, wrote, and edited the manuscript. All authors read and revised the final manuscript.

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frontiers | Frontiers in Aging Neuroscience

REVIEW
published: 03 May 2022
doi: 10.3389/fnagi.2022.890958

Neuroimmune Crosstalk Between the Peripheral and the Central Immune System in Amyotrophic Lateral Sclerosis

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OPEN ACCESS

Edited by:
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Reviewed by:
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The University of Queensland, Australia

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Specialty section:
This article was submitted to Neuroinflammation and Neuropathy, a section of the journal Frontiers in Aging Neuroscience

Received: *07 March 2022*
Accepted: *14 April 2022*
Published: *03 May 2022*

Citation:
Yu W, He J, Cai X, Yu Z, Zou Z and Fan D (2022) Neuroimmune Crosstalk Between the Peripheral and the Central Immune System in Amyotrophic Lateral Sclerosis. Front. Aging Neurosci. 14:890958. doi: 10.3389/fnagi.2022.890958

Amyotrophic lateral sclerosis (ALS) is a fatal disease characterized by the degeneration and death of motor neurons. Systemic neuroinflammation contributes to the pathogenesis of ALS. The proinflammatory milieu depends on the continuous crosstalk between the peripheral immune system (PIS) and central immune system (CIS). Central nervous system (CNS) resident immune cells interact with the peripheral immune cells *via* immune substances. Dysfunctional CNS barriers, including the blood–brain barrier, and blood–spinal cord barrier, accelerate the inflammatory process, leading to a systemic self-destructive cycle. This review focuses on the crosstalk between PIS and CIS in ALS. Firstly, we briefly introduce the cellular compartments of CIS and PIS, respectively, and update some new understanding of changes specifically occurring in ALS. Then, we will review previous studies on the alterations of the CNS barriers, and discuss their crucial role in the crosstalk in ALS. Finally, we will review the moveable compartments of the crosstalk, including cytokines, chemokines, and peripheral immune cells which were found to infiltrate the CNS, highlighting the interaction between PIS and CIS. This review aims to provide new insights into pathogenic mechanisms and innovative therapeutic approaches for ALS.

Keywords: amyotrophic lateral sclerosis, crosstalk, peripheral immunity, CNS barriers, CNS immunity

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease typically characterized by adult-onset dysfunction of both upper and lower motor neurons (MNs). The incidence rates of this fatal disease were 1.38 (urban China), 1.5 (United States), 2.08 (Europe) per 100,000 persons (Xu et al., 2020; Burchardt et al., 2022; Mehta et al., 2022), and most patients died within 3–5 years after disease onset (Chia et al., 2018). No clinical therapies have been proven effective except for riluzole

and edaravone, which can only delay disease progression (Chen et al., 2016; Scott, 2017; Shefner et al., 2020). The mechanisms underlying ALS pathogenesis are not yet fully understood. ALS is a multifaceted disease, and several mechanisms, including pathogenic gene mutations (Cervantes-Aragón et al., 2020), neuroinflammation (Beers and Appel, 2019), autophagy, mitophagy (French et al., 2018), necrosis (Yuan et al., 2019), aggregation of toxic proteins (Wei et al., 2017), dysfunction of energy metabolism (Vandoorne et al., 2018), and environmental factors (French et al., 2018), have been proven to participate in its pathogenesis.

Accumulating evidence indicates abnormalities in the immune system throughout ALS (Dutta et al., 2020; Theoharides and Tsilioni, 2020). Immune cells are activated and lead to a chronic proinflammatory microenvironment in both the peripheral and central nervous systems in ALS (Masrori et al., 2022). The pro-inflammation in ALS is systemic, and crosstalk exists between the peripheral immune system (PIS) and the central immune system (CIS). To date, crosstalk has not been well defined. With the development of insights into the understanding of ALS, researchers have realized the importance of the continuous interaction and communication of these two systems. CNS resident immune cells and peripheral immune cells interact with each other *via* immune molecules. Dysfunctional CNS barriers, including the blood–brain barrier (BBB) and the blood–spinal cord barrier (BSCB), open the gate for "crosstalk" and are also regulated by the inflammatory environment. As a result, chronic systemic inflammation contributes to the death of MNs, injuring motor neuron axons, and the dysfunction of neuromuscular junctions (Sweeney et al., 2019; Wu Y. et al., 2020; Pan and Nicolazzo, 2022; **Figure 1**).

MAJOR CHANGES OF RESIDENT IMMUNE CELLS IN AMYOTROPHIC LATERAL SCLEROSIS

Inflammation in Central Nervous System in Amyotrophic Lateral Sclerosis

Inflammation is widespread in the CNS in ALS (Beers and Appel, 2019; Liu et al., 2021). Glial cells, including microglia and astrocytes, trigger neuroinflammatory reactions, interact with infiltrated peripheral immune cells and eventually induce or accelerate neuronal death in CNS in ALS (Cragnolini et al., 2020). Microglia are the resident innate immune cells of the CNS, and mediate the neuroinflammation *via* the release of immune molecules including cytokines and chemokines. Microglia activation is heterogeneous and dependent on the nature of the pathological insult (Mattei and Notter, 2020). Researchers have categorized activated microglia into two opposite types: M1 (toxic or proinflammatory) or M2 (neuroprotective or anti-proinflammatory) microglia (Guo et al., 2022). However, researchers have recently realized that there is a continuum of phenotypes between M1 and M2 in ALS (Li et al., 2019), such as disease-associated microglia (DAM) (Krasemann et al., 2017; Dols-Icardo et al., 2020) and receptor-interacting protein kinase 1 (RIPK1)—regulated inflammatory microglia (RRIMs) (Mifflin et al., 2021). In general, accumulating studies have proven that microglia show an anti-inflammatory phenotype and protect MNs at the onset of the disease, while end-stage microglia shift to a proinflammatory phenotype and aggravate the neurodegeneration of MNs in ALS (Liu et al., 2021; Masrori et al., 2022). Astrocytes are the most common glial cells in the brain, maintain the CNS barriers (Signorile et al., 2021), secrete neurotrophic and neuroprotective factors, regulate neurotransmitter uptake and recycling, and promote neurogenesis (Gharbi et al., 2020). Studies have identified a role for astrocytes as immune modulators, as they may control the activation, migration, and proliferation of microglia (Sunnemark et al., 2005; Ouali et al., 2018).

Immune Activation in the Periphery in Amyotrophic Lateral Sclerosis

Peripheral immune abnormalities exist in ALS (McCombe et al., 2020). In general, the chronic peripheral immune response is proinflammatory in ALS. Lymphocytes, monocytes (including macrophages), neutrophils, natural killer (NK) cells, and mast cells (MCs) are peripheral resident immune cells. ALS patients were found to have elevated total leukocyte counts in blood (Murdock et al., 2017). In peripheral blood, most studies suggest decreased levels of neuroprotective CD4 T lymphocytes while the subgroup of CD4 T lymphocytes, regulatory T cells (Tregs), are reduced and dysfunctional in ALS patients. In ALS, the number of cytotoxic CD8 T lymphocytes in peripheral blood is controversial. NK T lymphocytes are thought to be harmful in ALS and are increased in peripheral blood in patients with ALS (Finkelstein et al., 2011; Perner et al., 2018; Giovannelli et al., 2020; Nishihara et al., 2020; Rolfes et al., 2021). B lymphocytes are merely discussed in ALS and studies suggest that they play a supplementary role in the pathogenesis of ALS (Naor et al., 2009; Pennati et al., 2018). Alterations in the proportion of monocytes were reported and circulating monocytes from ALS patients preferentially differentiated to a proinflammatory phenotype (Liu et al., 2016; Du et al., 2020). The numbers of neutrophils are increased in the peripheral blood and show a significant correlation with disease progression (Murdock et al., 2017; Leone et al., 2022). NK cells are innate immune cells and mediate cytotoxicity. Levels of NK cells in the blood of ALS patients are increased and could be pathogenic (Gustafson et al., 2017; Murdock et al., 2017). An increased number of circulating MCs was shown in ALS mice while there was a lack of evidence in ALS patients (Trias et al., 2018; Harcha et al., 2021).

Distal axonopathy is a recognized pathological feature of ALS (Nardo et al., 2016). Recruitment of activated MCs, macrophages, and neutrophils along the degenerating motor axons in sciatic nerves and skeletal muscle is observed in ALS (Chiu et al., 2009; Angelini et al., 2020; Trias et al., 2020). Peripheral immune cells can also infiltrate into CNS and exert an effect on motor neurons and glial cells, which will be discussed below. Peripheral immune cells have been increasingly discussed in their prognostic role. In this regard, with the development of technology and

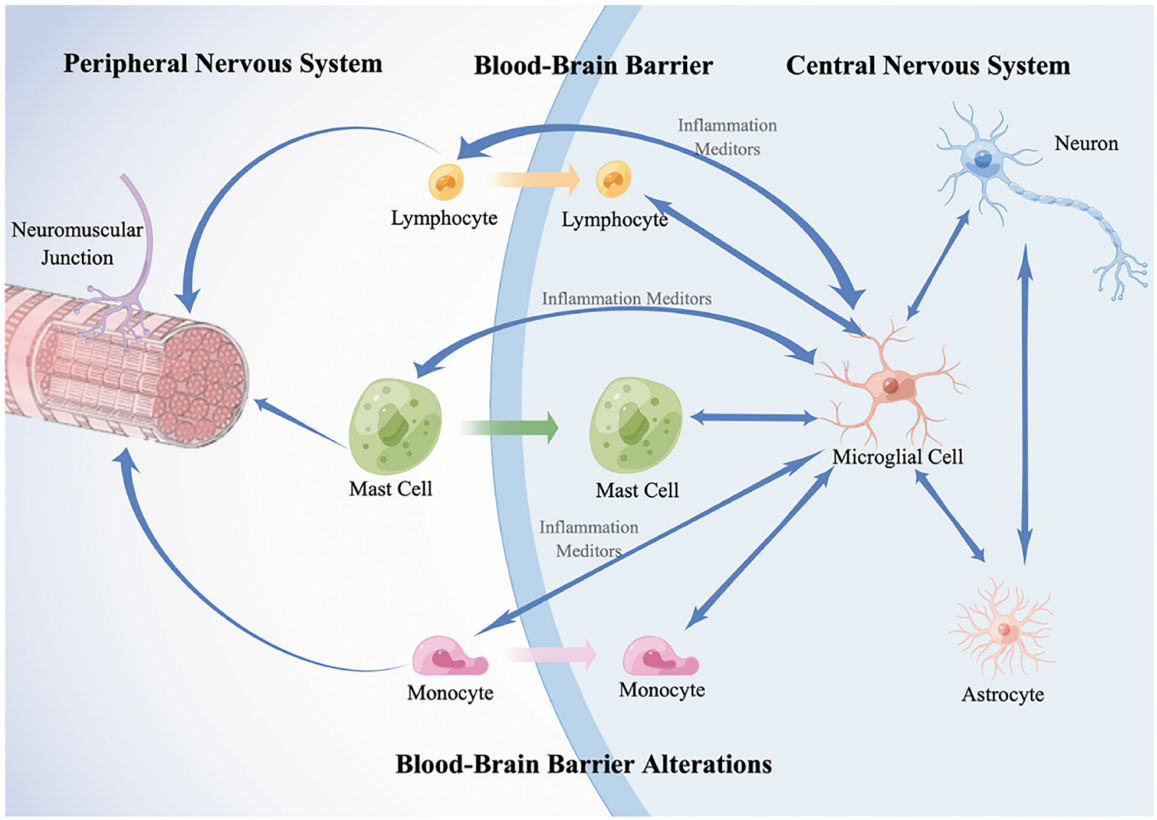

FIGURE 1 | A schematic diagram of immune crosstalk between PNS and CNS. In the CNS, resident immune cells, microglia, are activated and mediate the neuroinflammation by the release of proinflammatory or anti-inflammatory substances such as cytokines and interact with infiltrated peripheral immune cells; astrocytes control the activation, migration, proliferation of microglia. In the PNS, resident immune cells, including T lymphocytes, mast cells, and monocytes are activated and infiltrate along the peripheral motor nerve and neuromuscular junction. Meanwhile, they infiltrate into CNS triggered by microglia-derived inflammation mediators. In addition, dysfunction of CNS barriers, including the blood-brain barrier (BBB) and the blood-spinal cord barrier (BSCB), contribute to the infiltration of peripheral immune cells and accelerate the harmful interaction. As a result, inflammatory responses spread across the two systems contribute to the death of motor neurons (MNs), injuring MN axons, and the dysfunction of neuromuscular junctions. Double-headed arrows represent the communication of two cells. Blue single arrows represent that the cells release inflammatory mediators and influence their targets. Orange, green, and purple arrows represent that the peripheral cells infiltrate into the CNS, respectively.

understanding, researchers have turned to exploring a specific population or a single myeloid subpopulation to categorize or monitor patients (Murdock et al., 2017; Leone et al., 2022).

ALTERATION OF THE CENTRAL NERVOUS SYSTEM BARRIERS IN AMYOTROPHIC LATERAL SCLEROSIS

CNS barriers are formed by a layer of endothelial cells, connected by inter endothelial tight junctions (TJs), adhesion proteins, and cytoplasm (Bull et al., 2022). A basement membrane called the basal lamina (BL) ensheathed by pericytes and astrocytic end-feet supports endothelial cells and associated pericytes (Lochhead et al., 2020; Yu et al., 2020). They make up the physical barriers of the CNS while the biochemical barriers of CNS are imparted by various transport systems. Alterations in brain barriers have been observed in the early stage in ALS patients and mice, suggesting that the impairment may contribute to the pathogenesis (Bull et al., 2022). The alterations are summarized as follows: disruption of the integrity of physical barriers, function modulation of biochemical barriers, and secretion of neuroimmune-related substances by barrier cells in the immune response (Erickson and Banks, 2018; Kakaroubas et al., 2019; Gil-Martins et al., 2020). CNS barriers act as the center point in humoral-based communications between the CIS and PIS. A better understanding of how the integrity or function of CNS barriers is altered may provide approaches to terminate the harmful crosstalk in ALS.

Disruption of the Integrity of Physical Barriers in Amyotrophic Lateral Sclerosis

Multiple studies have found alterations of the ultrastructure of CNS barriers in ALS patients, including swelling and cytoplasmic vacuolization of microvascular endothelial cells, reduced pericyte coverage, and detachment of astrocyte end-feet processes from endothelial cells in the spinal cord of ALS patients (Miyazaki et al., 2011; Garbuzova-Davis et al., 2012; Yamadera et al., 2015).

Ultrastructural alterations have also been observed in the brain stem and cervical and lumbar spinal cords, but not in the motor cortex of ALS mice. The alterations have been noted to occur at the early disease stage and worsen with disease progression (Garbuzova-Davis et al., 2012; Winkler et al., 2013). TJs are formed by multiple proteins, such as zonula occludens-1 (ZO-1) and occludin, and prevent the paracellular movement of solutes (Winkler et al., 2013). A significant reduction in the expression of TJs and adhesion proteins such as ZO-1 and occludin was observed in the spinal cord of both ALS patients and mice (Pan and Nicolazzo, 2022). Despite the change in adhesion proteins, the morphological structures of TJs were found to be well preserved under electron microscopy in the spinal cord of postmortem ALS patients (Sasaki, 2015). Although morphological structures of TJs are preserved, the detection of endogenous proteins in the CNS suggests the increased paracellular permeability and leakiness of CNS barriers (Waters et al., 2021). Furthermore, BL thickening is observed in both ALS patients and mice. The detachment of endothelial cells exposes BL to plasma proteins, fibrin, and collagen IV within the BL, which then accumulate, leading to BL thickening (Sasaki, 2015). As BL abnormalities are detected in the early stage of ALS mice, these findings suggest that it may occur as a compensatory mechanism or a reparative process (Nguyen et al., 2021). Based on these findings, ultrastructural abnormalities and reduced expression of TJs adhesion proteins may contribute to compromised junctional integrity and an increase in paracellular permeability, permitting peripheral substances and cell access to the CNS. Therefore, it improves the communication of PIS and CIS, and accelerates the systemic proinflammation.

Functional Modulation of the Biochemical Central Nervous System Barriers

Biochemical CNS barriers are imparted by various transport systems, such as ATP-binding cassette (ABC) protein. They can effectively exclude various endogenous and exogenous toxins from the endothelial cells to maintain cellular homeostasis. The most well-studied ABC protein, P-glycoprotein (P-gp), is a major efflux transporter for small, lipid-soluble molecules expressed on CNS barriers (Gil-Martins et al., 2020). The expression and activity of P-gp are upregulated in both ALS patients and mice (Jablonski et al., 2012; Qosa et al., 2016; Chan et al., 2017; van Vliet et al., 2020). Tumor necrosis factor α (TNF-α) and growth factor-beta 1(TGF-β1) were shown to upregulate the expression and activity of P-gp in mice and rats (Dohgu et al., 2004; Bauer et al., 2007). As levels of TNF-α and TGF-β1 are increased in ALS patients and mice (Bougea, 2019; Tortelli et al., 2020), they are associated with the overexpression of P-gp. Moreover, astrocytes are also suspected to be responsible for the increased expression of P-gp in ALS dependent on ALS genotypes. For example, cocultured ALS-associated-mutant SOD1 astrocytes impacted P-gp in nearby endothelial cells by secreting soluble factors such as TNF-α, chemokines, and reactive oxygen species (ROS) (Ji et al., 2013). Meanwhile, ALS-associated mutant C9orf72 astrocytes have been shown to have no effects on endothelial P-gp expression (Mohamed et al., 2019). Additionally, the expression of breast cancer resistance protein (BRCP), another efflux transporter, is upregulated in ALS patients and mice (Jablonski et al., 2012; Chan et al., 2017; van Vliet et al., 2020). In general, the increased P-gp and BRCP abundance and activities at the CNS barriers suggest the modulation of interface functions of biochemical CNS barriers, which may ultimately influence the development of ALS.

Barrier Cells Secrete Neuroimmune-Related Substances in the Immune Response

Barrier cells, including endothelial cells, pericytes, and astrocytes, secrete neuroimmune-related substances in response to immune stimulation from peripheral or central immune cells. Brain endothelial cells (BECs) can constitutively secrete interleukin 6 (IL-6), prostaglandins, and nitric oxide in response to different stimuli (Iannucci et al., 2020; Charoensaensuk et al., 2021). As the number of pericytes is reduced in ALS (Winkler et al., 2013), its inflammatory-mediated role may also contribute to ALS pathologies. Compared to other barrier cells, pericytes are the most sensitive to TNF-α and can release IL-6 and macrophage inflammatory protein-1α (MIP-1α, also known as CCL3) in response (Matsumoto et al., 2014). Inflammatory reactive pericytes support neutrophil transmigration by the release of IL-8 and matrix metalloproteinase-9 (MMP-9), leading to the subsequent development of neuroinflammation (Pieper et al., 2013). Astrocytes are activated in the immune response in ALS. On the one hand, astrocytes control the activation, migration, and proliferation of microglia *via* multiple inflammatory factors, and secrete proteins such as MCP-1 which mediates monocyte migration to amplify neuroinflammation in the CNS (Ouali et al., 2018; Izrael et al., 2020). On the other hand, biochemical substances such as nitric oxide, vascular endothelial growth factors (VEGF), glial cell line-derived neurotrophic factor (GDNF), and MM-9 released from reactive astrocytes on barriers regulate the expression of TJ proteins and the proliferation of endothelial cells, thus influencing the integrity and permeability of CNS barriers (Spiller et al., 2019; Izrael et al., 2020; Takata et al., 2021; Qin et al., 2022). Therefore, barrier cells can not only transfer information from one side to the other side (such as PIS to CIS) but are also involved in mediating the inflammatory microenvironment.

THE CROSSTALK FROM THE PERIPHERAL IMMUNE SYSTEM TO THE CENTRAL NERVOUS SYSTEM CONTRIBUTES TO THE SYSTEMIC INFLAMMATORY MILIEU OF AMYOTROPHIC LATERAL SCLEROSIS

In ALS, injured MNs interact with glia, and they release certain levels of cytokines and chemokines, followed by the recruitment of innate and adaptive immune cells to infiltrate the CNS to promote inflammation. Proinflammatory signaling spreads from

CIS to PIS and from PIS to CIS, thereby contributing to the systemic inflammatory milieu of ALS.

Cytokines and Chemokines in Amyotrophic Lateral Sclerosis

Many cytokines and chemokines, such as IL-1, IL-6, TNF, and CC chemokine ligand 2 (CCL2), have been shown to cross CNS barriers while the barriers mediate their transport, penetration, and uptake (Zhao et al., 2020; Bull et al., 2022). On the one hand, due to the activation of immune cells, the levels of cytokines and chemokines are significantly changed in ALS (Sun et al., 2022). Their major roles in PIS or CIS in ALS are summarized in **Table 1**. On the other hand, elevated levels of proinflammatory mediators increase the permeability of the CNS barriers, act directly on their receptors to alter the function of resident cells, induce immune cell trafficking, and exacerbate barrier disruption and neuroinflammation (Wang et al., 2014; Banks, 2015; Erickson et al., 2020).

Central Nervous System Infiltration of Peripheral Immune Cells in Amyotrophic Lateral Sclerosis

Increasing evidence shows that many peripheral leukocytes are first activated in PIS and then migrate into the CNS in ALS

TABLE 1 | The major role of cytokines and chemokines in ALS.

Molecules	Secreting cells	Change in ALS	Role in the immune system	References
TNF-α	Macrophages, T lymphocytes, NK cells	Increased	Proinflammation: activation of immune cells	Tortarolo et al., 2017; Bougea, 2019
IL-1β	Monocytes, macrophages; M1 microglia	Increased	Proinflammation: activation of immune cells	Italiani et al., 2014; Sun et al., 2022
IL-6	Immune cells, endothelial cells, myocytes	Increased/unchanged	Proinflammation: activation of immune cells	Martinez-Merino et al., 2018; Pronto-Laborinho et al., 2019; Wosiski-Kuhn et al., 2019
IL-8/CXCL8	Monocytes, endothelial cells	Increased	Proinflammation: recruitment of neutrophils, activation of glial cells	Rusconi et al., 2017
IL-10	Monocytes, T lymphocytes, and B lymphocytes; immunosuppressive microglia (M2)	Increased/increased in the early stage and decreased during disease progression	Anti-inflammation: limiting excessive production of proinflammatory cytokines, ROS.	Batista et al., 2009; Noh et al., 2014; Strickland et al., 2020
IL-13	Th2 cells, CD4 cells, natural killer T cells, mast cells, basophils, eosinophils, and neurocytes	Increased	Controversial mechanism: proinflammation: enhancing MCP-1 expression in monocytes and macrophages; anti-inflammation: induce infiltration to the injured spinal cord and anti-inflammatory polarity of macrophages	Shi et al., 2007; Lu et al., 2016; Amo-Aparicio et al., 2021
IL-17a	Th17 cells, $CD8^+$ T cells, mast cells; astrocytes	Increased	Proinflammation	Fiala et al., 2010; Jin et al., 2021
IL-33	Multiple cells	Induced	Anti-inflammation: decreasing the proportion of $CD4^+$ and $CD8^+$ T cell populations, regulating mast cells function	Lin et al., 2012; Korhonen et al., 2019
G-CSF	Monocytes and macrophages	Induced	Dual mechanism: inducing mobilization of bone marrow cells from bone to the peripheral, stimulating proliferation, inducing the recruitment of microglia in the damaged areas	Salamone et al., 2020
CXCL13	MNs	Increased	Anti-inflammation	Trolese et al., 2020
CXCL12	Bone marrow stromal cells	Increased	Proinflammation: development of T and B lymphocytes, influencing survival of mature Lymphocytes, microglial pathology, and permeability of CNS barriers	Li and Ransohoff, 2008; Rabinovich-Nikitin et al., 2016
CX3CL1	MNs, microglia	Increased	Proinflammation: activation of microglia	Zhang et al., 2018
CCL2	MNs, microglia, astrocytes	Increased	Proinflammation: activation and recruitment of NK cells, T cells	Garofalo et al., 2020
CCL5	T lymphocytes,macrophages, endothelial cells	Increased	Proinflammation: proliferation and activation of T lymphocytes, monocytes	Perner et al., 2018
CCL18/MIP-4	DC	No changed	Proinflammation: attracting lymphocytes toward DC and activated macrophages, activation of microglia	Martinez-Merino et al., 2018

TNF-α, Tumor Necrosis Factor; IL, Interleukin; G-CSF, Recombinant Human Granulocyte-Colony Stimulating Factor; CCL, C-C Motif Ligand; CX3CL1, C-X3-C Motif Chemokine Ligand 1; CXCL, C-X-C Motif Chemokine Ligand; MIP-4, Macrophage Inflammatory Protein-4; DC, Dendritic Cell.

(Angelini et al., 2020). The regulation of leukocyte trafficking to the CNS is multifaceted and depends on the activation state of the leukocytes, TJ complexes at the endothelial interface, and the inflammatory microenvironment in the CNS and PNS (Congdon et al., 2019; Trolese et al., 2020; Marchetti et al., 2022). As peripheral leukocytes can be easily monitored, and intrathecal or intracerebroventricular is associated with several risks, targeting peripheral leukocytes may be feasible in ALS treatment. Therefore, a better understanding of how peripheral immune cells infiltrate into the CNS is needed.

T Lymphocytes

The infiltration of T lymphocytes in ALS is well-known (Rolfes et al., 2021). Chemokines and chemokine receptors are critical for parenchymal infiltration. The chronic inflammatory milieu induces the upregulation of leukocyte cell adhesion on the surface of endothelial cells, which binds to CD6 expressed on T lymphocytes, allowing their entry into the brain parenchyma (Larochelle et al., 2012). In addition, T lymphocyte-derived TNF-α and IL-17 induce the secretion of MM-9 in immune cells and MNs, facilitating T lymphocyte infiltration into the CNS (Song et al., 2015). A large amount of evidence highlights the differences between T-cell subsets and their specific mechanisms of entry into the CNS in ALS. For example, endothelial cells secrete chemokines such as CXCL9, CXCL10, CXCL11, CCL19, CCL21, and MCP-1 to recruit $CD4^+$ T cells through CNS barriers. Treg cells, which have an inhibitory effect on neuroinflammation, are activated and recruited to the CNS *via* CCL5/CCR5 and CCL6/CCR6 mechanisms to inhibit the activation of microglia in the early phase of the disease (Zhao et al., 2012; Beers et al., 2017). $CD8^+$ T cells show intense infiltration and induce MN death *via* MHC-I expressed in activated microglia and injured MNs (Coque et al., 2019; Liu et al., 2020).

Mast Cells

Findings in previous studies suggested that MCs play a role in early degeneration in the PNS and have a ripple effect on neuronal damage (Trias et al., 2018; Angelini et al., 2020). Later studies confirmed the infiltration of MCs in the spinal cord of ALS patients (Fiala et al., 2010; Kovacs et al., 2021). The expression of receptors on MCs is affected by IL-6, CCL5, and TNF-α released by activated microglia, resulting in the regulation of MC activation and CNS recruitment (Jones et al., 2019). Moreover, MCs can release proteases to TJs and extracellular matrix components, thus influencing the permeability and integrity of the BBB and leading to CNS invasion of MCs (Mattila et al., 2011; Jones et al., 2019).

Monocytes

Limited numbers of activated peripheral monocytes infiltrate the CNS and influence neuroinflammation in ALS (Chiot et al., 2020). Previous studies indicate an alteration in the proportion of monocytes in ALS (Mcgill et al., 2021). In patients with rapidly progressing ALS, monocytes in the peripheral circulation are usually in a proinflammatory state (Zhao et al., 2017). Recently, peripheral monocytes have been proven to infiltrate the CNS, which is related to improved motoneuron survival in ALS, but infiltration may be limited (Peake et al., 2017). In addition, monocyte-derived macrophages are activated in ALS. Activated macrophages exert neuroprotective functions by misfolding protein clearance during the disease (Chiu et al., 2009; Shiraishi et al., 2021). Macrophages also showed limited infiltration to the CNS. The evidence may suggest that the accumulating monocytes in the CNS were due to the proliferation of infiltrated cells instead of the infiltration of accumulated circulated monocytes (Chiot et al., 2020).

Other Immune Cells: Neutrophils, Natural Killer Cells

Few studies have discussed the role of neutrophils and NK cells in neuroimmune crosstalk. However, considering that there is a significant correlation between an increase in the number of neutrophils and NK cells in the peripheral blood and disease progression (Murdock et al., 2017; Leone et al., 2022), and their role in innate immune responses, it is believed to affect neuroinflammation of the CNS in complicated ways. For example, end-stage ALS mice showed a high NK cell frequency in the spinal cord (Finkelstein et al., 2011). NK cell-derived IFN-γ induces microglia toward an inflammatory phenotype, regulates the release of CCL2, a chemokine that can regulate CNS infiltration, from MNs, and impairs Treg cell migration (Garofalo et al., 2020). More studies are needed.

CONCLUSION

Previous investigations of neuroinflammation in ALS have mainly focused on the relationship between the two immune systems and ALS, respectively. Nevertheless, much less is discussed on the crosstalk between PIS and CIS in ALS, especially the role of the CNS barriers. In this review, we updated the understanding of the relationship between neuroinflammation and ALS. Crosstalk involving central immune cells and peripheral immune cells, CNS barriers, cytokines and chemokines was fully discussed. The dysfunction of all these elements contributed to the non-cellulous death of MNs. Crosstalk plays an important role in the systemic inflammatory milieu in ALS. It should be fully considered for mechanisms and treatment discovery in ALS.

CNS barriers play a crucial role in the crosstalk; thus, they may be a target when optimizing medicine use with ALS. For example, riluzole is a substrate for P-gp and BRCP expressed on CNS barriers so the drug efficacy may be negatively affected (Jablonski et al., 2014). Inhibitors of P-gp have been proven to improve drug delivery in ALS mice (Gil-Martins et al., 2020), but clinical trials should be conducted and more investigations are needed. In addition, a combination of possible CNS barriers-impaired medicine with other therapies may be beneficial. Angiopoietin-1 promotes angiogenesis in the CNS and reduces vascular permeability. The C16 peptide repairs vessels and inhibit transmigration and infiltration of leukocytes without the side effect of systemic immunosuppression. The roles of these two medicines have been well studied in animal models of CNS inflammation (Wu D. et al., 2020), but further experiments

in ALS are needed. Notably, the effect of neuroinflammation is dual, as it exerts a neurotoxic or neuroprotective effect during the disease. In conclusion, normalizing immune crosstalk and homeostasis instead of suppressing inflammation may provide a potential therapeutic target and direction for future study.

AUTHOR CONTRIBUTIONS

WY and JH wrote the manuscript and reviewed the literature under the supervision of DF. XC and ZY drafted the figure and table. ZZ revised the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

This work was funded by the National Natural Science Foundation of China (Grant Nos. 81873784, 82071426, and 81974197), the Clinical Cohort Construction Program of Peking University Third Hospital (Grant No. BYSYDL2019002), and Beijing Natural Science Foundation (Grant No. 7222215).

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SYSTEMATIC REVIEW
published: 06 May 2022
doi: 10.3389/fnagi.2022.888946

Association of Peripheral Blood Cell Profile With Alzheimer's Disease: A Meta-Analysis

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Background: Inflammation and immune dysfunction play significant roles in the pathogenesis of Alzheimer's disease (AD)-related dementia. Changes in peripheral blood cell profiles are a common manifestation of inflammation and immune dysfunction and have been reported in patients with AD or mild cognitive impairment (MCI). We systematically evaluated the association of peripheral blood cell counts and indices with AD or MCI through a meta-analysis.

Methods: We electronically searched sources to identify all case–control trials comparing peripheral blood cell counts and/or lymphocyte subsets between patients with AD or MCI and healthy controls (HCs). Meta-analyses were used to estimate the between-group standardized mean difference (SMD) and 95% confidence interval (CI).

Results: A total of 36 studies involving 2,339 AD patients, 608 MCI patients, and 8,352 HCs were included. AD patients had significantly decreased lymphocyte counts (SMD −0.345, 95% CI [−0.545, −0.146], $P = 0.001$) and significantly increased leukocyte counts (0.140 [0.039, 0.241], $P = 0.006$), neutrophil counts (0.309 [0.185, 0.434], $P = 0.01$), and neutrophil–lymphocyte ratio (NLR) (0.644 [0.310, 0.978], $P < 0.001$) compared to HCs. Similarly, significantly increased leukocyte counts (0.392 [0.206, 0.579], $P < 0.001$), NLR (0.579 [0.310, 0.847], $P < 0.001$), and neutrophil counts (0.248 [0.121, 0.376], $P < 0.001$) were found in MCI patients compared with HCs. A significantly decreased percentage of B lymphocytes (−1.511 [−2.775, −0.248], $P = 0.019$) and $CD8^+$ T cells (−0.760 [−1.460, −0.061], $P = 0.033$) and a significantly increased CD4/CD8 ratio (0.615 [0.074, 1.156], $P = 0.026$) were observed in AD patients compared to HCs. Furthermore, significant changes in hemoglobin level and platelet distribution width were found in patients with AD or MCI compared with HCs. However, no significant difference was found between AD or MCI patients and HCs in terms of platelet counts, mean corpuscular volume, red cell distribution width, mean platelet volume, and $CD4^+$ T, $CD3^+$ T, or natural killer cell counts.

Conclusion: Changes in peripheral blood cell profiles, particularly involving leukocyte, lymphocyte, neutrophil, and $CD8^+$ T cell counts, as well as the NLR and the CD4/CD8 ratio, are closely associated with AD. The diagnostic relevance of these profiles should be investigated in future.

Keywords: Alzheimer's disease, lymphocyte subsets, meta-analysis, mild cognitive impairment, peripheral blood

OPEN ACCESS

Edited by:
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Reviewed by:
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Specialty section:
This article was submitted to Alzheimer's Disease and Related Dementias, a section of the journal Frontiers in Aging Neuroscience

Received: *03 March 2022*
Accepted: *19 April 2022*
Published: *06 May 2022*

Citation:
Huang L-T, Zhang C-P, Wang Y-B and Wang J-H (2022) Association of Peripheral Blood Cell Profile With Alzheimer's Disease: A Meta-Analysis. Front. Aging Neurosci. 14:888946. doi: 10.3389/fnagi.2022.888946

INTRODUCTION

Alzheimer's disease (AD), a slowly progressive irreversible neurodegenerative disease, is characterized by memory loss of recent events or names as the early clinical symptom; impaired cognition and social function as later symptoms; and dysfunction in speaking, walking, or even swallowing as final symptoms (Reitz and Mayeux, 2014). AD accounts for 60–80% of dementia cases, and its prevalence continues to increase worldwide every year. It is estimated that the number of patients with AD in the USA will grow markedly from 5.8 million in 2020 to 13.8 million by the mid-twenty-first century (Zhang et al., 2021). In 2019, ∼$244 billion was spent on AD-related healthcare (Alzheimer's Association, 2021). Given the increasing concern about the aging population, healthcare costs for AD will continue to rise. AD ranks as the fifth-leading cause of death among people of all ages globally (Usman et al., 2021) and has no sufficiently effective treatment options. Unfortunately, numerous clinical trials in patients with AD have failed (Long and Holtzman, 2019). Thus, further studies are needed to understand this disease better.

The diagnostic criteria typically used for AD since 1984 are the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS–ADRDA) criteria, which combine neuropathological patterns and clinical manifestations (Dubois et al., 2007). In 1999, mild cognitive impairment (MCI) was introduced to define an intermediate state between normal cognition and dementia and represents a population at risk of developing AD (Bradfield, 2021).

Extracellular amyloid-β (Aβ) peptide deposition, as neuritic plaques, and intracellular hyperphosphorylated tau (p-tau) protein accumulation, as neurofibrillary tangles, remain key pathological changes in AD and are the primary neuropathological diagnostic criteria for AD (Winblad et al., 2016). Nevertheless, the pathological and clinical manifestations of AD has yet to be elucidated (Long and Holtzman, 2019). There is currently no effective prevention or treatment for Aβ or p-tau accumulation in clinical populations with AD. Other theories, including cholinergic disruption, vascular dysfunction, oxidative stress, immune dysregulation, and neuroinflammation, are emerging as supplementary explanations for this difficult problem (Firdaus and Singh, 2021).

Inflammation and immune dysregulation are involved in the generation of Aβ plaques and neurofibrillary tangles. Initially, the immune response reduces the plaque burden against neurodegeneration through the activation of microglial cells (Sayed et al., 2020). However, with prolonged inflammation, microglial cells lose their ability to clear the plaque (Wyss-Coray et al., 2002; Simard et al., 2006; Sayed et al., 2020). Subsequently, an increasing number of inflammatory cytokines are released, and the number of macrophages increase in an attempt to combat plaque deposition (Stalder et al., 2005; Krabbe et al., 2013). These cytokines and macrophages in turn increase amyloid precursor and p-tau protein levels, resulting in the formation of plaques and neurofibrillary tangles, which exacerbate neural degeneration (Liao et al., 2004; Quintanilla et al., 2004). In this process, the number of peripheral blood cells involved in inflammation and the immune response is increased, including lymphocytes, neutrophils, monocytes, platelets, and lymphocyte subsets (Xue et al., 2009; Chen et al., 2017; Dong et al., 2019; Kara et al., 2022).

Cerebral spinal fluid (CSF) Aβ and p-tau protein have been proven to be biomarkers for identifying older individuals at risk of developing dementia (Langa and Levine, 2014), but they are not widely used because of the difficulty and invasiveness of CSF sampling. Therefore, an easy but feasible way to obtain screening markers, such as peripheral blood cell profile, will be helpful in clinical practice.

Several studies have demonstrated significantly elevated peripheral neutrophil count and/or decreased peripheral lymphocyte count in patients with AD as well as MCI patients compared with age-matched healthy controls (HCs) (Rembach et al., 2014; Chen et al., 2017; Kalelioglu et al., 2017; An et al., 2019; Dong et al., 2019). Some studies have reported an elevated neutrophil-to-lymphocyte ratio (NLR) in those patients (Rembach et al., 2014; Kalelioglu et al., 2017; An et al., 2019; Dong et al., 2019), which has been used to indicate the prognosis of many inflammatory or immune diseases. Changes in the population of other peripheral blood cells were also observed in AD or MCI, including leukocytes, red blood cells (RBCs), and platelets, and their relevant indices such as hemoglobin level, mean corpuscular volume (MCV), red cell distribution width (RDW), mean platelet volume (MPV), and platelet distribution width (PDW) (Chang et al., 2007; Öztürk et al., 2013; Wang et al., 2013; Koç et al., 2014; Liang et al., 2014; Chen et al., 2017; An et al., 2019; Dos Santos and Pardi, 2020). In terms of lymphocyte subsets, several studies have reported an increased percentage of $CD4^+$ T cells, decreased percentage of $CD8^+$ T cells, and increased CD4/CD8 ratio in patients with AD compared to age-matched HCs (Pirttilä et al., 1992; Shalit et al., 1995; Lombardi et al., 1999; Richartz-Salzburger et al., 2007; Schindowski et al., 2007; Xue et al., 2009; Zhang et al., 2013). Nevertheless, some studies have reported non-significant or even contradictory results. Thus, to date, no consistent conclusion about the changes in peripheral blood cell profiles has been reached.

To better understand the associations between peripheral blood cell profiles and AD related dementia, it is necessary to make a comprehensive meta-analysis. To the best of our knowledge, this study is the first meta-analysis to compare peripheral blood cell counts and indices between patients with AD or MCI and age-matched HCs.

METHODS

Search Strategy

We systematically searched the PubMed, Cochrane Library, Embase, and Web of Science databases for studies published up to January 20, 2022. The search terms used were: "Alzheimer," "mild cognitive impairment," "blood cell count," "lymphocyte subsets," "neutrophil," "eosinophil," "basophil" "lymphocyte," "NLR," "monocyte," "white blood cell," "leucocyte," "red blood cell," "erythrocyte," "hemoglobin," "platelet," "PLR," "mean corpuscular volume," "red cell distribution width," "mean platelet volume," "platelet distribution width," "$CD4^+$," "$CD8^+$," "$CD4^+/CD8^+$," "$CD3^+$," "B lymphocyte," and "NK cell." Relevant conference

abstracts and presentations were also searched. Two independent authors screened all articles.

Selection Criteria

Eligible studies were case–control studies describing the association between peripheral blood cell counts or indices or lymphocyte subsets and AD and/or MCI patients, with data expressed as the mean and standard deviation, and wherein the number of patients was available. Non-comparative studies, duplicated studies, case reports, opinions, editorials, review articles, or studies with insufficient data to allow meta-analysis were excluded.

Data Collection and Quality Assessment

The following data were collected from the identified studies: authors, publication year, country, disease, number of subjects, median age, diagnostic criteria, Mini-Mental State Examination (MMSE) scores, and peripheral blood cell indices or lymphocyte subsets. The Agency for Healthcare Research and Quality (AHRQ) criteria were used to assess the methodological quality of the included cross-sectional case–control studies, with scores ranging from 0 to 11 points (Chou et al., 2018). Screening of studies, data extraction, and quality assessment were conducted by two researchers independently. Disagreements were resolved by discussion.

Statistical Analysis

Absolute numbers of peripheral blood cell parameters and percentages of lymphocyte subsets were continuous outcomes, expressed as the standardized mean difference (SMD) with corresponding 95% confidence intervals (CIs). Meta-analysis was performed using STATA software (version 12.0; StataCorp, College Station, TX, USA). In addition, a funnel plot was constructed to evaluate symmetry to visually assess publication bias, and sensitivity analyses were performed for the outcome with the leave-one-out approach to exclude potential bias induced by single study. All tests were two-sided, and statistical significance was set at $P < 0.05$. I-squared statistics were used to assess heterogeneity. I-squared statistic >50% or $P < 0.10$ was defined as significant heterogeneity among studies. The fixed-effects model was used in the absence of heterogeneity, and the random-effects model was used when heterogeneity was present among studies.

RESULTS

Study Selection and Characteristics

A total of 1,312 publications were screened from databases and conference abstract compilations through an initial database search. After reviewing the titles and abstracts, 102 potential articles were identified and reviewed in detail. Finally, 36 studies were considered to be suitable for our meta-analysis according to the stipulated inclusion and exclusion criteria (**Figure 1**).

In total, 2,339 AD patients, 608 MCI patients, and 8,352 HCs were identified in the 36 identified studies. Thirty-four studies were included in the comparisons of AD patients and HCs, while six studies compared MCI patients and HCs. These studies were used for meta-analysis of peripheral blood cell counts and relevant indices, including leukocyte count; neutrophil count; NLR, lymphocyte count; monocyte count; RBC count; hemoglobin level; MCV; RDW; platelet count; MPV; PDW; percentage of $CD4^+$ T cells, $CD8^+$ T cells, $CD3^+$ T cells, B lymphocytes, and natural killer (NK) cells; and the CD4/CD8 ratio. Few of these studies had analyzed eosinophils, basophils, MCH, or PLR. The characteristics and quality of the included studies are summarized in **Table 1**.

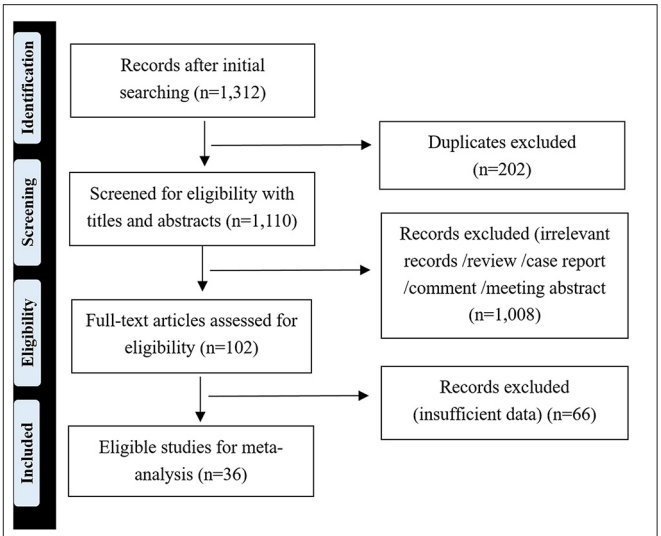

FIGURE 1 | Overview of literature search and selection.

Association of Peripheral Leucocytes With AD or MCI Patients

Total leukocyte counts were first analyzed, and then the subclasses of leukocytes were explored, including polymorphonuclear cells (PMNs) and peripheral blood mononuclear cells (PBMCs). The meta-analysis results are presented in **Table 2**.

Nine studies (involving 1,621 subjects) compared the peripheral leukocyte count between patients with AD and HCs. AD patients had a significantly higher leukocyte count than HCs (SMD: 0.140; 95% CI: 0.039–0.241; $P = 0.006$), without significant heterogeneity among the studies (I-squared = 46.2%, $P = 0.062$) (**Figure 2A**). Two studies (involving 455 subjects) analyzed the peripheral leukocyte count differences between MCI patients and HCs. The peripheral leukocyte counts were significantly higher in the MCI group than in the HC group. The overall pooled SMD was 0.392 (95% CI: 0.206–0.579, $P < 0.001$). There was no significant heterogeneity among the studies (I-squared = 46.2%, PP = 0.173) (**Figure 2B**).

PMNs

PMNs include neutrophils, eosinophils, and basophils. However, only neutrophils were analyzed in several studies. The NLR was also analyzed.

TABLE 1 | Characteristics of included studies.

References	Country	Disease	Number of subjects	Median age (years)	Diagnostic criteria	MMSE scores	Peripheral blood cell or lymphocyte subset	Scores*
Kara et al. (2022)	Turkey	AD	AD: 94 HC: 61	AD: 74.2 ± 9.6 HC: 65.7 ± 4.6	DSM-IV	N/A	WBC, N, L, M, PLT, NLR	9
Sun et al. (2022)	China	AD	AD: 127 HC: 100	AD: 67.36 ± 10.2 HC: 68.05 ± 8.73	NINCDS-ADRDA	AD: 11.50 ± 4.56 HC: 27.29 ± 2.09	M	9
Dos Santos and Pardi (2020)	Brazil	AD	AD: 60 HC: 60	AD: N/A HC: ≥60	N/A	N/A	HGB, PLT	8
Du et al. (2020)	China	MCI	MCI: 85 HC: 85	MCI: 70.0 ± 4.77 HC: 70.05 ± 4.73	Peterson's	MCI: 22.07 ± 1.3 HC: 27.25 ± 1.98	HGB, MCV	8
Dong et al. (2019)	China	AD and MCI	AD: 56 MCI: 57 HC: 59	AD: 69.04 ± 9.05 MCI: 70.67 ± 9.26 HC: 68.12 ± 5.81	NINCDS-ADRDA	N/A	WBC, N, L, M, PLT, NLR, RBC, MCV, RDW, MPV, PDW	6
An et al. (2019)	China	MCI	MCI: 186 HC: 153	MCI: 73.10 ± 3.29 HC: 71.19 ± 3.32	N/A	MCI: 20.38 ± 2.13 HC: 23.96 ± 1.59	N, L, NLR, HGB, WBC, PLT	7
Kalelioglu et al. (2017)	Turkey	AD and MCI	AD: 31 MCI: 30 HC: 31	N/A	DSM-IV	N/A	N, L, PLT, NLR	8
Chen et al. (2017)	China	AD	AD: 92 HC: 84	AD: 69.95 ± 10.63 HC: 70.6 ± 5.39	NINCDS-ADRDA	AD: 15.09 ± 4.56 HC: 29.08 ± 1.00	L, RBC, HGB, MPV, MCV, PDW	8
Min and Min (2016)	Korea	AD	AD: 49 HC: 4,639	AD: ≥60 HC: ≥60	N/A	N/A	HGB	6
Rembach et al. (2014)	Australia	AD and MCI	AD: 205 MCI: 130 HC: 759	AD: 78.99 ± 8.4 MCI: 76.25 ± 7.5 HC: 70.57 ± 6.98	AD: NINCDS-ADRDA MCI: Peterson's	AD: 20 ± 5.27 MCI: 26.5 ± 2.66 HC: 29 ± 1.19	N, L, NLR	6
Koç et al. (2014)	Turkey	AD	AD: 109 HC: 81	AD: 76.74 ± 8.99 HC: 75.32 ± 8.42	NINCDS-ADRDA; DSM-IV	AD: 19.27 ± 4.87	PLT, MPV	6
Zhang et al. (2013)	USA	AD	AD: 41 HC: 31	AD: 77.9 ± 7.7 HC: 75.4 ± 9.5	NINCDS-ADRDA	AD: 24.5 ± 2.1	CD4/CD8, $CD4^+$%, $CD8^+$%	7
Wang et al. (2013)	China	AD and MCI	AD: 120 MCI: 120 HC: 120	AD: 72.8 ± 3.6 MCI: 72.9 ± 3.5 HC: 73.7 ± 4.2	AD: NINCDS-ADRDA MCI: Peterson's	AD: 14.5 ± 2.2 MCI: 24.8 ± 0.8 HC: 27.9 ± 1.5	PLT, MPV, PDW	8
Liang et al. (2014)	China	AD	AD: 110 HC: 150	AD: 73.4 ± 4.0 HC: 72.7 ± 3.9	NINCDS-ADRDA	AD: 15.2 ± 3.1 HC: 27.8 ± 1.6	PLT, MPV, PDW	7
Westman et al. (2013)	Sweden	AD	AD: 50 HC: 50	AD: 77.5 ± 6.9 HC: 74.0 ± 8.0	NINCDS-ADRDA; DSM-IV	AD: 19.9 ± 3.1 HC: N/A	WBC, HGB, L	5
Koçer et al. (2013)	Turkey	AD	AD: 89 HC: 104	AD: 75 (46–88) HC: 72 (60–86)	NINCDS-ADRDA	AD: 18 (6–26) HC: N/A	MPV	5
Öztürk et al. (2013)	Turkey	AD	AD: 197 HC: 133	AD: 76.22 ± 6.92 HC: 71.68 ± 5.3	NINCDS-ADRDA; DSM-IV	AD: 15.79 ± 5.33 HC: 26.75 ± 3.27	WBC, HGB, RDW, PLT	6

(Continued)

TABLE 1 | Continued

References	Country	Disease	Number of subjects	Median age (years)	Diagnostic criteria	MMSE scores	Peripheral blood cell or lymphocyte subset	Scores*
Kuyumcu et al. (2012)	Turkey	AD	AD: 241 HC: 175	AD: 76.53 ± 6.00 HC: 71.95 ± 5.40	NINCDS-ADRDA; DSM-IV	AD: 18.32 ± 7.94 HC: 27.08 ± 3.29	WBC, PLT, HGB, NLR	5
Yesil et al. (2012)	Turkey	AD	AD: 126 HC: 286	AD: 76.2 ± 6.8 HC: 75.2 ± 6.3	NINCDS-ADRDA; DSM-IV	AD: 20.1 ± 7.2 HC: 26.0 ± 3.4	WBC, HGB, PLT, MPV	5
Shah et al. (2011)	USA	AD	AD: 113 HC: 768	AD: 85.9 ± 6.3 HC: 80.0 ± 7.4	NINCDS-ADRDA	AD: 25.9 ± 2.6 HC: 28.2 ± 1.8	HGB, MCV, RDW	6
Xue et al. (2009)	China	AD	AD: 48 HC: 30	AD: 74.5 ± 9.8 HC: 71.7 ± 8.9	NINCDS-ADRDA; DSM-IV	AD: 17.2 HC: 29.8	$CD3^+$%, $CD4^+$%, $CD8^+$%, B lymphocyte%, NK cell%	8
Larbi et al. (2009)	Canada	AD	AD: 12 HC: 6	AD: 75.4 ± 7.1 HC: 74.0 ± 3.8	NINCDS-ADRDA; DSM-IV	AD: 25 HC: 30	WBC, HGB, PLT	7
Bonotis et al. (2008)	Greece	AD	AD: 23 HC: 21	AD: 76.35 ± 6.9 HC: 71.23 ± 4.4	NINCDS-ADRDA	AD: 19.76 ± 2.2 HC: 28.33 ± 1.3	$CD4^+$%, $CD8^+$%, CD4/CD8	5
Speciale et al. (2007)	Italy	AD	AD: 51 HC: 51	AD: 72.2 ± 8.8 HC: 69.1 ± 8.2	NINCDS-ADRDA	AD: 17.57 ± 6.19 HC: 28.5 ± 1.03	B lymphocyte%	7
Richartz-Salzburger et al. (2007)	Germany	AD	AD: 43 HC: 34	AD: 70.9 ± 8.2 HC: 67.5 ± 7.3	NINCDS-ADRDA	AD: 17.9 HC: N/A	$CD3^+$%, $CD4^+$%, $CD8^+$%, CD4/CD8, B lymphocyte%, NK cell%	6
Schindowski et al. (2007)	Germany	AD	AD: 24 HC: 34	AD: 73.4 ± 3.5 HC: 71.5 ± 4.6	NINCDS-ADRDA	AD: 18.8 ± 1.12 HC: N/A	$CD4^+$%, $CD8^+$%, CD4/CD8	5
Chang et al. (2007)	China	AD	AD: 21 HC: 23	AD: 76 ± 3 HC: 77 ± 4	NINCDS-ADRDA; DSM-IV	AD: 24.1 ± 0.4 HC: 28.9 ± 0.9	WBC, RBC, HGB, MCV, PLT	6
Schindowski et al. (2006)	Germany	AD	AD: 23 HC: 25	AD: 72.2 ± 3.1 HC: 74.0 ± 4.9	NINCDS-ADRDA	AD: 19.1 ± 1.48 HC: N/A	$CD3^+$%, B lymphocyte%, NK cell%	8
Armanini et al. (2003)	Italy	AD	AD: 23 HC: 23	AD: 67 (49–89) HC: 66 (49–89)	NINCDS-ADRDA; DSM-IV	AD: 20 (15–24) HC: N/A	$CD4^+$%, $CD8^+$%, CD4/CD8	6
Lombardi et al. (1999)	Spain	AD	AD: 45 HC: 45	AD: 69 (63–76) HC: 71 (61–79)	NINCDS-ADRDA; DSM-III-R	N/A	$CD3^+$%, $CD4^+$%, $CD8^+$%, NK cell%	8
Song et al. (1999)	Belgium	AD	AD: 15 HC: 16	AD: 78.4 ± 10.3 HC: 75.3 ± 8.8	DSM-IV	AD: 8.0 ± 6.7 HC: N/A	L, N, WBC, M	5
Shalit et al. (1995)	Israel	AD	AD: 12 HC: 13	AD: 76.2 ± 7.4 HC: 75.2 ± 6.1	NINCDS-ADRDA	AD: 15.7 ± 1.97 HC: 28.85 ± 0.39	L, $CD4^+$%, $CD8^+$%	5
Inestrosa et al. (1993)	Chile	AD	AD: 18 HC: 32	AD: 69.2 ± 1.5 HC: 67.8 ± 1.4	NINCDS-ADRDA	N/A	PLT	5
Pirttilä et al. (1992)	Finland	AD	AD: 33 HC: 35	AD: 65.5 ± 6.9 HC: 61.8 ± 6.8	NINCDS-ADRDA	N/A	L, $CD4^+$%, $CD8^+$%, CD4/CD8, B lymphocyte%	6
Ikeda et al. (1991)	Japan	AD	AD: 13 HC: 13	AD: 61 ± 8 HC: 57 ± 7	NINCDS-ADRDA; DSM-III-R	N/A	$CD4^+$%, $CD8^+$%, CD4/CD8	5
Araga et al. (1990)	Japan	AD	AD: 25 HC: 22	AD: 78.0 (59–93) HC: 77.9 (65–88)	DSM-III-R	N/A	$CD4^+$%, $CD8^+$%, CD4/CD8	6

**Agency for Healthcare Research and Quality (AHRQ) scores for assessing the methodological quality of included studies. AD, Alzheimer's disease; DSM, Diagnostic and Statistical Manual of Mental Disorder; HC, healthy control; HGB, hemoglobin; L, lymphocyte; M, monocyte; MCI, mild cognitive impairment; MCV, mean corpuscular volume; MMSE, Mini Mental State Examination; MPV, mean platelet volume; N, neutrophil; N/A, not applicable; NINCDS–ADRDA, National Institute of Neurologic and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association; NK cell, natural killer cell; NLR, neutrophil-lymphocyte ratio; PDW, platelet distribution width; PLT, platelet; RBC, red blood cell; RDW, red cell distribution width; WBC, white blood cell (leucocyte).*

TABLE 2 | Summary of meta-analyses.

Blood indices	Number of studies	Patients with AD or MCI	Patients with HC	Effect estimates		Heterogeneity estimates		
				SMD [95% CI]	*P*-value	I-squared	*P*-value	Model
AD vs. HC								
Leucocyte	9	812	809	0.140 [0.039, 0.241]	0.006	46.20%	0.062	Fixed
Neutrophil	5	401	926	0.309 [0.185, 0.434]	<0.001	0.00%	0.712	Fixed
NLR	5	627	1,085	0.644 [0.310, 0.978]	<0.001	86.50%	<0.001	Random
Lymphocyte	9	588	1,108	−0.345 [−0.545, −0.146]	0.001	59.60%	0.011	Random
Monocyte	4	292	236	−0.318 [−0.707, 0.072]	0.11	76.30%	0.005	Random
$CD4^+$ T cell%	11	328	301	0.468 [−0.166, 1.102]	0.148	92.70%	<0.001	Random
$CD8^+$ T cell%	11	328	301	−0.760 [−1.460, −0.061]	0.033	93.70%	<0.001	Random
CD4/CD8	8	223	213	0.615 [0.074, 1.156]	0.026	86.20%	<0.001	Random
$CD3^+$ T cell%	4	159	134	−1.763 [−4.405, 0.879]	0.191	98.50%	<0.001	Random
B Lymphocyte%	5	174	175	−1.511 [−2.775, −0.248]	0.019	96.00%	<0.001	Random
NK cell%	4	159	134	−0.111 [−1.149, 0.927]	0.834	94.50%	<0.001	Random
RBC	3	169	166	−0.520 [−1.289, 0.250]	0.186	90.50%	<0.001	Random
Hemoglobin	10	961	6,224	−0.347 [−0.563, −0.131]	0.002	81.10%	<0.001	Random
MCV	4	282	934	0.213 [−0.197, 0.623]	0.309	83.50%	<0.001	Random
RDW	3	366	960	0.250 [−0.200, 0.700]	0.276	89.60%	<0.001	Random
Platelet	13	1195	1,217	0.071 [−0.175, 0.318]	0.57	86.90%	<0.001	Random
MPV	7	702	884	−0.247 [−0.988, 0.495]	0.514	97.90%	<0.001	Random
PDW	4	378	413	−1.195 [−1.796, −0.595]	<0.001	93.20%	<0.001	Random
MCI vs. HC								
Leucocyte	2	243	212	0.392 [0.206, 0.579]	<0.001	46.20%	0.173	Fixed
Neutrophil	4	403	1,002	0.248 [0.121, 0.376]	<0.001	26.10%	0.255	Fixed
NLR	4	403	1,002	0.579 [0.310, 0.847]	<0.001	70.80%	0.016	Random
Lymphocyte	4	403	1,002	−0.209 [−0.515, 0.096]	0.179	78.10%	0.003	Random
Hemoglobin	2	271	238	−0.869 [−1.927, 0.189]	0.107	96.40%	<0.001	Random
MCV	2	141	144	−0.104 [−0.441, 0.234]	0.546	50.90%	0.153	Random
Platelet	4	393	363	−0.073 [−0.217, 0.070]	0.315	47.10%	0.129	Fixed
MPV	2	176	179	−0.342 [−1.899, 1.215]	0.667	97.80%	<0.001	Random
PDW	2	176	179	−0.446 [−1.485, 0.593]	0.4	95.20%	<0.001	Random

AD, Alzheimer's disease; CI, confidence interval; HC, healthy control; MCI, mild cognitive impairment; MCV, mean corpuscular volume; MPV, mean platelet volume; NK cells, natural killer cells; NLR, neutrophil-lymphocyte ratio; PDW, platelet distribution width; RBC, red blood cell; RDW, red cell distribution width; SMD, standardized mean difference.

Neutrophil Count

Five studies (involving 1,327 subjects) analyzed the peripheral neutrophil count differences between AD patients and HCs. The AD group had a significantly higher peripheral neutrophil count than did the HC group. The overall pooled SMD was 0.309 (95% CI: 0.185–0.434, $P < 0.001$). There was no significant heterogeneity among the studies (I-squared = 0.0%, $P = 0.712$) (**Figure 2C**).

Four studies (involving 1,405 subjects) analyzed the peripheral neutrophil count differences between patients with MCI and HCs. The peripheral neutrophil count was statistically significantly higher in the MCI patients than in the HC group. The overall pooled SMD was 0.248 (95% CI: 0.121–0.376, $P < 0.001$). There was no significant heterogeneity among the studies (I-squared = 26.1%, $P = 0.255$) (**Figure 2D**).

NLR

Five studies, including 1,712 subjects, analyzed the NLR differences between AD patients and HCs. The NLR was statistically significantly higher in the AD group than in the HC group. The overall pooled SMD was 0.644 (95% CI: 0.310–0.978, $P < 0.001$), with significant heterogeneity among the studies (I-squared = 86.5%, $P < 0.000$) (**Figure 3A**).

Four studies, including 1,405 subjects, compared the NLR between MCI patients and HCs. Similarly, the MCI group had a significantly higher NLR than the HC group. The overall pooled SMD was 0.579 (95% CI: 0.310–0.847, $P < 0.001$), with significant heterogeneity among the studies (I-squared = 70.8%, $P = 0.016$) (**Figure 3B**).

PBMCs

The total PBMC count, including lymphocytes, monocytes, T cells with different surface antigens, B lymphocytes, and NK cells,

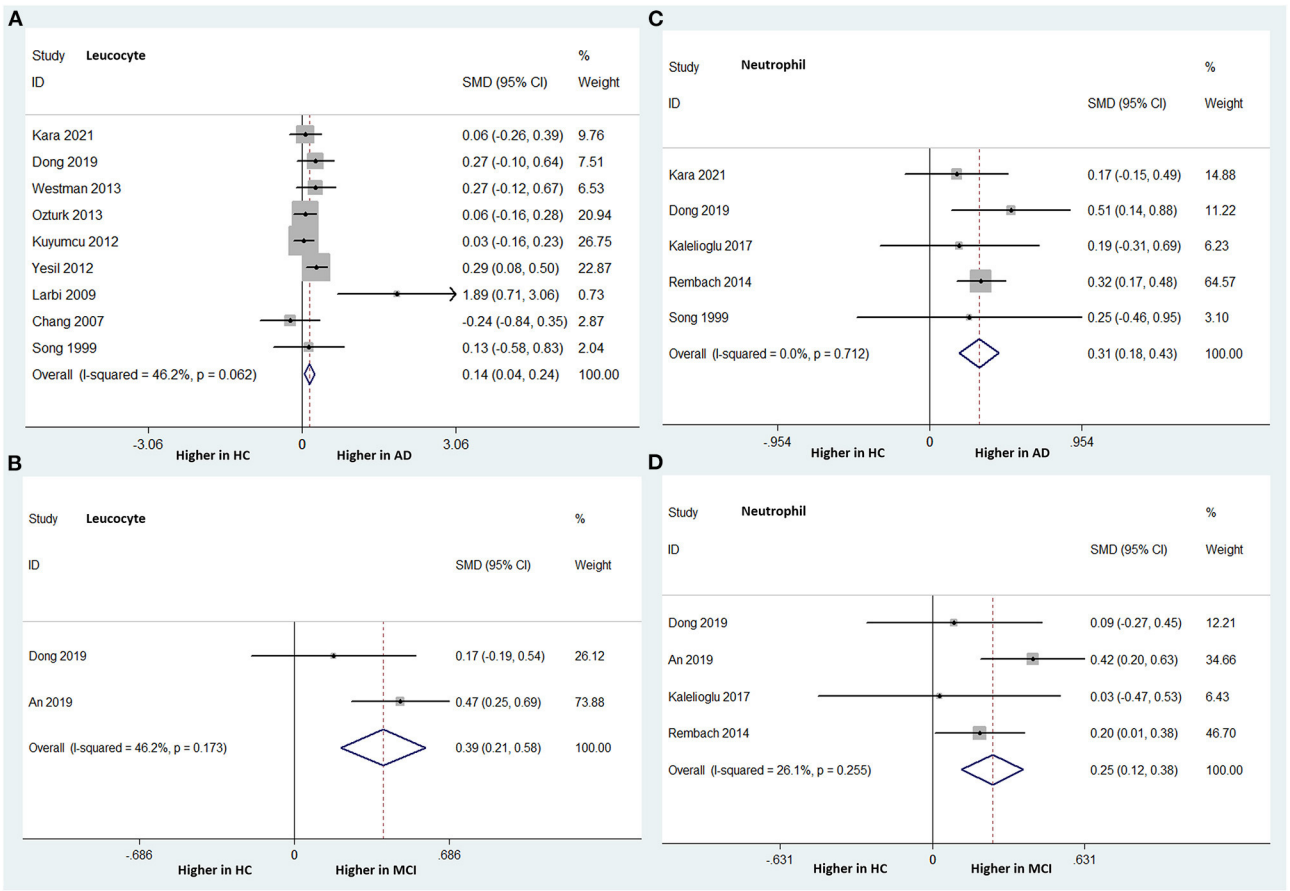

FIGURE 2 | Forest plots of standardized mean differences (SMDs) for leucocyte counts in Alzheimer's disease (AD) **(A)** and mild cognitive impairment (MCI) **(B)**, neutrophil counts in AD **(C)** and MCI **(D)**, compared to healthy controls (HCs).

was analyzed to compare the differences between patients with AD or MCI and HCs.

Lymphocyte Count

Nine studies (involving 1,696 subjects) analyzed the peripheral lymphocyte count differences between patients with AD and HCs. The AD group had significantly lower peripheral lymphocyte counts than did the HC group. The overall pooled SMD was −0.345 (95% CI: −0.545 to −0.146, $P = 0.001$) (**Figure 3C**). There was significant heterogeneity among the studies (I-squared = 59.6%, $P = 0.011$).

Four studies (involving 1,405 subjects) compared the peripheral lymphocyte counts between patients with MCI and HCs. The peripheral lymphocyte count was relatively lower in the MCI group than in the HC group; however, the difference was not statistically significant. The overall pooled SMD was −0.209 (95% CI: −0.515–0.096, $P = 0.179$) (**Figure 3D**). There was significant heterogeneity among the studies (I-squared = 78.1%, $P = 0.003$).

$CD4^+$ and $CD8^+$ T Cell Percentages and CD4/CD8 Ratio

Eleven studies (involving 328 patients with AD and 301 HCs) analyzed the $CD4^+$ T cell percentage differences. There was no statistically significant difference between the two groups in this parameter (SMD: 0.144; 95% CI: −0.657–0.945; $P = 0.724$) (**Figure 4A**).

The same 11 studies compared the $CD8^+$ T cell percentages between patients with AD and HCs. A significantly lower percentage of $CD8^+$ T cells was found in patients with AD than in HCs (SMD: −0.760; 95% CI: −1.460 to −0.061; $P = 0.033$). There was significant heterogeneity among these studies (I-squared = 93.7%, $P < 0.001$) (**Figure 4B**).

Eight studies (involving 436 patients) compared the CD4/CD8 ratios between AD patients and HCs. The AD group had a significantly higher CD4/CD8 ratio than did the HC group (SMD: 0.615; 95% CI: 0.074–1.156; $P = 0.026$), with significant heterogeneity (I-squared = 86.2%, $P < 0.001$) (**Figure 4C**).

The number of studies on these indices between patients with MCI and HCs was insufficient to perform a meta-analysis.

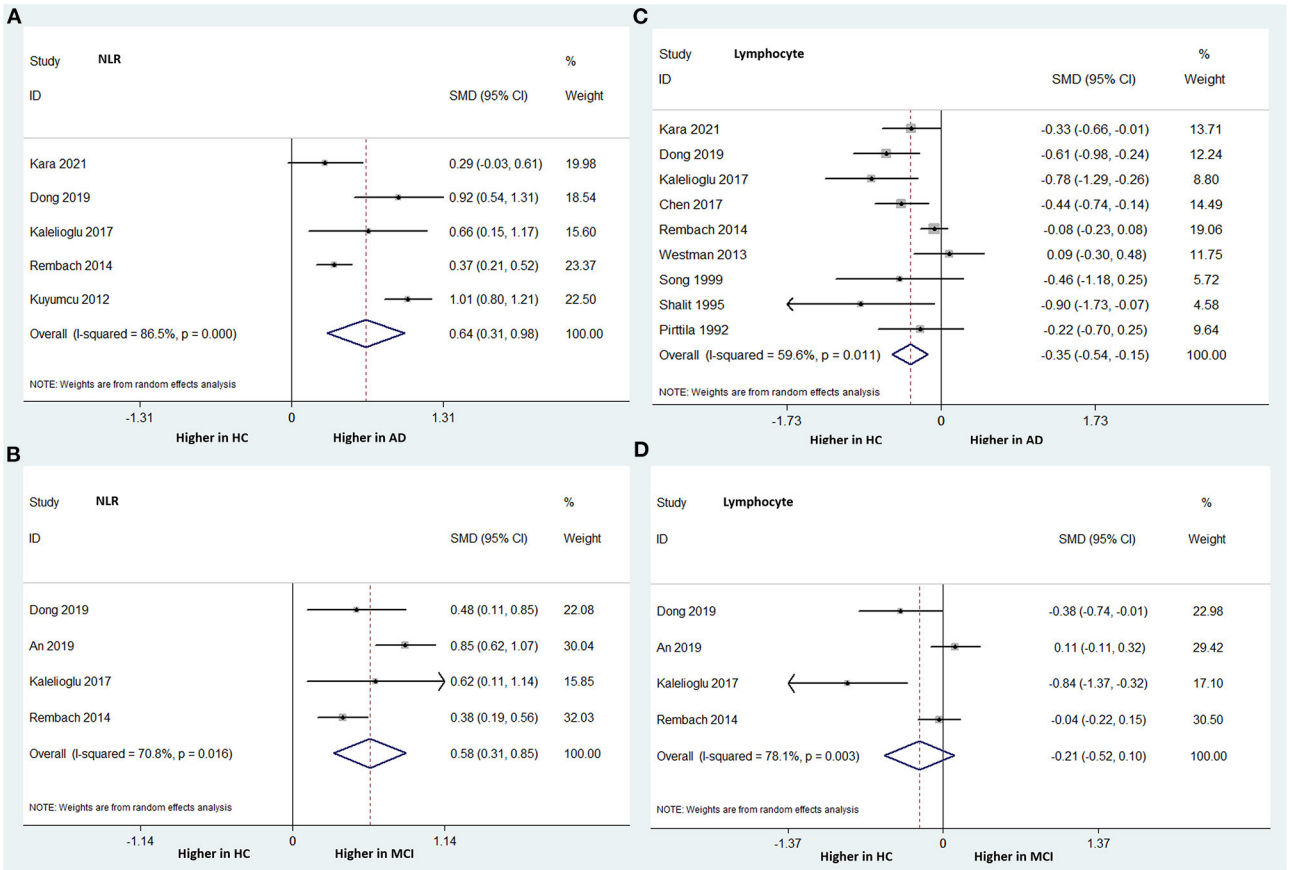

FIGURE 3 | Forest plots of standardized mean differences (SMDs) for the neutrophil–lymphocyte ratio (NLR) in Alzheimer's disease (AD) **(A)** and mild cognitive impairment (MCI) **(B)**, and lymphocyte counts in AD **(C)** and MCI **(D)**, compared to healthy controls (HCs).

B, NK, and CD3$^+$ T Cell Percentages

Five studies (involving 349 subjects) analyzed the differences in the percentage of B lymphocytes between patients with AD and HCs. We found that the AD group had a significantly lower percentage of B lymphocytes than did the HC group. The overall pooled SMD was −1.511 (95% CI: −2.775 to −0.248, P = 0.019) (**Figure 4D**). There was significant heterogeneity among the studies (I-squared = 96.0%, $P < 0.001$).

There was no significant difference in the CD3+ T cell or NK cell percentage between patients with AD and HCs. The number of studies on these indices between patients with MCI and HCs was insufficient to perform a meta-analysis.

Monocyte Count

Four studies (involving 528 subjects) compared peripheral monocyte counts between patients with AD and HCs. The AD group had a relatively lower peripheral monocyte count than did the HC group; however, the difference was not statistically significant. The overall pooled SMD was −0.318 (95% CI: −0.707–0.072, P = 0.179). There was significant heterogeneity among the studies (I-squared = 76.3%, P = 0.005).

The number of studies on peripheral monocyte count differences between MCI patients and HCs was insufficient to allow meta-analysis.

Association of Peripheral RBCs With AD or MCI Patients

Total RBC counts and correlated indices, such as hemoglobin level, RDW, and MCV, were analyzed. The meta-analysis results are presented in **Table 2**.

RBC Counts

Three studies (involving 335 subjects) compared the peripheral RBC counts between patients with AD and HCs. The AD group had a relatively lower RBC count than did the HC group; however, the difference was not statistically significant. The overall pooled SMD was −0.520 (95% CI: −1.289 to 0.250, P = 0.186). There was significant heterogeneity among these studies (I-squared = 90.5%, $P < 0.001$). Few studies compared the RBC counts between MCI and HC groups.

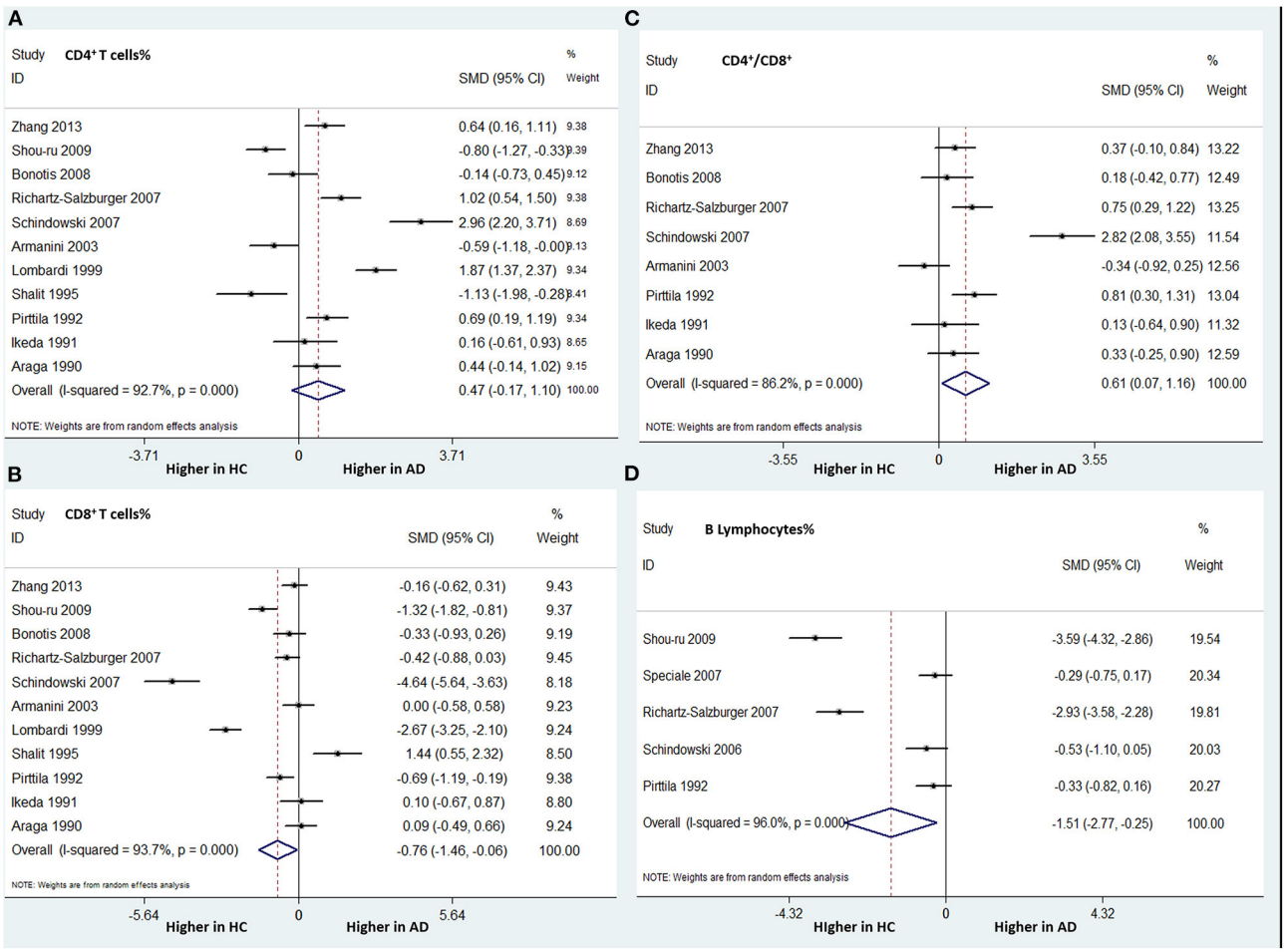

FIGURE 4 | Forest plots of standardized mean differences (SMDs) for percentage of $CD4^+$ T cells **(A)** and $CD8^+$ T cells **(B)**, the CD4/CD8 ratio **(C)**, and percentage of B lymphocytes **(D)** in Alzheimer's disease (AD), compared to healthy control (HCs).

Hemoglobin, RDW, and MCV

Ten studies (involving 961 patients with AD and 6,224 HCs) compared the peripheral hemoglobin levels between AD patients and HCs. In contrast to the RBC results, patients with AD had significantly lower hemoglobin levels than did HCs (SMD −0.347; 95% CI: −0.563 to −0.131; $P = 0.002$) (**Figure 5A**). There was significant heterogeneity among the studies (I-squared = 81.1%, $P < 0.001$). However, no difference was found in hemoglobin levels between patients with MCI and HCs.

There was no significant difference regarding RDW and MCV between the AD or MCI and HC groups.

Platelets in AD or MCI Patients

Platelet counts and correlated indices, such as PDW and MPV, were analyzed. The meta-analysis results are presented in **Table 2**.

Platelet Count

Thirteen studies were included in the comparison of peripheral platelet count between patients with AD and HCs, but no difference was found between the two groups (SMD 0.071; 95% CI: −0.175–0.318; $P = 0.57$) (**Figure 5B**). Additionally, there was no difference in the platelet counts between patients with MCI and HCs.

PDW and MPV

Four studies (involving 791 subjects) compared the peripheral PDW between AD patients and HCs. Patients with AD had a significantly lower PDW than did HCs (SMD −1.195; 95% CI: −1.796 to −0.595; $P < 0.001$) (**Figure 5C**). There was significant heterogeneity among the studies (I-squared = 93.2%, $P < 0.000$). There was no difference in PDWs between patients with MCI and HCs.

Furthermore, there was no significant difference between the AD, MCI, and HC groups regarding the MPV.

Subgroup Analyses Based on Region

Based on the region of population in these studies, we divided them into Asian and European. Subgroup meta-analyses of peripheral blood cell comparisons were performed when there

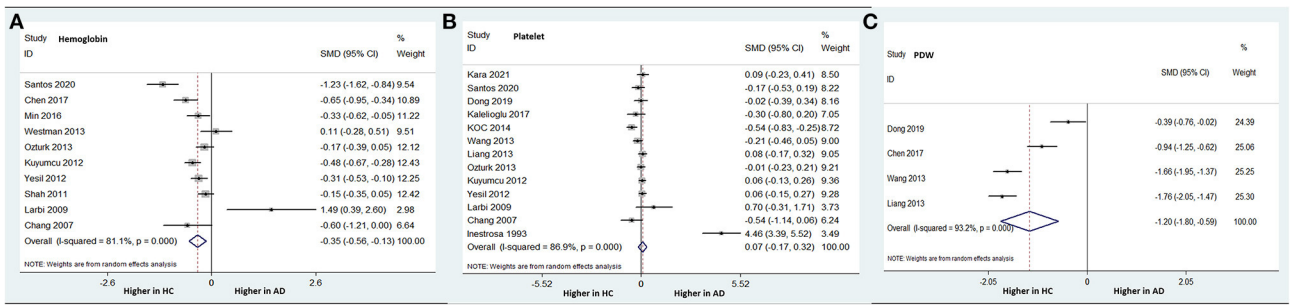

FIGURE 5 | Forest plots of standardized mean differences (SMDs) for hemoglobin level **(A)**, platelet count **(B)**, and platelet distribution width (PDW) **(C)** in Alzheimer's disease (AD) compared to healthy controls (HCs).

were at least two studies of one region. Minor differences were found in the results (**Table 3**).

Study Quality, Publication Bias, and Sensitivity Analyses

We assessed the methodological limitations of all the included studies using the AHRQ scale. All studies scored more than 5, indicating that these studies presented moderate or high-quality results (**Table 1**). A low publication bias was observed in the funnel plot. Sensitivity analysis for each peripheral blood cell comparison between AD and HCs was performed, and no significant heterogeneity from any study was found (**Supplementary File 1**).

DISCUSSION

To gain a better understanding of the association of circulating inflammatory and immune blood cell profiles with the severity of AD-related dementia, we here performed a meta-analysis on 36 studies that included a total of 2,339 AD patients, 608 MCI patients, and 8,352 HCs, comparing not only AD patients and HCs but also MCI patients and HCs. To date, no meta-analysis on this topic has been conducted. According to our data, compared to HCs, patients with AD had significantly increased neutrophil, leukocyte, and $CD4^+/CD8^+$ T cell counts and NLR, as well as decreased lymphocyte counts, hemoglobin levels, PDW, and percentage of $CD8^+$ T cells and B lymphocytes. Furthermore, significantly elevated neutrophil and leukocyte counts and NLR were observed in MCI patients compared with HCs. Our results suggest that significant alterations in peripheral inflammatory cells and lymphocyte subsets may be associated with the pathogenesis of AD and MCI.

Significant alterations in the population of neutrophils, leukocytes, and lymphocytes and NLR in AD patients, reflecting the body's inflammation, stress, and immune response, suggest that lymphocyte and neutrophil proliferation may be influenced by pathophysiological mechanisms of AD, including oxidative stress reactions, immune dysregulation, and neuroinflammation (Pluta et al., 2018). As key inflammatory cells, neutrophils can increase in number by some inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α) and interleukin-9, which are involved in the pathogenesis of AD (Cowburn et al., 2004; Holmes et al., 2011). Baj et al. found that chronic release of TNF-α by microglia could lead to the accumulation of Aβ and p-tau (Baj and Seth, 2018). Increased neutrophil counts, in turn, stimulate T cells through upregulation of antigen presentation, resulting in increased activation of neutrophils and release of TNF-α in a bi-directional manner (Dong et al., 2019). Moreover, activated neutrophils exacerbate neurodegenerative diseases by damaging the blood–brain barrier (BBB). Thus, neuroinflammation is exacerbated as increasing numbers of inflammatory cells and cytokines migrate across the compromised BBB (Baik et al., 2014; Pietronigro et al., 2017; Sayed et al., 2020). With more peripheral neutrophils and lymphocytes migrating into the central nervous system and with elevated neutrophil production in the circulation, the peripheral NLR is elevated significantly in patients with AD and MCI. According to our results, similar changes in the neutrophil count and NLR were also found in MCI patients compared to HCs, but there was no significant decrease in lymphocyte count in the former. This result might represent the slow progressive nature of the neurodegenerative disease. We did not perform a direct comparison between AD and MCI patients because of the limited number of relevant studies. Future research on the different grades of AD-related dementia is required.

Earlier case–control studies on lymphocyte subsets delivered conflicting results, and no consistent conclusion has been reached regarding lymphocyte subset distribution. In the present meta-analysis, we confirmed the increased peripheral CD4/CD8 ratio as well as the decreased percentage of $CD8^+$ T cells and B lymphocytes in patients with AD compared to HCs. Decreased $CD8^+$ T cell percentage was found in patients with AD but not in those with Parkinson's disease or vascular dementia, indicating that $CD8^+$ T cells may be involved in the pathogenesis of AD (Pirttilä et al., 1992; Lombardi et al., 1999). $CD8^+$ T cell infiltration is closely related to p-tau accumulation in human AD patients and mouse models (Merlini et al., 2018; Stojić-Vukanić et al., 2020). The decrease in the percentage of circulating $CD8^+$ T cells may be attributed to the fact that these cells are recruited into the brain from the periphery, leading to AD progression in the central nervous system. $CD8^+$ T cells are located near microglia and are involved in neuronal processes both in patients

TABLE 3 | Subgroup meta-analyses based on region.

Blood indices	European			Asian			Total		
	Number of studies	SMD [95% CI]	*P*-value	Number of studies	SMD [95% CI]	*P*-value	Number of studies	SMD [95% CI]	*P*-value
AD vs. HC									
Leucocyte	7	0.141 [0.035, 0.248]	0.009	2	0.128 [−0.185, 0.440]	0.423	9	0.140 [0.039, 0.241]	0.006
Lymphocyte	7	−0.279 [−0.507, −0.052]	0.016	2	−0.505 [−0.739, −0.271]	0.000	9	−0.345 [−0.545, −0.146]	0.001
Monocyte	2	−0.129 [−1.288, 1.030]	0.827	2	−0.379 [−0.829, 0.070]	0.098	4	−0.318 [−0.707, 0.072]	0.11
$CD4^+$ T cell%	8	0.672 [−0.088, 1.432]	0.083	3	−0.091 [−0.922, 0.739]	0.829	11	0.468 [−0.166, 1.102]	0.148
$CD8^+$ T cell%	8	−0.908 [−1.830, 0.015]	0.054	3	−0.396 [−1.399, 0.607]	0.439	11	−0.760 [−1.460, −0.061]	0.033
CD4/CD8	6	0.740 [0.047, 1.432]	0.036	2	0.257 [−0.205, 0.719]	0.275	8	0.615 [0.074, 1.156]	0.026
Hemoglobin	6	−0.166 [−0.382, 0.051]	0.134	3	−0.496 [−0.714, −0.278]	0.000	10	−0.347 [−0.563, −0.131]	0.002
Platelet	7	−0.054 [−0.243, 0.135]	0.576	4	−0.102 [−0.310, 0.106]	0.337	13	0.071 [−0.175, 0.318]	0.570
MPV	3	0.247 [−0.263, 0.758]	0.342	4	−0.619 [−1.831, 0.592]	0.316	7	−0.247 [−0.988, 0.495]	0.514
MCI vs. HC									
Neutrophil	2	0.176 [0.002, 0.351]	0.048	2	0.248 [0.121, 0.376]	<0.001	4	0.330 [0.144, 0.516]	<0.001
NLR	2	0.404 [0.229, 0.580]	<0.001	2	0.692 [0.337, 1.047]	<0.001	4	0.579 [0.310, 0.847]	<0.001
Lymphocyte	2	−0.400 [−1.185, 0.385]	0.318	2	−0.110 [−0.580, 0.361]	0.648	4	−0.209 [−0.515, 0.096]	0.179

AD, Alzheimer's disease; CI, confidence interval; HC, healthy control; MCI, mild cognitive impairment; MPV, mean platelet volume; NLR, neutrophil-lymphocyte ratio; SMD, standardized mean difference.

and in animal models (Merlini et al., 2018; Unger et al., 2018; Gate et al., 2020; Stojić-Vukanić et al., 2020). The production of B lymphocytes decreases with age (Stephan et al., 1998). Our analysis showed a more evident decrease in B lymphocytes in AD patients than in age-matched HCs, suggesting that ongoing AD may exacerbate aging characteristics.

The decrease in hemoglobin levels in patients with AD can be explained by multiple influencing factors. Decreased hemoglobin level in the blood reduces cerebral blood perfusion, resulting in neuroinflammation- and oxidative stress-mediated production of brain Aβ (Babiloni et al., 2014; Salminen et al., 2017; Raz et al., 2019), which exacerbates neurodegenerative diseases. Weiss et al. found that the RDW level was elevated by inflammation through iron metabolism impairment, inhibition of proliferation of erythroid progenitor cells, and release of immature RBCs (Weiss and Goodnough, 2005). The RDW is strongly associated with circulating inflammatory markers, such as high-sensitivity C-reactive protein, and erythrocyte sedimentation rate (Lippi et al., 2009). Decreased RBC counts and hemoglobin levels and increased RDW and MCV were found in AD and MCI patients in some studies (Chang et al., 2007; Öztürk et al., 2013; Du et al., 2020). However, no statistical evidence of association was shown in our analysis of RBC counts, RDW, or MCV, except for the significantly decreased hemoglobin level in AD patients compared to HCs. This result may be attributed to the fact that the number of included relevant studies on these indices was small, while there were as many as 10 studies for the meta-analysis of hemoglobin.

Similar to the results of previous studies, there was no statistically significant change in PLT counts or MPV values in patients with AD or MCI in this meta-analysis. We found significantly reduced PDW levels in patients with AD compared with HCs. Some studies have reported that the number of coated platelets, a subset of activated platelets, is positively associated with AD progression (Prodan et al., 2007, 2008). Jaremo et al. showed that densities of platelets were significantly different in patients with AD and age-matched HCs (Järemo et al., 2012). Thus, platelet heterogeneity may account for inconsistent results. The reason for the significantly reduced PDW in AD is unclear. PDW can be used to indicate variations in platelet size and differentiate thrombocytopenia categories (Borkataky et al., 2009). The cytokines and chemokines released by abnormal platelet activation may be involved in AD pathogenesis through neuroinflammation, such as macrophage inflammatory protein-1a, RANTES (Iarlori et al., 2005), platelet endothelial cell adhesion molecule-1, and intercellular adhesion molecule-1

(Nielsen et al., 2007). Future studies on the association of AD-related dementia with different types of PLTs, relevant indices (MPV and PDW), and cytokines are needed.

Some of the peripheral blood indices, such as the NLR, MPV, and PDW, have been explored as diagnostic biomarkers for AD. Dong et al. reported an NLR cutoff point of 2.35 for differentiating patients with AD from HCs, with a sensitivity and specificity of 83 and 54%, respectively (Dong et al., 2019). Kuyumcu et al. established a similar NLR cutoff of 2.48, with a sensitivity and specificity of 70 and 80%, respectively (Kuyumcu et al., 2012). Similar but lower sensitivities and specificities were determined for differentiating MCI patients from HCs (An et al., 2019; Dong et al., 2019). Owing to the limited number of studies using receiver operator characteristic curves, we could not perform a meta-analysis to explore the value of these indices as diagnostic biomarkers.

The limitations of our meta-analysis are as follows: First, heterogeneity was identified in several comparisons, possibly because of varying ages or different stages of AD among the studies. Stratified analysis could not be performed because of insufficient and inconsistent stage criteria for AD. Second, all included studies were cross-sectional and inevitably subject to selection bias. The duration of AD, treatment of AD, and combined chronic diseases were confounding factors. Third, few studies have explored the differences between AD and MCI patients, so that there was not enough data to perform a meta-analysis for any peripheral blood cell or index between AD and MCI. Fourth, the results of comparisons between MCI and HCs were not so convincing due to the limited numbers of studies (2–4 studies) included for them. To clarify the association between the severity of AD-related dementia and peripheral inflammatory and immune alterations, well-designed studies are needed in future.

CONCLUSION

Our work supports the theory that peripheral inflammatory and immune profiles are associated with AD. According to our data, patients with AD had significantly increased leukocyte and neutrophil counts, increased NLR and CD4/CD8 ratio, as well as decreased lymphocyte counts, hemoglobin levels, PDW, and percentage of $CD4^+$ T cells and B lymphocytes. Furthermore, significantly elevated neutrophil and leukocyte counts and NLR were observed in MCI patients compared with HCs. Future studies should focus on the diagnostic value of these peripheral blood cells and indices for AD-related dementia.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Materials**, further inquiries can be directed to the corresponding authors.

AUTHOR CONTRIBUTIONS

L-TH, Y-BW, and J-HW conceived and designed the study. L-TH and J-HW took full responsibility for data collecting, performed the meta-analysis, systematic review, and drafted the manuscript. C-PZ, Y-BW, and J-HW helped revise the manuscript. All authors have read and approved the final manuscript.

FUNDING

This work was funded by National Key R&D Program of China (Nos. 2018YFC2002100 and 2018YFC2002103).

ACKNOWLEDGMENTS

The authors thank all the medical staff who contributed to the maintenance of the medical record database.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnagi.2022.888946/full#supplementary-material

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frontiers | Frontiers in Aging Neuroscience

ORIGINAL RESEARCH
published: 12 May 2022
doi: 10.3389/fnagi.2022.910261

Effects of Sport Stacking on Neuropsychological, Neurobiological, and Brain Function Performances in Patients With Mild Alzheimer's Disease and Mild Cognitive Impairment: A Randomized Controlled Trial

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Objective: To investigate the effects of sport stacking on the overall cognition and brain function in patients with mild Alzheimer's disease (AD) and mild cognitive impairment (MCI).

Methods: A single-blind randomized controlled design was performed using sport stacking for 30 min, 5 days/week for 12 weeks. Forty-eight subjects with mild AD or MCI were randomly divided into the sport stacking group (T-mAD = 12, T-MCI = 12) and the active control group (C-mAD = 11, C-MCI = 13). Auditory Verbal Learning Test (AVLT), Alzheimer's Disease Cooperative Study–Activities of Daily Living scale (ADCS-ADL), Geriatric Depression Scale (GDS-30), and Pittsburgh Sleep Quality Index (PSQI) were performed, the level of amyloid β-protein-40 (Aβ-40), Aβ-42, brain-derived neurotrophic factor (BDNF), insulin-like growth factor-1(IGF-1), tumor necrosis factor-alpha (TNF-α), Interleukin-6 (IL-6), and soluble trigger receptor expressed on myeloid cells 2 (sTREM2) in plasma were tested, and brain functional connectivity in resting state and activation under finger movement task were analyzed by functional near-infrared spectroscopy (fNIRS).

Results: Thirty-nine patients completed the trial. After 4 weeks, we found a significant increase in AVLT score in T-MCI (6.36 ± 5.08 vs. −1.11 ± 4.23, $p = 0.004$), and T-mAD group (4.60 ± 4.77 vs. −0.11 ± 2.89, $p = 0.039$). After 12 weeks, there was a significantly improved in AVLT (9.64 ± 4.90 vs. −0.33 ± 6.10, $p = 0.002$) and ADCS-ADL (3.36 ± 3.59 vs. −1.89 ± 2.71, $p = 0.003$) in T-MCI. There was a significant improvement in AVLT (5.30 ± 5.42 vs. 0.44 ± 2.40) in T-mAD ($p < 0.05$). Plasma levels of BDNF

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Specialty section:
This article was submitted to Alzheimer's Disease and Related Dementias, a section of the journal Frontiers in Aging Neuroscience

Received: *01 April 2022*
Accepted: *20 April 2022*
Published: *12 May 2022*

Citation:
Yang Z, Zhang W, Liu D, Zhang S-s, Tang Y, Song J, Long J, Yang J, Jiang H, Li Y, Liu X, Lü Y and Ding F (2022) Effects of Sport Stacking on Neuropsychological, Neurobiological, and Brain Function Performances in Patients With Mild Alzheimer's Disease and Mild Cognitive Impairment: A Randomized Controlled Trial. Front. Aging Neurosci. 14:910261. doi: 10.3389/fnagi.2022.910261

were upregulated in both T-MCI and T-mAD, and IGF-1 increased in T-MCI ($P < 0.05$) compared to the control groups. The functional connectivity in MCI patients between DLPFC.R and SCA.R, SMA.L, and SCA.R was decreased. In contrast, in mAD patients, the brain regional function connection was increased between DLPFC.R and Broca's.L. The activation of channel 36 located in the left primary somatosensory cortex was significantly increased after 12-week training, which was correlated with the improved AVLT and the increase of BDNF.

Conclusion: Our findings suggested that sport stacking is effective for patients with MCI and mild AD, possibly through increasing the expression of neuroprotective growth factors and enhancing neural plasticity to improve neurocognitive performance.

Clinical Trial Registration: https://www.ClinicalTrials.gov, ChiCTR.org.cn, identifier: ChiCTR-2100045980.

Keywords: sport stacking, AD, MCI, Alzheimer's disease, mild cognitive impairment, neuropsychological, neurobiological, fNIRS

INTRODUCTION

Mild cognitive impairment (MCI) is an intermediate state between normal aging and dementia, with about 15% would progress to dementia in 2 years (Petersen et al., 2018), and one-third (32%) develop Alzheimer's disease (AD) within 5 years (Ward et al., 2013). As predicted, the number of people with dementia will reach to 78 million by 2030 (Gauthier et al., 2021). However, there is still a lack of effective pharmacological treatment today for AD, the most common cause of dementia. In recent years, non-pharmacologic treatments have drawn wide attention by showing their significant role in improving and maintaining cognitive function, quality of life, and daily function in patients with different severity of cognitive impairment. Moreover, a recent review found non-pharmacologic treatments seemed to be more effective than pharmacologic treatments for agitation and aggression in people with dementia (Watt et al., 2019). Aerobic exercise, the mainly studied physical activity among non-pharmacological treatments, has been shown to enhance the cognitive function of people with AD, reduce the risk of AD and other dementia, and delay the onset or progression (Groot et al., 2016; Hamer et al., 2018; Song et al., 2018). However, regular aerobic exercise may be difficult for dementia patients to adhere (Yágüez et al., 2011) for poor interest (Maltais et al., 2019) and low participation of the elderly (Padala et al., 2017).

Sport stacking is a new sport that began in the early 1980s. Participants use 12 specialized cups with both hands to make a pyramid ("up stacking") and then return the cups into stacks ("down stacking"). The whole process must be in predetermined sequences (Hart et al., 2005). Sport stacking could be seen more suitable for patients with cognitive impairment because it is combined with the game and physical activity, which can trigger a high willingness to participate (Park, 2017). Previous studies showed that sport stacking was beneficial in many aspects, such as hand-eye coordination (Hart et al., 2006), reaction time (Liggins et al., 2007), bilateral coordination (Rhea et al., 2006), and dual hemispheric brain activity (Hart and Bixby, 2005). Some studies have applied sport stacking to stroke patients and found significant improvement in reaction time (Tretriluxana et al., 2014). Despite its beneficial role for cognitive improvements in other diseases, there is still a lack of evidence for the effect of sport stacking on people with dementia.

Growing evidence suggests that AD is a multifactorial disease that affects the central nervous system and systemic processes (Morris et al., 2014). More specifically, increased inflammation and production of reactive oxygen species (ROS) might also play an essential role in the pathophysiology of MCI and AD (Gilgun-Sherki et al., 2001; Rosenberg, 2005; Koyama et al., 2013). Evidence shows that physical activity appears to have a positive effect on inflammation, oxidation, and neurotrophic biomarkers by enhancing the antioxidant activity of plasma and reducing the serum expression of proinflammatory cytokines, which may affect the destructive effects of oxidative stress and inflammation in nerve tissue (Stigger et al., 2019). Physical exercise has been shown to produce an increase in brain-derived neurotrophic factor (BDNF) and variable response to insulin-like growth factor-1 (IGF-1) (Anderson-Hanley et al., 2018). However, the evidence for the effect of sport stacking on these biomarkers as well as AD biomarkers is unknown.

Functional Near-Infrared Spectroscopy (fNIRS) is a new brain mechanism functional imaging technology which can perform advanced cognition and interactive behavior in natural situations. It makes up for the limitations of detection tools such as single-photon emission computed tomography (SPECT), positron emission tomography (PET), and functional magnetic resonance imaging (fMRI) (Liu et al., 2011; Yeung and Chan, 2020). fNIRS can evaluate the activation of different brain regions by observing the changes of oxygenated hemoglobin (Oxy-Hb), deoxyhemoglobin (Deoxy-Hb), and total hemoglobin (Total-Hb) concentration curves in different brain regions during the cognitive process. Hoshi (2011) indicated that monitoring the changing trend of blood oxygen concentration in the prefrontal cortex while completing cognitive tasks could objectively reflect the subjects' cognitive level. Since the abilities of language

comprehension, execution of an action, working memory, and movements are needed in fulfilling sport stacking, the brain regions of interest (ROI) were selected in corresponding to these functions. Recent studies have shown that the dorsolateral prefrontal cortex (DLPFC) is a crucial area for processing various behavioral tasks, and specifically, the right DLPFC (DLPRC.R) modulates the direction of these tasks (Xia et al., 2021) and semantic cognition (Herbet et al., 2018). Broca's area, a prefrontal region that was demonstrated to not only be involved in language production and comprehension but also play a role in several non-language-related functions such as working memory, execution, and perception of action (Clos et al., 2013; Kepinska et al., 2018). In addition, the subcentral area (SCA), also known as the subcentral motor cortex, and left supplementary motor area (SMA.L) are correlated with hand movements and grip (White et al., 2013; Auer et al., 2018; Eichert et al., 2021). Therefore, we select DLPFC.R, SCA.R, SMA.L, and Broca's area as the ROIs in this study.

This study aimed to investigate the effects of sport stacking on the neurocognitive performances, molecular biomarkers, and brain function performances in patients with mild AD and MCI. We hypothesized that sport stacking would effectively improve the cognitive function of patients with mild AD and MCI, potentially through divergent molecular factors (e.g., neuroprotective growth factors and cytokines) and the changes in activation of brain areas examined by fNIRS.

MATERIALS AND METHODS

Study Design

The current study was designed as a single-blind randomized controlled trial (RCT) of 12 weeks of sport stacking vs. a non-exercise and clinic routine management control group. Participants were included from May 2021 to September 2021. The Medical Ethics Committee of the First Affiliated Hospital of Chongqing Medical University approved the research protocol. The study was conducted in compliance with the Declaration of Helsinki's ethical standards (World Medical Association, 2013). It was registered on the Chinese Clinical Trial Registry (Registration No.: ChiCTR2100045980). Participants all agreed to participate in the study and gave written informed consent.

Participants

Eligible elderly participants with a clinical diagnosis of dementia (DSM-IV) or NINCDS-ADRDA Alzheimer's Criteria would be recruited from the geriatric memory clinic at the First Affiliated Hospital of Chongqing Medical University between May 2021 and September 2021. Eligibility criteria for inclusion were: 1) between 60 and 90 years of age; 2) a Clinical Dementia Rating (CDR) score of 0.5 or 1; 3) at least 3 months of stable doses if receiving antidementia medication or mood-stabilizing medication; 4) basic communication skills and normal vision and hearing; and 5) informed consent. Exclusion criteria were: 1) severe psychiatric illness and the use of antidepressants; 2) alcohol or drug abuse; 3) participation in exercise more than twice weekly on a regular basis; and 4) any medical condition precluding participation in the exercise program (e.g., severe cardiovascular, musculoskeletal, or neurological disease).

Sample Size

Cognition (ADAS-Cog) was the primary outcome measure. We calculated that a sample size of 40 participants would provide 80% power (at a two-tailed α-level of 0.05) for detecting differences between groups for an effect size of 0.46 in the ADAS-Cog (Orrell et al., 2012). Assuming a 20% attrition rate, the study recruited a total of 48 participants (24 per group). Sample size calculation was performed using G*Power 3.1.9 (Faul et al., 2007).

Randomization and Blind

Forty-eight patients who met the inclusion criteria were randomized 1:1 ratio to receive either the intervention (sport stacking) or control (clinic routine management) *via* a computer-generated randomization sequence by a statistician. Allocation concealment was ensured since the randomization was performed by a research assistant who was not involved in the assessment or intervention. This was a single-blind study in which study participants were blinded to the group allocation.

Procedure

In the sport stacking group, participants and their caregivers were taught how to stack the cups in a specific sequence with the correct technique using the lesson plans recommended in the Speed Stacks® instructor guide (Speed Stacks, 2014) by an experienced instructor at the geriatric clinic or online from baseline to the 3rd month.

Participants needed a set of stacking tools to practice, including 12 cups, a timer, and a stacking mat. To facilitate the self-practice at home, a set of audiovisual videos showing the skills trained in all lessons was provided as a reminder to guide the participants' self-practice. The whole training lessons consist of 3 patterns, including 3–3–3, 3–6–3, and the Cycle. Because of the characteristics of elderly participants with dementia, three lessons were divided into 7 stages, including 3, 3–3–3, 6, 3–6–3, 3–6–3 & 6–6, 1–10–1, and the Cycle. The difficulty of the stages increased per level to ensure that the training remained cognitively challenging. Participants were asked to practice sport stacking at least 30 min a day and at least 5 days a week for a total of 3 months at home. Meanwhile, the participants were asked to record each time they finished sport stacking and the duration of their daily self-practice at home. The caregivers were encouraged to assist the participants in the practice and compliance recording process. The participants or their caregivers were asked to videotape the participants' self-practice on the first and last day of each week to the researcher for collecting feedback. These videos could be used to check the participants' mastery of the sport stacking mode and correct their inaccuracies promptly. Adherence to the intervention was calculated by the number of pages of the self-recording logbook and the videos of participants' self-practice.

In the control group, all participants (MCI and mAD) received routine management from the Memory Clinic, mainly including (1) Regular medication; (2) Basic health education (medication, diet, exercise, etc.).

Experimental Procedure and Measurements

The neuropsychological evaluations were administrated at three timepoints, namely baseline before randomization, immediately after 4 weeks and after 12 weeks of sport stacking training. Blood tests and fNIRS measurements were conducted at baseline and 12 weeks. The assessments were carried out at the Memory Clinic, Department of Geriatrics, The First Affiliated Hospital of Chongqing Medical University. Under certain special circumstances, the neuropsychological evaluations of some of the participants were carried out online. And trained research assistants were blind to group allocation.

Neuropsychological Evaluation

The Mini-mental State Examination (MMSE; Folstein et al., 1975), and the Alzheimer's Disease Assessment Scale-Cognitive Subscale (ADAS-Cog; Mohs et al., 1983) were used to evaluate the global cognition, behavior, and ability to manage daily life. The Auditory Verbal Learning Task (AVLT; Arnáiz et al., 2004) was used to assess their memory. We used the clock drawing test (CDT; Sunderland et al., 1989) to assess executive functions. The 30-Geriatric Depression Scale (GDS-30; Yesavage et al., 1982) was used to rate the severity of depressive symptoms. The Alzheimer's Disease Cooperative Study–Activities of Daily Living scale (ADCS-ADL; Galasko et al., 1997) was used to assess activities of daily living function of patients with AD and MCI. The Pittsburgh Sleep Quality Index (PSQI; Buysse et al., 1989) was used to measure sleep quality.

Neurobiological Measurements

A 10-mL blood sample was obtained by a qualified nurse from the antecubital vein prior to and after the intervention. The blood samples were drawn to analyze serum amyloid β-protein (e.g., Aβ-40, Aβ-42), neuroprotective growth factors (e.g., BDNF, IGF-1), cytokines (e.g., TNF-α, IL-6), and markers of microglia activation and reactive oxygen species (e.g., sTREM2) levels. The blood samples were centrifuged at 3,000 rpm for 15 min, and serum was isolated and kept at −80°C until the next step. The levels of serum amyloid β-protein, neuroprotective growth factors, inflammatory cytokines, and plasma sTREM2 were analyzed using the Human ELISA kit (Shanghai Jianglai Industrial Limited by Share Ltd., Shanghai, China).

fNIRS Tasks, Acquisition and Analysis

Resting State and Finger Movement Task

The 8-min resting state at the sitting position took place in a sound-attenuated room. During the resting state recording, the participants were required to stay still and keep their eyes closed without falling asleep.

The finger movement task refers to a simple finger sequence (SFS) (Anwar et al., 2016), which requires the participants to sequentially tap the index, middle, ring, and the fourth finger against the thumb on both hands simultaneously. A block design was used in which subjects were asked to perform the finger movement task (SFS) for 20 s followed by 20 s rest for 6 repeat times (**Figure 1A**).

fNIRS Acquisition

In this experiment, the hemoglobin concentrations were measured using a multi-channel fNIRS system (NirScan, Danyang Huichuang Medical Equipment Co. Ltd, China). The sampling frequency was 11 Hz, and the wavelengths were 760 and 850 nm, according to the requirements of the internationally-used 10/20 electrode distribution system. We used the FPz channel (10/20 international system) as the center of the middle probe; a total of 31 SD probes (consisting of 15 sources and 16 detectors) with a fixed 3-cm inter-probe distance were placed to cover each subject's bilateral prefrontal cortex (PFC) and temporal cortices, and the lowest probes were positioned along with the Fp1–Fp2 line (**Figures 1B,C**). A total of 48 NIRS channels were established. The channels and the corresponding brain regions are consistent with a previous study (Liu et al., 2022).

Pre-processing

We used the NirSpark software package v1.7.3 (Danyang Huichuang Medical Equipment Co. Ltd, China) to analyze NIRS data. Data were preprocessed via the following steps. Motion artifacts were corrected by a moving SD and a cubic spline interpolation method. All differential path-length factors (DPF) were set to 6.0. According to previous studies (Li et al., 2021; Zhang et al., 2021), a bandpass filter with cut-off frequencies of 0.01–0.20 Hz was used to minimize noise, global trends, and biological signals (e.g., respiration and cardiac activity). The modified Beer-Lambert law was applied to convert optical densities into changes in Oxy-Hb and Deoxy-Hb concentrations. We used Oxy-Hb as our primary indicator in the following analysis because the Oxy-Hb signal generally has a better signal-to-noise ratio than Deoxy-Hb (Strangman et al., 2002).

Block Average

SFS block waveforms were calculated with a pre-baseline range set of 0–10 s and a block range set of 0–40. We used a 20 s task period of constructing phrases as the time window to analyze mean Oxy-Hb during the task and compare 20 s task period with 20 s rest period to analyze the Oxy-Hb change between task and baseline.

GLM Analysis

The generalized linear model (GLM) was used to analyze the Oxy-Hb time series data. The *t*-test was performed on the baseline and task state signals for each channel of each subject. The canonical hemodynamic response function (HRF) with time and dispersion derivative was selected as the basic function of the GLM. Through calculating the match between the experimental HRF value and the designs, the GLM can derive a value of activation coefficient β value representing the intensity of activation triggered by the task in the subject's cerebral cortex.

Functional Connectivity Analysis

The regions of interest (ROI) were selected as right dorsolateral prefrontal cortex (DLPFC.R) (channel 26, 28, and 40), left

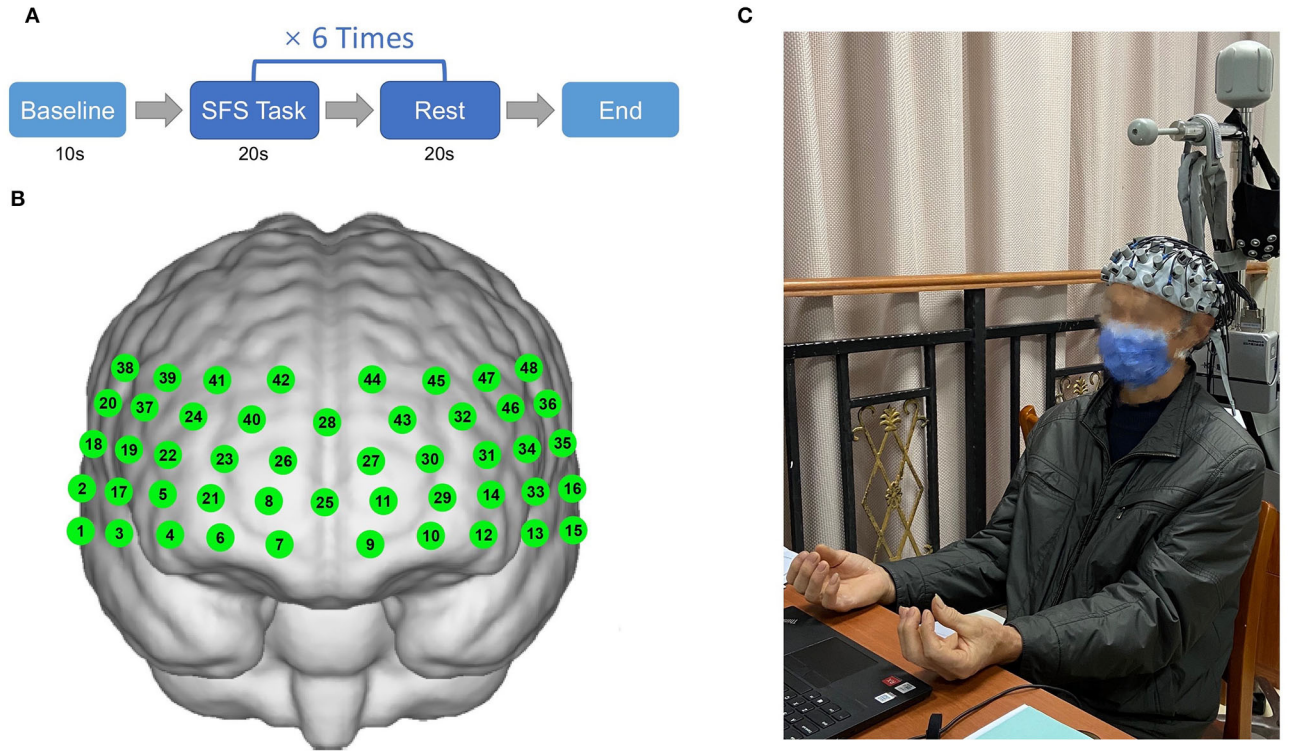

FIGURE 1 | Illustration of finger movement task (simple finger sequence) paradigm **(A)** and positions of 48 channels **(B,C)** (Informed consent was obtained from the patient).

Broca's Area (Broca's. L) (channel 30, 31, and 32), left supplementary motor area cortex (SMA.L) (channel 46), and right subcentral area (SCA.R) (channel 17). For each resting-state dataset, functional connectivity (FC) was analyzed by performing Spearman's correlation between the time series of each channel-to-channel pair, resulting in a 48 × 48 matrix of R-values (**Supplementary Figure 1**).

Statistical Analysis

All statistical analyses were performed by IBM SPSS ver. 26.0 (IBM Corp., Armonk, USA). The NirSpark software package v1.7.3, GraphPad Prism 9, and Photoshop software were used to generate figures and graphs. The Shapiro–Wilk test and the Levene test were used to determine the normality and homoscedasticity of the data. All data expressed as mean ± standard deviation (SD). All the (outcome) variables were analyzed for differences between control and intervention groups using independent-sample Student's *t*-tests and the Mann–Whitney *U*-test for continuous variables, and Fisher's exact tests for categorical variables. The between-group difference in the change of score of the continuous variables from baseline to follow-up at 4 and 12 weeks were analyzed by using delta(Δ)-linear mixed models (Δ = change from baseline), where "change from baseline" represents the difference between the baseline and follow-up groups for each diagnosis, after adjusted for the premeasurement covariates (age and sex) for primary and secondary endpoint outcomes. Unpaired *t*-tests were used to compare mean and Oxy-Hb changes between mild AD and MCI cohorts, and paired *t*-tests were used to compare the *R*-values of FC and β-value of task activation between baseline and 12-week follow-up in T-mAD and T-MCI group. A $p < 0.05$ was considered statistically significant, and all *p*-values were two-tailed. The statistical results were corrected for multiple comparisons across channels by the false discovery rate (FDR). Additionally, we calculated the Pearson correlation coefficient between changes in brain region activation (β-value), BDNF levels, and neuropsychological performances (AVLT) in the T-mAD group.

RESULTS

A total of 48 patients participated in the study, and 39 patients completed the study (21 in the intervention group and 18 in the control group). During this period, 39 patients completed neuropsychological assessments, 32 patients gave blood samples, and 29 completed measurements of fNIRS at baseline and 12-week follow-up (mAD = 15, MCI = 1 4).

Characteristics of the Study Population

A total of 48 eligible patients, 23 with MCI and 25 with mild AD, were enrolled in the study. Twenty-four were assigned to the intervention group (IG) and 24 to the control group (CG). Before the end of the trial, 9 participants withdrew (2 loss of interest; 2 refused to hospital; 2 no time; 1 poor health; 1 unable to contact

and 1 other reasons), resulting in a drop-out rate of 18.8%. Thirty-Nine patients [26 female (66.7%); mean (SD) age, 73 (6.9) years] completed the study at last (IG: $n = 21$; CG: $n = 18$). The Consolidated Standards of Reporting Trials (CONSORT) flowchart outlining the number of participants from screening to study completion at 12-week follow-up is shown in **Figure 2**.

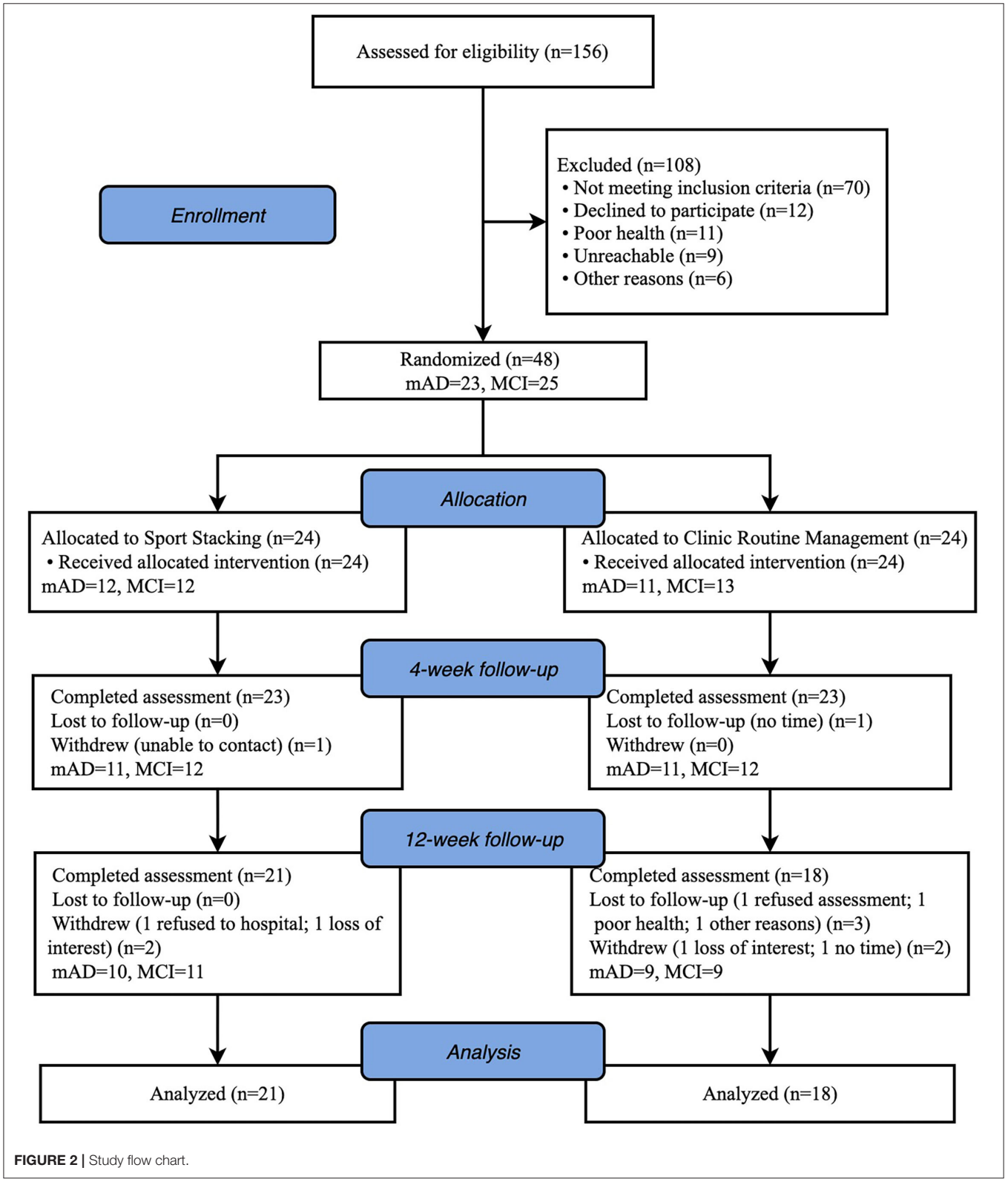

FIGURE 2 | Study flow chart.

TABLE 1 | Baseline characteristics of the participants ($n = 39$).

	MCI ($n = 20$)			mAD ($n = 19$)		
	Control group ($n = 9$)	Intervention group ($n = 11$)	p-value	Control group ($n = 9$)	Intervention group ($n = 10$)	p-value
Sex distribution (female/male), *n* (%)	4/5	7/4	0.653[c]	7/2	8/2	1.000[c]
Age (years)	72.0 (8.1)	70.5 (5.4)	0.617[a]	75.9 (8.0)	73.1 (5.2)	0.374[a]
Education (years)	9.6 (4.9)	9.7 (3.3)	0.766[b]	9.3 (3.0)	8.8 (3.9)	0.744[a]
Marital status						
Single/divorced/separated/widowed, *n* (%)	1 (11.1%)	3 (27.3%)	0.591[c]	3 (33.3%)	3 (30.0%)	1.000[c]
Married, *n* (%)	8 (88.9%)	8 (72.7%)		6 (66.7%)	7 (70.0%)	
AD medication						
Yes	4 (44.4%)	2 (18.8%)	0.336[c]	9 (100.0%)	10 (100.0%)	1.000[c]
No	5 (55.6%)	9 (81.2%)		0 (0.0%)	0 (0.0%)	
MMSE score	25.4 (2.4)	26.6 (3.0)	0.131[b]	19.8 (3.2)	17.8 (3.2)	0.156[b]
ADAS-cog score	12.2 (5.7)	9.8 (8.3)	0.131[b]	19.4 (9.3)	21.9 (6.0)	0.486[a]
AVLT immediate recall score	20.1 (7.0)	21.5 (5.2)	0.629[a]	12.1 (5.7)	11.8 (5.0)	0.657[a]
AVLT delay recall score	3.8 (3.7)	6.1 (3.6)	0.171[a]	2.0 (3.0)	1.0 (1.3)	0.661[b]
CDT score	13.4 (1.8)	13.3 (2.2)	0.941[b]	9.2 (3.9)	9.1 (2.6)	0.936[a]
ADCS-ADL score	57.7 (8.1)	61.7 (3.1)	0.331[b]	53.1 (8.8)	51.4 (9.7)	0.693[a]
GDS-30 score	8.0 (4.8)	5.5 (4.2)	0.152[b]	7.1 (2.3)	5.4 (3.0)	0.188[a]
PSQI score	5.9 (4.3)	6.4 (4.0)	0.801[a]	6.7 (2.9)	6.1 (3.7)	0.515[a]

[a] *Independent sample t-test.*
[b] *Mann–Whitney U-test.*
[c] *Fisher's exact test.*
MCI, Mild cognitive impairment; mAD, mild Alzheimer's disease; MMSE, Mini-mental state examination; ADAS-cog, Alzheimer's Disease Assessment Scale– Cognitive Subscale; AVLT, Auditory Verbal Learning Test; CDT, Clock Drawing Test; ADCS-ADL, Alzheimer's Disease Cooperative Study–Activities of Daily Living scale; GDS-30, 30-item Geriatric Depression Scale; PSQI, Pittsburgh Sleep Quality Index.

There were no significant statistical differences in characteristics or neuropsychological assessment results between the groups at baseline. Baseline characteristics of the study population are shown in **Table 1**.

Neuropsychological Tests

As shown in **Tables 2**, **3**, after the 4-week intervention, there was a significant improvement in AVLT in T-MCI (6.36 ± 5.08 vs. −1.11 ± 4.23) and T-mAD (4.60 ± 4.77 vs. −0.11 ± 2.89) compared with the corresponding control group ($P < 0.05$). After the 12-week intervention, there was a significantly improved AVLT (9.64 ± 4.90 vs. −0.33 ± 6.10) and ADCS-ADL (3.36 ± 3.59 vs. −1.89 ± 2.71) in T-MCI, and there was a significant improvement in AVLT (5.30 ± 5.42 vs. 0.44 ± 2.40) in T-mAD compared with the corresponding control group ($p < 0.05$).

Neurobiological Tests

As shown in **Tables 4**, **5**, most inflammatory markers remained unchanged after sport stacking. After the 12-week intervention, there was a significant improvement in BDNF in T-MCI (41.6 ± 24.3 vs. −7.5 ± 55.2 ng/ml) and T-mAD (29.9 ± 33.4 vs. −23.5 ± 25.5 ng/ml), as compared with the corresponding control group ($p < 0.05$). We found a significantly increased IGF-1 (2.7 ± 5.3 vs. −4.7 ± 11.6 ng/ml) and IL-6 (0.5 ± 0.2 vs. 1.0 ± 0.2 pg/ml) in T-MCI compared with the control group ($p < 0.05$).

Inter-cohort fNIRS Analysis

We firstly compared the mean Oxy-Hb concentration during the SFS task between mild AD cohort and MCI cohort, as well as the Oxy-Hb change between task and baseline. As is presented in **Figures 3A,B,D**, compared to MCI patients, significant lower Oxy-Hb concentrations during the task were exhibited in mild AD patients in channel 2, 4, 8, and 43 [mean with 95% CI, mAD vs. MCI, CH2: −0.0058 (−0.0205, 0.0088) vs. 0.0348 (0.0122, 0.0573), $p = 0.006$; CH4: −0.0054 (−0.0229, 0.01200) vs. 0.0329 (0.0137, 0.0521), $p = 0.019$; CH8: −0.0006 (−0.0142, 0.0129) vs. 0.0408 (0.0235, 0.0581), $p = 0.008$; CH43: −0.0053 (−0.0137, 0.0032) vs. 0.0354 (0.0178, 0.0530), $p = 0.0004$; all after FDR corrected]. Next, the Oxy-Hb change (task-rest) during SFS task in mild AD and MCI cohorts were shown in **Figures 3C,E**. Compared with MCI subjects, mild AD patients showed significant lower differences of Oxy-Hb level between the task and rest in channel 25 and channel 43 [mean with 95% CI, mAD vs. MCI, CH25: −0.0042 (−0.0208, 0.0126) vs. 0.0397 (0.0228, 0.0567), $p = 0.0005$; CH43: −0.0001 (−0.0101, 0.0098) vs. 0.0287 (0.0156, 0.0418), $p = 0.0007$; all after FDR corrected].

Functional Connectivity Change After Intervention

In both T-MCI and T-mAD groups, the changes of R-values among every two ROIs for each subject between baseline and 12-week follow-up were compared by paired *t*-test and were

TABLE 2 | Adjusted comparison of neuropsychological outcomes between changes from baseline at 4 and 12 weeks after intervention in patients with MCI ($n = 20$).

Outcomes	Control group ($n = 9$)			Intervention group ($n = 11$)			4-week adjusted estimate (95% CI)[a]; *P*-value	12-week adjusted estimate (95% CI)[a]; *P*-value
	Baseline	Change from baseline at 4 weeks	Change from baseline at 12 weeks	Baseline	Change from baseline at 4 weeks	Change from baseline at 12 weeks		
MMSE	25.44 (2.35)	0.33 (1.66)	−0.11 (1.83)	26.64 (2.98)	−0.09 (1.38)	−0.09 (1.81)	−0.7 (−2.1–0.7); 0.301	−0.02 (−1.9–1.8); 0.982
ADAS-cog	12.23 (5.71)	−1.19 (4.45)	−1.97 (3.32)	9.83 (8.30)	−2.98 (5.08)	−3.36 (5.29)	−2.0 (−6.9–2.9); 0.398	−1.3 (5.9–3.3); 0.552
AVLT immediate recall	20.11 (7.01)	−1.11 (4.23)	−0.33 (6.10)	21.45 (5.22)	6.36 (5.08)	9.64 (4.90)	7.4 (2.7–12.1); 0.004	9.6 (4.0–15.1); 0.002
AVLT delay recall	3.78 (3.67)	1.11 (3.14)	1.56 (2.24)	6.09 (3.56)	1.82 (4.19)	4.00 (3.29)	0.7 (−3.2–4.5); 0.718	2.6 (−0.3–5.6); 0.074
CDT	13.44 (1.81)	−2.00 (2.83)	−3.22 (3.42)	13.27 (2.24)	−0.36 (2.01)	−0.18 (2.56)	1.4 (−0.9–3.6); 0.222	2.5 (−0.3–5.3); 0.072
ADCS-ADL	57.67 (8.11)	−0.22 (1.92)	−1.89 (2.71)	61.73 (3.13)	0.36 (1.69)	3.36 (3.59)	0.4 (−1.3–2.1); 0.661	5.4 (2.2–8.7); 0.003
GDS-30	8.00 (4.80)	0.00 (2.45)	0.22 (2.77)	5.45 (4.16)	−0.09 (2.95)	−0.82 (3.46)	−0.03 (−2.8–2.8); 0.980	−1.1 (−4.3–2.2); 0.497
PSQI	5.89 (4.31)	0.00 (1.87)	0.44 (2.70)	6.36 (3.96)	0.64 (2.66)	−1.27 (2.76)	1.1 (−0.9–3.2); 0.266	−1.2 (−3.8–1.3); 0.319

[a] *Controlling for age and sex.*

MMSE, Mini-mental state examination; ADAS-cog, Alzheimer's disease assessment scale–cognitive subscale; AVLT, auditory verbal learning test; CDT, clock drawing test; ADCS-ADL, Alzheimer's disease cooperative study–activities of daily living scale; GDS-30, 30-item geriatric depression scale; PSQI, Pittsburgh sleep quality index.

TABLE 3 | Adjusted comparison of neuropsychological outcomes between changes from baseline at 4 and 12 weeks after intervention in patients with mild AD ($n = 19$).

Outcomes	Control group ($n = 9$)			Intervention group ($n = 10$)			4-week adjusted estimate (95% CI)[a]; *P*-value	12-week adjusted estimate (95% CI)[a]; *P*-value
	Baseline	Change from baseline at 4 weeks	Change from baseline at 12 weeks	Baseline	Change from baseline at 4 weeks	Change from baseline at 12 weeks		
MMSE	19.78 (3.19)	0.89 (2.26)	0.33 (2.45)	17.80 (3.22)	0.60 (2.99)	1.40 (2.88)	−0.5 (−3.3–2.3); 0.707	1.1 (−1.7–4.0); 0.423
ADAS-cog	19.39 (9.33)	−1.07 (6.60)	−1.70 (7.51)	21.93 (6.03)	−2.44 (4.72)	−5.04 (5.17)	−0.7 (−6.3–4.9); 0.786	−3.3 (−10.1–3.5); 0.319
AVLT immediate recall	12.11 (5.73)	−0.11 (2.89)	0.44 (2.40)	11.83 (4.99)	4.60 (4.77)	5.30 (5.42)	4.3 (0.3–8.4); 0.039	4.9 (0.4–9.4); 0.035
AVLT delay recall	2.00 (2.96)	0.67 (1.41)	1.00 (2.18)	1.00 (1.33)	0.00 (1.49)	1.80 (2.57)	−0.7 (−2.2–0.8); 0.345	1.0 (−1.3–3.3); 0.383
CDT	9.22 (3.90)	0.22 (3.07)	−0.67 (3.84)	9.10 (2.56)	1.60 (3.34)	1.00 (2.94)	0.8 (−2.4–3.9); 0.601	1.2 (−2.3–4.6); 0.479
ADCS-ADL	53.11 (8.81)	−0.11 (3.72)	2.33 (6.24)	51.40 (9.67)	0.10 (6.40)	2.10 (8.60)	0.1 (−5.2–5.4); 0.968	−0.8 (−8.1–6.5); 0.817
GDS-30	7.11 (2.32)	1.44 (4.16)	2.33 (6.38)	5.40 (3.03)	0.10 (2.69)	−0.70 (2.40)	−1.8 (−5.4–1.7); 0.280	−3.4 (−8.4–1.5); 0.158
PSQI	6.73 (2.85)	1.33 (2.00)	1.56 (3.24)	6.10 (3.67)	−0.2 (2.57)	−0.4 (2.12)	−1.8 (−4.1–0.5); 0.108	−1.9 (−4.7–0.8); 0.158

[a]*Controlling for age and sex.*

MMSE, mini-mental state examination; ADAS-cog, Alzheimer's disease assessment scale–cognitive subscale; AVLT, auditory verbal learning test; CDT, clock drawing test; ADCS-ADL, Alzheimer's disease cooperative study–activities of daily living scale; GDS-30, 30-item geriatric depression scale; PSQI, Pittsburgh sleep quality index.

corrected by FDR. As is shown in **Figure 4A**, compared to baseline, patients with MCI ($n = 10$) showed significant decrease of FC between SCA.R and SMA.L (mean of R, baseline vs. follow-up: 0.5040 vs. 0.2744, $p = 0.038$, FDR corrected), and between SCA.R and DLPFC.R (mean of R, baseline vs. follow-up: 0.3454 vs. 0.1786, $p = 0.038$, FDR corrected) after sport stacking training for 12 weeks. As for T-mAD group (n=8), a significant increase of FC was analyzed between DLPFC.R and Broca's.L after sport stacking intervention (mean of R, baseline vs. follow-up: 0.1085 vs. 0.3727, $p = 0.024$, FDR corrected) (**Figure 4B**).

Change of Brain Activation After Intervention

In SFS task fNIRS analysis, the β value derived from GLM from each channel represents cortical activation. We compared the β value of each channel for each T-MCI, T-mAD, C-MCI and C-mAD subjects between baseline and 12-weeek follow-up. We only found that in T-mAD group, CH36 area was significantly activated after sport stacking intervention, which overlaps cortex of left supramarginal gyrus (SMG.L) [mean with 95% CI of β, baseline vs. follow-up: 0.0119 (0.0004, 0.02329) vs. 0.0535 (0.03401, 0.0730), $p = 0.0003$, FDR corrected] (**Figure 5**), however, no significant differences between baseline and follow-up were found in the other three groups. Further, we correlated the changes of β value in CH36 of T-mAD subjects with their improved performance of ALVT (difference value) and changes of BDNF levels by Pearson Correlation analysis, and we found that the change of β value in CH36 was correlated with BDNF levels (coefficient value $r = -0.780$, $p = 0.039$) and with changes of AVLT scores ($r = -0.875$, $p = 0.004$), and AVLT performance was also correlated with increasement of BDNF levels ($r = 0.763$, $p = 0.046$). No significant differences were found in T-MCI, C-MCI and C-mAD groups, and no significant differences were found between T-MCI and C-MCI groups, and between T-mAD and C-mAD groups at follow-up.

DISCUSSION

The present study was aimed at investigating the effects of sport stacking on cognitive performances in individuals with mild AD and MCI. There was apparent evidence of improved cognitive function in tests of AVLT and ADCS-ADL. Our results showed that the intervention effectively improved episodic memory of patients with mild AD and MCI and improved the activities of living of patients with MCI. Moreover, this 12-week sport stacking added to usual care successfully increased the expression of some neuroprotective growth factors, including BDNF in both mild AD and MCI patients and IGF-1 in MCI subjects. More importantly, we found the functional connectivity in MCI patients between DLPFC.R and SCA.R as well as between SMA.L and SCA.R decreased after training. In contrast, in mild AD patients, the brain regional function connection was increased between DLPFC.R and Broca's L. In addition, the activation of channel 36, which was located in the left primary

TABLE 4 | Adjusted comparison of neurobiological measurements between changes from baseline at 12 weeks after intervention in patients with MCI ($n = 16$).

	Control group ($n = 6$)		Intervention group ($n = 10$)		Between-group difference (95%CI)[a]	*P*-value
	Baseline	Change from baseline at 12 weeks	Baseline	Change from baseline at 12 weeks		
Aβ-40 (pg/ml)	263.9 (71.0)	−44.8 (23.0)	247.3 (57.2)	−69.1 (39.6)	−18.7 (−56.8, 19.4)	0.305
Aβ-42 (pg/ml)	61.9 (27.2)	3.5 (48.7)	44.2 (6.9)	8.6 (16.6)	12.6 (−21.4, 46.6)	0.434
Aβ42/Aβ40	0.23 (0.08)	0.1 (0.2)	0.19 (0.06)	0.1 (0.1)	0.1 (−0.1, 0.3)	0.470
BDNF (ng/ml)	159.7 (65.9)	−7.5 (55.2)	110.9 (39.3)	41.6 (24.3)	56.6 (12.8, 100.4)	0.016
IGF-1 (ng/ml)	17.2 (9.2)	−4.7 (11.6)	10.1 (2.1)	2.7 (5.3)	9.4 (0.5, 18.2)	0.040
TNF-a (pg/ml)	3.7 (1.1)	1.1 (0.5)	4.0 (1.3)	0.7 (0.8)	−0.3 (−1.1, 0.5)	0.405
IL-6 (pg/ml)	2.1 (0.3)	1.0 (0.2)	2.4 (0.3)	0.5 (0.2)	−0.4 (−0.6, −0.2)	0.001
sTREM-2 (pg/ml)	19.4 (13.3)	−4.9 (18.6)	11.3 (2.9)	0.1 (6.6)	7.9 (−5.4, 21.2)	0.220

[a]*Controlling for age and sex.*

Aβ-40, amyloid β-protein-40; BDNF, brain-derived neurotrophic factor; IGF-1, insulin-like growth factor-1; TNF-α, tumor necrosis factor-alpha; IL-6, Interleukin-6; sTREM2, soluble trigger receptor expressed on myeloid cells 2.

TABLE 5 | Adjusted comparison of neurobiological measurements between changes from baseline at 12 weeks after intervention in patients with mild AD ($n = 16$).

	Control group ($n = 7$)		Intervention group ($n = 9$)		Between-group difference (95%CI)[a]	*P*-value
	Baseline	Change from baseline at 12 weeks	Baseline	Change from baseline at 12 weeks		
Aβ-40 (pg/ml)	212.0 (55.9)	−29.0 (43.5)	219.4 (49.1)	−25.2 (35.7)	−1.2 (−47.0, 44.6)	0.954
Aβ-42 (pg/ml)	40.6 (5.2)	5.9 (10.5)	57.9 (30.3)	−3.8 (31.0)	−8.9 (−38.9, 21.2)	0.531
Aβ42/Aβ40	0.20 (0.02)	0.07 (0.09)	0.26 (0.10)	0.03 (0.16)	−0.03 (−0.2, 0.1)	0.696
BDNF (ng/ml)	137.9 (29.1)	−23.5 (25.5)	120.1 (25.8)	29.9 (33.4)	50.5 (13.9, 87.0)	0.011
IGF-1 (ng/ml)	10.0 (1.6)	−0.03 (3.32)	14.2 (8.2)	−1.5 (8.5)	−1.2 (−9.3,6.9)	0.752
TNF-a (pg/ml)	3.8 (1.1)	0.8 (0.3)	3.8 (0.6)	0.3 (0.4)	−0.4 (−0.9, 0.02)	0.060
IL-6 (pg/ml)	2.3 (0.7)	1.2 (0.7)	2.4 (0.1)	0.7 (0.7)	−0.5 (−1.3, 0.4)	0.243
sTREM-2 (pg/ml)	9.7 (1.9)	0.2 (4.6)	18.0 (12.7)	−6.1 (13.0)	−6.4 (−18.9, 6.2)	0.291

[a]*Controlling for age and sex.*

Aβ-40, amyloid β-protein-40; BDNF, brain-derived neurotrophic factor; IGF-1, insulin-like growth factor-1; TNF-α, tumor necrosis factor-alpha; IL-6, Interleukin-6; sTREM2, soluble trigger receptor expressed on myeloid cells 2.

somatosensory cortex, was significantly increased after 12-week of sport stacking, and this increased activation was correlated with the improved cognitive function (AVLT) as well as the increased level of BDNF. These findings support the hypothesis that sport stacking would improve the cognitive function of patients with mild AD and MCI and that sport stacking would have an upregulating effect on anti-inflammatory cytokines and neuronal plasticity.

Sport stacking, which combines game and exercise, is a new sport. Our study indicated that a significant increase in the score of AVLT was measured in the sport stacking group after 12 weeks which means our training could improve all patients' episodic memory. Our results were consistent with that reported in a randomized controlled study of Hagovska and Nagyova (2017), which showed that cognitive training combined with physical training could significantly improve AVLT scores, indicating greater improvement of the memory in older people with mild cognitive impairment. A systematic study of Law et al. (2014) presented similar results in the improvement of general cognitive functions and memory in older adults after the intervention of combined exercise and cognitive training. Similar studies in the literature also found improvements in the previously mentioned cognitive domains (Phirom et al., 2020). In addition, there is growing evidence that the combination of physical and cognitive activities may have synergistic effects (Kraft, 2012; Gheysen et al., 2018). Although physical exercise contributes to plasticity, cognitive activities lead to changes in plasticity (Fissler et al., 2013). This combined benefit from exercise and cognitive stimulation would be consistent with previous animal research, which showed that this cognitive benefit had been found to be from different mechanisms (cell proliferation and cell survival, respectively, Olson et al., 2006; Fabel et al., 2009). And this combined-effect hypothesis indicated that simultaneous exercise and cognitive interventions could further increase cognitive

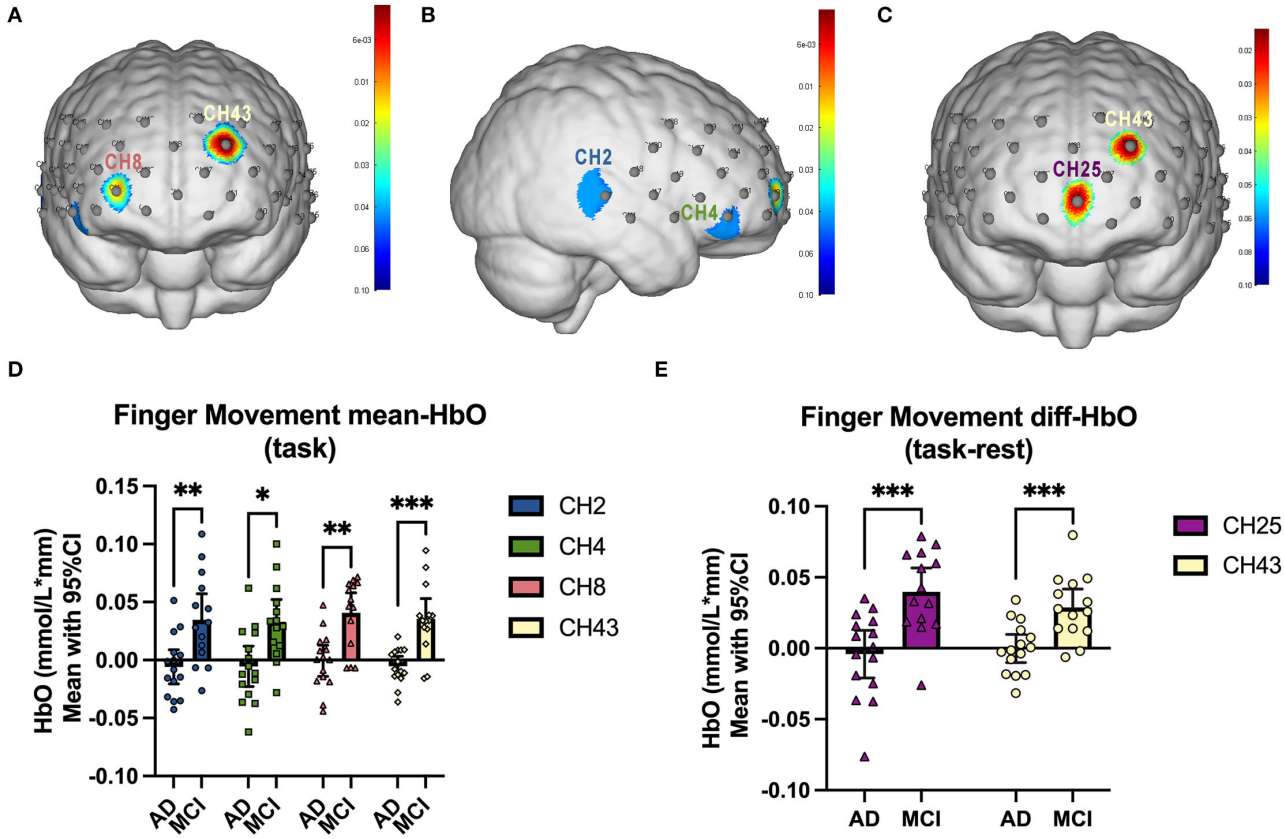

FIGURE 3 | fNIRS comparison between mAD and MCI subjects. **(A,B)** The front and right lateral view of significant channels in mean Oxy-Hb comparison, and the statistics were presented in **(D)**. **(C)** The front view of significant channels in diff-Oxy-Hb (task-rest) comparison, and the statistics were presented in **(E)**. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

benefits. Similarly, in line with previous studies, these findings showed that when physical exercise was cognitively challenging, the cognitive benefits were greater than traditional exercise (Anderson-Hanley et al., 2012).

Besides improvements in cognitive performances, activities of living of patients with MCI, as assessed with the ADCS-ADL, ameliorated. Sport stacking is a coordinated exercise of hands and eyes. Hand movement can improve hand function, stimulate brain function, reduce the occurrence and development of brain-related diseases (Geng, 2012), and enhance the learning ability of students with intellectual disabilities (Qu et al., 2012). Nyberg et al. found that finger tapping can stimulate the motor cortex in the brain (Nyberg et al., 2006). Finger exercise can also maintain or improve the ability of daily living and self-care and handling tools in patients with dementia (Zhang et al., 2010). Wang and Kui (2014) also suggested that finger movement could improve ADL by promoting blood circulation in the brain and the central nervous system, thereby improving brain function.

However, we did not find significant mAD results in the intervention group for ADL and other cognitive domains, probably due to the short duration of our intervention, while the overall score of patients with mild AD is changing in the direction of improvement. There is evidence that the severity of neurocognitive impairment can regulate the cognitive effect of combined cognitive and sports training (Bamidis et al., 2015). The increase in the severity of neurocognitive impairment may decrease the effect of the intervention (Bamidis et al., 2015). This can be explained by a reduction in the structural brain capacity of participants with more severe neurocognitive impairment (e.g., reduced number of neurons and synapses), which may result in limited resources for training-induced benefits (Bamidis et al., 2015). As a result, it may be more difficult to induce cognitive benefits in people with dementia than in people with MCI or healthy older people.

Our results indicate that the concentration of BDNF in peripheral blood increased significantly in all patients who participated in 12-week sport stacking, and the concentration of IGF-1 in peripheral blood only increased significantly in patients with MCI. Consistent with the results of our study, previous research has suggested that multicomponent exercise could increase BDNF concentrations (Wang et al., 2020), and physical exercise has been shown to increase IGF-1 levels in patients with MCI (Baker et al., 2010). Exercise-related upregulation of BDNF and IGF-1 may help to offset the age-related decline in synaptogenesis, neurogenesis, angiogenesis,

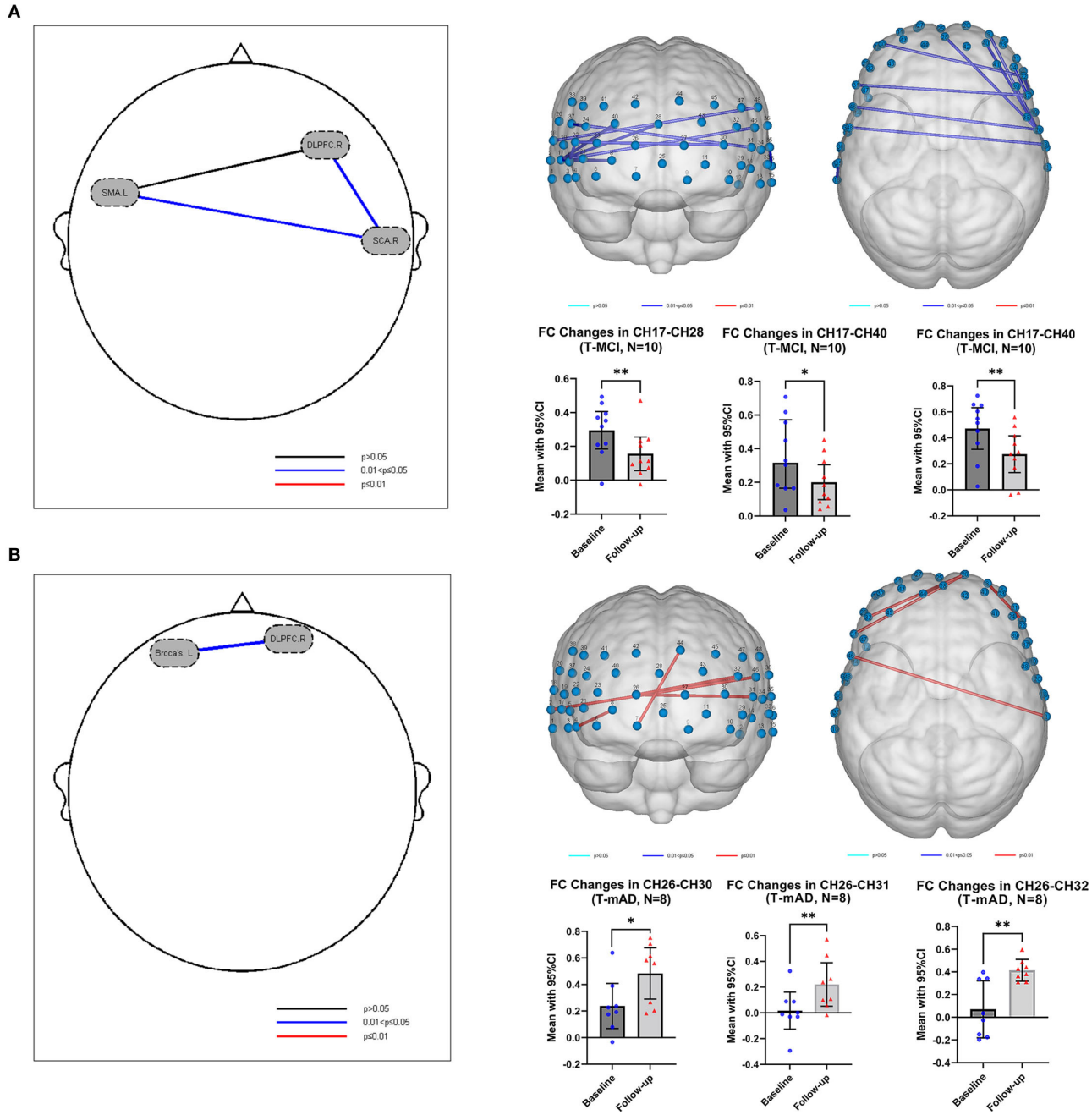

FIGURE 4 | Functional connectivity change after intervention. **(A)** The functional connectivity change in T-MCI ($n = 10$) after sport stacking training for 12 weeks. **(B)** The functional connectivity change in T-mAD ($n = 8$) after sport stacking training for 12 weeks. Each dot represents the FC value of each channel in T-MCI and T-mAD participants at baseline and at follow-up. *$p < 0.05$. No significant differences between intervention groups and control groups were found. **$p < 0.01$.

synaptic plasticity, and learning and memory, thus making the brain more flexible in dealing with age-related structural and functional neurodegeneration (Cotman and Berchtold, 2002, 2007). Together, these findings suggest that the production of BDNF and IGF-1 may be a mechanism responsible for cognitive improvement after sport stacking. In addition, our results show that non-strenuous exercise games such as sport stacking can improve BDNF and IGF-1 levels, which is of great significance for the exercise program of the elderly because the health status of the elderly is often unable to do strenuous exercise and hard to stick to.

Regarding IL-6, our results indicate that the change of IL-6 concentration in blood serum increased in both intervention groups, whereas only changes from the MCI group became statistically significant. Consistent with the results of our study, Behrendt et al. showed that both open and closed skill exercises

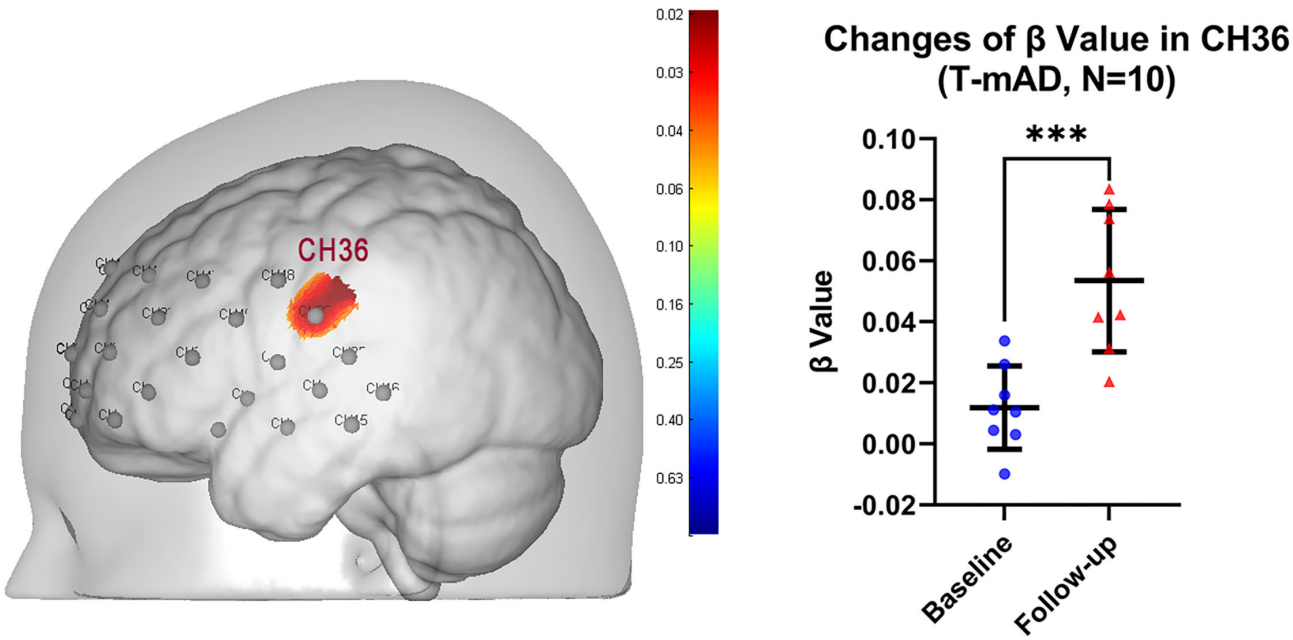

FIGURE 5 | Change of brain activation after intervention in T-mAD subjects. CH36 area was significantly activated after sport stacking intervention (left) and statistical results (right). ***$p < 0.001$. Each dot represents the individual β value of T-mAD participants in CH36 at baseline and at follow-up. No significant brain activation differences in T-MCI subjects were found, and no significant differences between intervention groups and control groups were found.

were equally efficient in acutely increasing the IL-6 serum levels in healthy older adults (Behrendt et al., 2021). However, Forti et al. (2014) showed results, contrary to ours, that IL-6 levels significantly decreased after 12 weeks of strength training in 20 older adults. It is reported that peripheral IL-6 concentration increases sharply during physical exercise and returns to the baseline level within 24 h (Fischer, 2006). In this case, IL-6 is considered to have anti-inflammatory effects and may be a factor in reducing the risk of chronic inflammation and neurological diseases by exercising regularly (Funk et al., 2011; Smart et al., 2011). As IL-6 plays multiple roles in a variety of biological processes of the human body (Norman et al., 2018; Ellingsgaard et al., 2019), further research is needed to better understand the relationship between IL-6 and exercise and its impact on neurocognitive processes.

In this study, the differences of mean and difference of Oxy-Hb concentration between mild AD and MCI subjects indicated that the cortical blood flow and neuronal activity in mAD patients were significantly reduced, consistent with previous studies (Herrmann et al., 2008; Haberstumpf et al., 2022). One of the important findings of this study is the different outcomes of FC analysis in T-mAD and T-MCI during resting state, compared with baseline and 12-week follow-up. There was a decrease of FC between DLPFC.R and other ROIs in MCI, while an increase was displayed in subjects with mild AD. The DLPFC is an associative cortical region that is often described as a functional hub enabling a host of higher-order processes, including working memory (McKendrick et al., 2014), mentalizing, attention, and response inhibition (Rodrigo et al., 2014). In functional studies of healthy participants, faster processing speeds have been related to reduced directed functional connectivity and activation of the DLPFC (Motes et al., 2018). Based on these studies and our results, we speculated that the decrease of FC in between ROIs in MCI patients may correlate with the neuronal plasticity changes after sport stacking. Although DLPFC activation is commonly observed to increase in a parametric manner with workload (Ayaz et al., 2012), increased DLPFC activation may also occur as a compensatory mechanism to reductions in available neural resources (Stuss and Knight, 2002) or alternatively, inefficient utilization of neural resources (Neubauer and Fink, 2009). which may indicate that patients with MCI may have higher FC values between these ROIs at baseline because of functional compensation in these brain areas, but after sport stacking intervention, the neural remodeling, while improving cognitive function, also regulated the compensation of brain regions and thus reduced FC between ROIs were found. Our study also found the increased activation in CH36, overlapping the cortex of SMG.L, in mild AD subjects under the SFS task and was correlated with increased cognitive performance and upregulated level of BDNF. A previous study has demonstrated that SMG.L is involved in goal orientated cognition (Smallwood et al., 2021). This may prove that sport stacking may arouse a multi-system effect to improve cognition through enhancing neuronal plasticity and boosting the production of BDNF.

The present study has some limitations. Firstly, the sample size in our study is small and it might lead to statistical errors. In order to explore the further impact of sport stacking on the elderly with dementia and avoid statistical errors in the process of data

analysis, future studies should expand the sample size; secondly, the period of our intervention was only 12 weeks, and we did not see any significant improvement in outcome indicators other than episodic memory in all patients, probably because the intervention time was relatively short; thirdly, although sport stacking was confirmed to be effective for individuals with MCI and AD, larger trials comparing sport stacking with other active control interventions such as aerobic exercise need to confirm or refute our findings. Finally, our results are restricted to patients with mild AD and MCI and cannot be generalized to those with more dementia types.

Although our study had these limitations, results still suggested the effectiveness of sport stacking and its benefits on participants' memory and activities of daily living, possibly via upregulation of BDNF and IGF-1. What's more, our study provides a new method for non-pharmaceutical treatment for patients with early stages of cognitive impairment.

CONCLUSION

Our findings suggested that sport stacking is effective for patients with MCI and mild AD, possibly through increasing the expression of neuroprotective growth factors and enhancing neural plasticity to improve neurocognitive performance.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of the First Affiliated Hospital of Chongqing Medical University. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

FD, YLü, ZY, WZ, YT, JL, DL, JY, and HJ contributed to study design, implementation, and interpretation. ZY, WZ, JS, YLi, and XL contributed to the management of data. ZY, DL, and WZ contributed to the analysis and interpretation of neuropsychological data. ZY, S-sZ, and YT contributed to biomarker data analysis and interpretation. WZ contributed to fNIRS data analysis and interpretation. ZY and WZ contributed to the drafting of the manuscript. FD, YLü, ZY, and WZ contributed to the critical revision of the manuscript. All the authors contributed to the data collection. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by the Intelligent Medicine Project of Chongqing Medical University (Grant No. ZHYX2019003) and the Nursing Research Fund Project of the First Affiliated Hospital of Chongqing Medical University (Grant No. HLJJ2015-06 and HLJJ2020-06). This study was supported by the Geriatric Memory Clinic at the First Affiliated Hospital of Chongqing Medical University, the Department of Histology and Embryology and Laboratory of Stem Cell and Tissue Engineering in Chongqing Medical University, Chongqing, China.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnagi.2022.910261/full#supplementary-material

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frontiers | Frontiers in Aging Neuroscience

SYSTEMATIC REVIEW
published: 18 May 2022
doi: 10.3389/fnagi.2022.890816

Pathological Role of Natural Killer Cells in Parkinson's Disease: A Systematic Review

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Parkinson's disease (PD) is one of the common neurodegenerative diseases that is characterized by selective degeneration of dopaminergic neurons in the substantia nigra, and misfolding of α-synuclein into aggregates is thought to contribute to its pathology. Studies have shown that immune-inflammatory responses are involved in the development of PD and play an important role in α-synuclein scavenge. Natural killer (NK) cells are first responders in immune cells and can directly promote immune defense mechanisms by cytotoxicity and by secreting cytokines. Recent discoveries suggest that NK cells are increasingly recognized in the pathological features of PD. However, the mechanisms underlying it have not been fully understood. In this review, we systematically retrieved and evaluated published evidence about the functions of NK cells in PD. We find alterations in the number of NK cells and cytotoxicity during the progression of PD, and it seems that NK cells play a neuroprotective role in PD pathogenesis, which may further reveal novel targets for the management and treatment of PD.

Keywords: Parkinson's disease, α-synuclein, natural killer cells, natural killer cell cytotoxicity, neuroinflammation

OPEN ACCESS

Edited by:
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Specialty section:
This article was submitted to Parkinson's Disease and Aging-related Movement Disorders, a section of the journal Frontiers in Aging Neuroscience

Received: *06 March 2022*
Accepted: *14 April 2022*
Published: *18 May 2022*

Citation:
Zhang L, Zhang Y and Fan D (2022) Pathological Role of Natural Killer Cells in Parkinson's Disease: A Systematic Review. Front. Aging Neurosci. 14:890816. doi: 10.3389/fnagi.2022.890816

INTRODUCTION

Parkinson's disease (PD) is one of the fastest-growing neurological diseases in terms of prevalence and mortality and is the second most common degenerative disease in the elderly with an increasing burden on healthcare systems (Poewe et al., 2017; Brakedal et al., 2021; Feigin et al., 2021). It is principally characterized by motor features, such as postural instability, bradykinesia, tremor, and rigidity. In addition, non-motor symptoms, such as hyposmia, anxiety, depression, and cognitive dysfunction, are also found to be increasingly prevalent over the course of PD development, which are gradually being paid more attention (Schapira et al., 2017). Evidence has demonstrated that progressive and selective loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc) is the main cause of motor symptoms, but the clinical diagnosis of the pathological changes that had already occurred before the manifestation of PD poses a challenge to the early diagnosis and treatment of PD (Gruden et al., 2011; Poewe et al., 2017).

At present, it is generally believed that the abnormal accumulation of α-synuclein (α-syn) is one of the important etiological factors leading to the degeneration of dopaminergic neurons, which is closely related to the formation of the Lewy body (Vekrellis et al., 2011; Poewe et al., 2017). In addition, extensive neuroinflammation characterized by the innate and adaptive immune response is found to contribute to the extensive degeneration of dopaminergic neurons (Tan et al., 2020; Harms et al., 2021). Remarkably, the neurotoxicity of α-syn, neuroinflammation, and neurodegeneration can trigger a series of pathophysiological mechanisms both locally and systemically in PD (Wong and Krainc, 2017; Tan et al., 2020; Harms et al., 2021).

Recent advances in the field of neuroinflammation demonstrate that complex interactions between CNS-resident cells and peripheral immune cells are involved in the PD pathogenesis (Iba et al., 2020; Tan et al., 2020; Harms et al., 2021). However, the mechanism involved in the recruitment of peripheral cells into the CNS and whether it is a passive migration or active participation are still unclear.

Natural killer (NK) cells are members of the innate lymphoid cells (ILCs) family, and represent 5-20% of the peripheral lymphocytes in humans (Abel et al., 2018; Zitti and Bryceson, 2018). A variety of inhibitory or activating receptors are expressed on NK cells, which regulate cell functions by binding to specific ligands and play crucial roles in cellular signal transduction. When the cells downregulate the expression of major histocompatibility complex class I (MHC-I) molecules or upregulate the expression of NK cell-activating receptor ligands abnormally, they may become the immunological attack targets for NK cells. The mechanism of cytotoxicity of NK cells can be basically classified into three types: direct release of the lytic molecules such as perforin and granzyme, activation of the extrinsic apoptotic pathway through Fas ligand (FasL) and TNF-related apoptosis-inducing ligand (TRAIL), and antibody-dependent cell-mediated cytotoxicity (ADCC) effects (Abel et al., 2018; Prager and Watzl, 2019). In addition, NK cells can produce and respond to different cytokines and play a crucial role in immunomodulation (Zitti and Bryceson, 2018).

It has been reported that NK cells are capable of interacting with α-syn in the animal models of PD and modulating neuroinflammation in neurodegenerative diseases (Poli et al., 2013; Earls et al., 2020; Garofalo et al., 2020). The existence of a link between PD and NK cells had been originally suspected due to two main reasons One is that NK cell activity was found to be related to neurological diseases, particularly demyelinating diseases in the previous studies, and researchers speculated whether similar pathological changes or immune interactions might also be present in patients with PD (Bokor et al., 1993). On the other hand, epidemiological studies provided evidence for the fact that patients with PD were more likely to have a lower incidence of cancer, probably because increased activity of natural killer cells provides defense against the infiltration of tumor cells (Mihara et al., 2008). Based on these findings, several basic and clinical studies focused on the pathological role of NK cells and performed the beneficial exploration and the attempt in this context. However, research in this field is still in its infancy, and some of the findings might be controversial. Therefore, we conducted a systematic review of the relevant literature to investigate the alterations of NK cells in PD and discuss how NK cells can influence the disease onset and progression.

MATERIALS AND METHODS

This systematic review was reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.

Literature Search

The search of PubMed, Embase, and Web of Science databases (from inception up to January 2022) was performed by two authors (ZL and ZYS) independently. The search strategy involved selecting the most relevant Medical Subject Headings (MeSH) and title/abstract text keywords used in combination or alone: "Parkinson's disease," "NK cell," "natural killer cell," "alpha-synucleinopathy," and "dopaminergic nerve cell" (detailed search strategy is described in **Supplementary Table 1**).

Selection Criteria

To prove the eligibility of the studies, the following inclusion and exclusion criteria were formulated:

Inclusion criteria were as follows: (1) articles published in English, (2) original articles regarding animals and humans (peripheral lymphocyte analysis) and *in vitro* studies (cell cultivation) of natural killer cells where relevant, and (3) the research theme focused on "Parkinson's disease." Exclusion criteria were as follows: (1) case report, case series, letter, poster, commentary, proceedings, laboratory science studies, review, and systematic review, (2) repeatedly published literature, unpublished data, or findings reported in abstract form only, and (3) literature of poor quality.

Data Extraction

Data extraction from eligible articles was performed by two investigators (ZL and ZYS) independently. The following clinical information was extracted from the study: publication, region of the population, animal models of PD, number of patients and healthy controls, gender statistics, age at diagnosis, disease duration, disease severity, and the main results.

Quality Assessment and Risk of Bias

Quality assessment was carried out by two authors (ZL and ZYS) independently. Modified Collaborative Approach to Meta-Analysis and Review of Animal Data from Experimental Studies (CAMARADES) and Newcastle-Ottawa Scale (NOS) were used as quality assessment tools for the animal experimental studies and clinical studies separately. Ten items are included in the modified CAMARADES tool: sample size calculation, random allocation to treatment or control, allocation concealment, blinded assessment of outcome, appropriate animal model, use of anesthetic without significant intrinsic neuroprotective activity, statement of control of temperature, compliance with animal welfare regulations, peer-reviewed publication, and statement of potential conflict of interests. Seven items are included in the NOS tool: adequate case definition, representativeness of the cases, selection of controls, definition of controls, comparability of cases and controls on the basis of the design or analysis (study controls for age or any additional factors), ascertainment of exposure, and same method of ascertainment for cases and controls and non-response rate. The total scores of the two scales are 10 and 9, respectively. The final scores of each study are described in **Supplementary Tables 2, 3**. Any discrepancies were resolved through discussion. If an agreement could not be reached, the disagreements were resolved with the help of a third author.

RESULTS

A total of 408 records were generated from the database search after removing the duplicates. After screening for title and abstract, 388 records were excluded. Full-text of the remaining 20 articles was assessed, and finally, a total of 12 studies were eligible for the systematic review after full-text screening (**Figure 1**).

Animal Studies

We identified three studies on animals according to the inclusion criteria, and the main findings are summarized in **Table 1**. Two studies used a preformed fibril (PFF) α-syn injection model in C57BL/6J mice and transgenic mice that overexpressed human A53T α-syn mutant protein separately, and one study used a 6-hydroxydopamine (6-OHDA) injection model in Wistar rats. These two models are commonly used in the study of PD. For the PFF α-syn model, PFF α-syn acts as a template and induces the endogenous α-syn to accumulate into misfolded phosphorylated pathological aggregates in the substantia nigra (Earls et al., 2020). In the 6-OHDA model, 6-OHDA (a neurotoxin) is absorbed by dopaminergic neurons rich in monoamine oxidase in the substantia after injection, which can further convert into free radicals and cause damage to neurons; 6-OHDA does not induce the formation of α-syn aggregates or Lewy-like inclusions in PD (Grembecka et al., 2021).

The first study (Earls et al., 2019) showed that intrastriatal injection of PFF α-syn could induce Lewy body–like pathology, and the percentage of NK cells in the mononuclear immune cells at 5 months post injection (p.i.) was relatively increased in the CNS parenchyma, which was confirmed by the flow cytometry analysis. In addition, characterization of the leukocytes in the peripheral blood showed that the number of NK cells decreased, but the percentage of the NK cell population did not show significant changes.

In the second study, Earls et al. confirmed the presence of NK cells in the SNs of both postmortem PD patients and M83 Tg mice based on immunohistochemistry (IHC) markers (Earls et al., 2020). More importantly, obvious clinical motor deficits and an increased number of p-α-syn inclusions within the striatum, substantia nigra pars compacta (SNpc), and brainstem were observed in PFF α-syn–injected M83 Tg mice after systemic NK cell depletion, which indicated the protective role of NK cells in PD. Moreover, researchers carried out cell-based assays and found the bidirectional effects between extracellular α-syn and NK cells: NK92 cells and primary human NK cells could internalize the extracellular α-syn and scavenge it through the endosome and lysosome pathway, while extracellular α-syn aggregates attenuated NK cell cytotoxicity (NKCC) and IFN-γ secretion without causing any significant changes in NKG2A and NKG2D receptors on the cell surface. It is an interesting phenomenon that NK cells are not aberrantly activated.

For the third study (Grembecka et al., 2021), we extracted information regarding the comparison between the PD model and the blank controls. It seems that the number of NK (CD3-CD161a+) cells in PBMC did not show any significant difference between the 6-OHDA groups and the controls (without *p*-values recorded). Furthermore, they observed elevated peripheral NKCC levels after 6-OHDA microinjection into the SNpc, which means NK cells might be actively involved in the progression of PD.

Clinical Studies

Nine clinical studies explored the relationship between NK cells and sporadic Parkinson's disease according to the inclusion criteria, and the basic information and important findings of these studies are summarized in **Tables 2**, **3**. Most of the included studies provided basic information regarding the subject number, gender, age at visit/onset of patients, disease duration, and H&Y/UPDRS-III (part III of the Unified Parkinson's Disease Rating Scale) assessment. NK cells are mainly characterized based on the cell number, cell cytotoxicity, and changes in the cell surface receptors.

Number of NK Cells in PD

Seven studies analyzed NK cell count in the peripheral blood. One of them found no differences between the patients and controls (Stevens et al., 2012), five of them reported a higher percentage of NK cells in the patients with PD (Mihara et al., 2008; Niwa et al., 2012; Cen et al., 2017; Sun et al., 2019; Huang et al., 2021), and in one study, Tian et al. divided NK cells into three clusters and reported that only C32 (CD56+, CD16+, CD57–, and CD28–) and C27 (CD56+, CD16+, CD57+, and CD28–) clusters increased (Tian et al., 2022). Furthermore, we noticed that the markers used to identify NK cells from the PBMC were quite different. CD16, a typical NK cell marker and also known as FCγRIII, is an activation receptor that mediates ADCC effects. CD56, known as nerve cell-associated adhesion molecule (NCAM), is considered as a functional indicator of human NK cells and separates the cells into precursor and mature populations based on its expression. In some studies, cells labeled by CD16 or CD56 were used as markers, while in others, those cells that expressed both CD16 and CD56 were recognized as NK cells. Mihara et al. and Niwa et al. reported the occurrence of the inhibitory NKG2A cells and the activating NKG2D cells, which indicated that the percentage of NKG2A+ cells among CD3-CD56+ NK cells was lower and the percentage of NKG2D+ cells in the peripheral lymphocytes was higher in the PD group ($p < 0.05$) (Mihara et al., 2008; Niwa et al., 2012).

NKCC in PD

Experiments and bioinformatics analysis were used to analyze the cytotoxicity in NK cells. Only two studies measured NKCC activity against the K562 target cells through the LDH cytotoxic assay and the calcein acetoxymethyl ester release assay, and both of them found no significant differences between the patients with PD and the controls (Mihara et al., 2008; Niwa et al., 2012). Additionally, the former study provided information that NKCC increased as the disease progressed in PD (Mihara et al., 2008). On the other hand, from the perspective of bioinformatics analysis, Huang et al. carried out the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis and revealed that NK cell-mediated cytotoxic pathways were enriched in PD based on the blood expression profile (Huang et al., 2021).

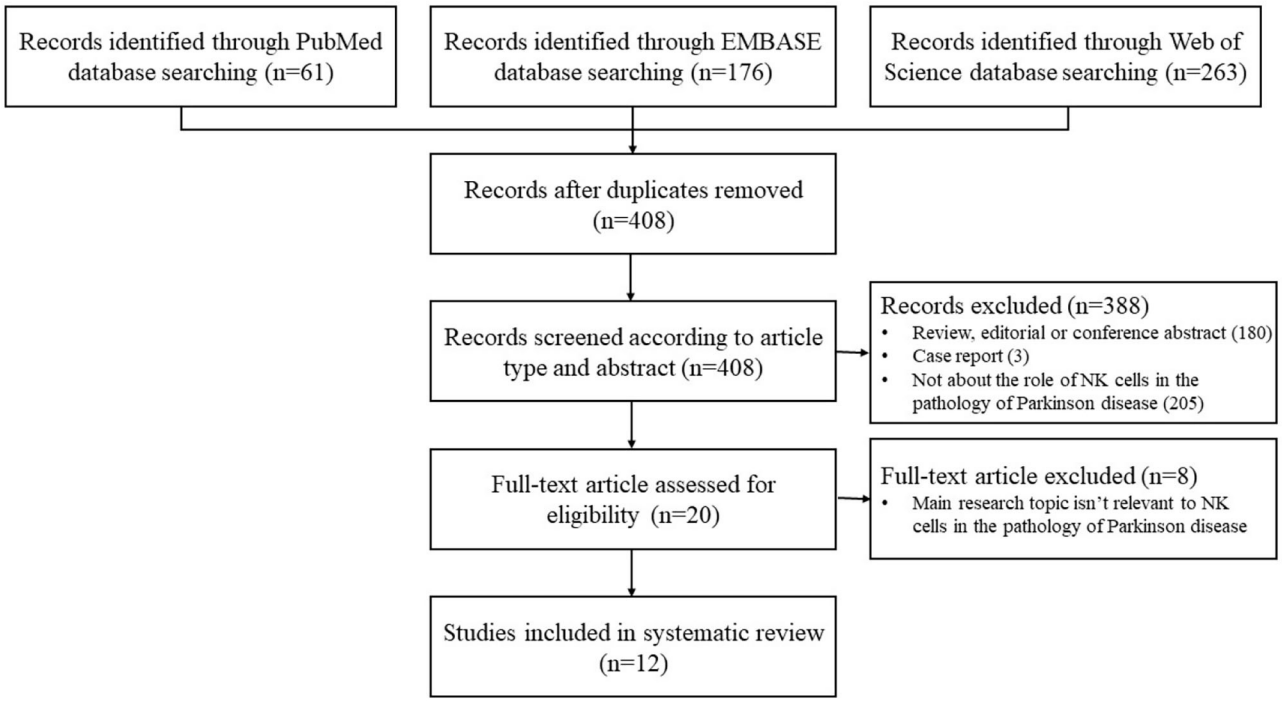

FIGURE 1 | Flowchart of the study selection.

Correlation of NK Cells With Clinical Phenotypes

Since aging is a common predisposing factor for Parkinson's disease, stratification by age at disease onset needs to be considered (Pagano et al., 2016). Two studies analyzed the subset of NK cells in the early-onset Parkinson's disease (EOPD) and late-onset Parkinson's disease (LOPD). Cen et al. observed that the percentage of NK cells in peripheral blood did not differ significantly between the two groups ($p = 0.067$) (Cen et al., 2017). Tian et al. showed a more detailed comparison between early- or late-onset patients and the age-matched controls. For the less toxic NK cells, CD32 (CD56+, CD16+, CD57–, and CD28–) clusters were significantly higher in the EOPD/LOPD group, while CD27 (CD56+, CD16+, CD57+, and CD28–) clusters were significantly higher only in the LOPD group. Cytotoxic CD29 (CD56+, CD16+, CD57+, and CD28+) clusters were lower in the LOPD group when compared to the control group, with no statistical difference between the groups. Furthermore, it was found that C27 cell clusters increased as the disease was prolonged in LOPD (Tian et al., 2022).

In addition, the disease severity may also be associated with the NK cells. Two studies explored the relationship between NK cells and disease progression measured by UDPRS and Mini-Mental State Examination (MMSE) scales. According to Niwa et al., the percentage of CD16+ cells and CD56+ cells was not correlated with the UPDRS ($p > 0.1$) and UPDRS-III scores ($p > 0.1$) in the 29 patients diagnosed with sporadic PD (Niwa et al., 2012). However, an increase in C32 clusters was found to correspond to increased UPDRS and UPDRS-III scores but decreased MMSE scores in the patients with PD (Tian et al., 2022).

In addition, other markers of NK cells were found to have an impact on the clinical manifestations. Using the bioinformatics tools, Anderson et al. analyzed the interaction between polymorphic killer immunoglobulin-like receptors (KIR) and human leukocyte antigen (HLA) class I ligands in patients with PD (Anderson et al., 2020). KIR3DL1, a member of the KIR family, conducts inhibitory signals depending on two immunoreceptor tyrosine-based inhibition motifs (ITIM) and binds to HLA-B molecules containing the Bw4 phenotype. The researchers found that high expression of KIR3DL1 alleles in combination with HLA-Bw4/Bw4i reduced gait difficulties and rigidity in patients, while weak KIR3DL1/HLA interactions were found to be associated with a higher risk of rigidity. Thus, they speculated that high expression levels of inhibitory KIR to reduce NK cell-mediated inflammation may reduce the severity of symptoms and have a protective effect in PD.

DISCUSSION

This is the first systematic review to evaluate the influence of NK cells in the development of Parkinson's disease based on the data from both foundation medicine and clinical medicine, and several issues should be considered critically and seriously.

TABLE 1 | Main findings of animal studies.

No	Author	Year	Animals	Models	Main results	PMID
1	Earls	2019	C57BL/6J mice	PFF α-syn injection model	• Relative percentage of NK cells in PFF α-syn mice higher than monomer controls at 5 months; • Decreased numbers of NK cells in the blood of PFF α-syn mice compared with monomer controls; • No major changes in cytokines or chemokines in the serum of PFF α-syn mice compared with the monomer controls	31796095
2	Earls	2020	Transgenic mice (M83) overexpressing human A53T α-syn mutant protein	PFF α-syn injection model	Using the PFF α-Syn Mouse Model • NK Cells are found in the brains of the model; • NK cell depletion increased hind limb clasping and exacerbated motor deficits and motor function in the mice; • NK cell-deficient mice displayed significantly increased p-α-syn inclusions within the striatum, SNpc and brainstem; • Using the human NK Cell • NK92 cells efficiently internalized various sizes of α-syn (monomers, oligomers, and higher molecular weight fibrils) in a dose-dependent manner; • NK cells internalized α-syn in the cytoplasmic compartment and α-syn colocalized with both Rab7 and LC3B (the endosome and lysosome pathway marker); • Only α-syn aggregates significantly attenuated NK cell cytotoxicity in a dose-dependent manner; • Extracellular α-syn aggregates significantly decreased IFN-γ secretion in NK 92 cells; • The levels of NKG2A, and NKG2D receptors on NK cells were not altered by extracellular α-syn aggregate treatments;	31900358
3	Grembecka	2021	Wistar rats	6-OHDA injection model	• The NKCC was higher in 6-OHDA groups (dopamine depleted groups) in comparison to controls using a 51Cr release assay; • It seems that percentage of NK(CD3- CD161a+) cells in PBMC between 6-OHDA and control groups didn't show a significant difference	32648088

NK Cell Numbers and NKCC in PD

Evidence showed that NK cells were found in the brains of patients with PD (Earls et al., 2020), and the percentage of NK cells increased in the CNS parenchyma prior to the dopaminergic neuronal degeneration (Earls et al., 2019), which indicated infiltration of NK cells in the CNS during PD progression. From the point of ethics and experimental convenience, NK cells in the peripheral blood were appropriate and easy to obtain and could also give us information about pathogenesis. However, analysis of the cell counts and cell cytotoxicity had produced some contradictory results in both clinical studies and animal studies.

Most groups reported increased NK cells (Mihara et al., 2008; Niwa et al., 2012; Cen et al., 2017; Sun et al., 2019; Huang et al., 2021; Tian et al., 2022) in patients, while some researchers showed that the number of NK cells did not change significantly when compared to the corresponding controls (Stevens et al., 2012; Earls et al., 2019; Grembecka et al., 2021). We speculated that cohort/model differences, PD subtype, disease duration, or time delay between the clinical measurements of the disease and the pathological changes are the possible influencing factors, and the mechanisms underlying altered immune profiles still need to be explored.

Moreover, peripheral blood mononuclear cells (PBMCs) were shown as a basic reference in most studies. PBMCs are cell groups that include blood cells with a round nucleus (i.e., lymphocytes, monocytes, NK cells, or dendritic cells) (Sen et al., 2017). Among the PBMCs, components other than NK cells may also be altered in the pathological process of PD. For example, changes in monocyte subpopulations were observed in the early and late PD (Harms et al., 2021; Tian et al., 2022). Therefore, it might be better to measure the absolute value of the NK cells for comparison rather than considering only the percentage of cells.

As for the NKCC, Huang et al. confirmed enriched NK cell-mediated cytotoxic pathways in the peripheral blood using Gene Set Enrichment Analysis (GSEA), supporting a disease-associated cytotoxic response of NK cells in PD (Huang et al., 2021). Also, NK cells may be actively involved in the acute injury of dopaminergic neurons, since increased peripheral NKCC was found in rats injected with 6-OHDA for several weeks (Grembecka et al., 2021). However, some studies revealed no significant changes in NKCC targeting the K562 cells lacking

TABLE 2 | Basic information of clinical studies.

No	Author	Year	Subjects	Gender (M/F)	Age (means±SD)	Disease duration (means±SD)	Disease severity (means±SD)	PMID
1	Mihara	2008	20 patients 20 HC	PD: 9/11 HC: 11/9	PD: 70.7 ± 7.8 HC: 67.6 ± 9.4	5.4 ± 3.4	H&Y score: 2.9 ± 0.8	17702627
2	Niwa	2012	29 patients 30 HC	PD: 17/12 HC: 16/14	PD: 70.4 (mean) HC: 68.9 (mean)	6.38 ± 4.07	H&Y score: 2.63 ± 0.92 UPDRS III score: 23.92 ± 15.81	21929737
3	Stevens	2012	88 patients 77 HC	PD: 56/32 HC: 39/38	PD: 69 ± 9 HC: 67 ± 10	6 ± 5	H&Y score: 2 ± 0.7 UPDRS III score: 21 ± 10	22910543
4	Cen	2017	268 patients 268 HC	PD: 156/112 HC: 168/100	PD: 60.59 ± 11.11 HC: 59.41 ± 11.11	EOPD: 3.94 ± 2.32 LOPD: 4.95 ± 4.10	H&Y score: EOPD: 1.47 ± 0.51 LOPD: 1.93 ± 0.88 UPDRS III score: EOPD: 11.00 ± 4.63 LOPD: 12.89 ± 7.88	28791571
5	Sun	2019	127 patients 148 HC	PD: 75/52 HC: 84/64	PD: 62.7 ± 12.5 HC: 60.3 ± 13.4	4.4 ± 4.1	UPDRS III score: 17.9 ± 9.2	31930038
6	Anderson, K. M.	2020	1,314 patients 1,978 HC	-	-	-	-	32709660
7	Huang	2021	205 patients 233 HC	-	-	-	-	34483877
8	Konstantin Nissen	2022	78 patients 28 HC	PD: 39/39 HC: 15/13	age at visit: PD: 66.6 ± 9.5 HC: 64.2 ± 7.4 age at onset: PD: 60.4 ± 9.2	4.5 (IQR: 3-9)	H&Y score: 2 (median) UPDRS III score: 27.84 ± 14.29 MoCA: 26 (median)	35026420
9	Tian	2022	22 patients 18 HC	PD: 12/10 HC: 13/5	EOPD: 40.30 LOPD: 64.23 YHC: 40.30 EHC: 68.25	EOPD: 2.44 LOPD: 1.8	H&Y score: EOPD: 1.31 LOPD: 1.44 UPDRS III score: EOPD: 15.38 LOPD: 21.63	35013369

ligands of the inhibitory NKG2A receptors (Mihara et al., 2008; Niwa et al., 2012). Given the fact that alterations in the expression levels of NKG2A receptors on NK cells were inconsistent between different studies (Mihara et al., 2008; Niwa et al., 2012), a possible approach involving the measurement of NKCC activity would be an influencing factor in the final results.

Notably, researchers for the first time observed that extracellular α-syn aggregates are internalized and degraded in the NK cells, and a more intriguing finding is that NKCC decreased obviously in a dose-dependent manner after the interaction between α-syn aggregates and NK cells (Earls et al., 2020). It seemed that the internalization of α-syn modulated the cytotoxicity of NK cells in a non-activated way. Recent studies have demonstrated that microglia can ingest and degrade extracellular α-syn with high efficiency, but consequently, α-syn fibrils are likely to induce apoptosis of microglia by increased reactive oxygen species (ROS) production and mitochondrial network disintegration (Choi et al., 2020; Scheiblich et al., 2021). In this case, whether α-syn exhibits toxicity in the NK cells and how α-syn aggregates affect the NKCC after the phagocytosis are still unclear.

In addition, researchers also observed increased CD16 expression on mature NK cells and speculated that an enhanced ADCC effect was primed in patients with PD (Konstantin Nissen et al., 2022). Since information about antibodies and target cells is unknown, cytotoxicity through ADCC is not measured in an intuitive way, and hence more number of experiments are needed to prove this hypothesis. Therefore, some doubts remain about the measurement of NKCC. To further elucidate these findings, mechanisms and further validations are encouraged to identify the cytotoxicity of NK cells more accurately and comprehensively in the dynamic development of PD in patients.

NK Cells and Age at Onset in PD

Previous studies demonstrated that older age at onset was associated with a more severe motor phenotype and higher H&Y stage and UPDRS-III score, and similar results were observed by Cen et al. and Tian et el. (Szewczyk-Krolikowski et al., 2014; Pagano et al., 2016). Possibly, it can be explained by a greater dopaminergic dysfunction in patients with older age at onset, but the mechanisms are still unclear. Evidence suggests that genetic variation plays an important role in determining the age of onset

TABLE 3 | Main findings of clinical studies.

No	Author	Main results
1	Mihara	• In peripheral lymphocytes: the percentage of NK cells (CD3-CD56+) of the PD group was higher • NK activity was not significantly different between the PD and HC groups; • The correlation between NK activity of PD patients and disease duration was positive; • The percentage of NKG2A+ cells among CD3-CD56+ NK cells in the PD group was statistically lower; • No significant difference between both groups in the percentage of NKG2D+ cells amongCD3-CD56+ NK cells
2	Niwa	• In peripheral lymphocytes: the percentage of CD16+ and CD56+ cells was significantly higher in patients with PD • No significant difference between both groups in the NK cell activity; • The percentages of CD16+ and CD56+ cells were not correlated with the UPDRS and UPDRS III score;
3	Stevens	In PBMC: no changes in the numbers of CD56+ cells in PD patients
4	Cen	In PBMC: • The percentage of NK cells in peripheral blood from PD patients was higher than that from the control group; • The percentage of NK cells did not differ between the early-onset groups and the late-onset group
5	Sun	In peripheral blood lymphocytes: • The percentage of NK cells (CD3-CD16+CD56+) in PD patients was significantly higher than that in healthy controls; • People with NK cells deviating from the reference range had an increased risk of PD
6	Anderson, K. M.	• High expression KIR3DL1 alleles in combination with HLA-Bw4/Bw4i reduce gait difficulties and rigidity in Parkinson's disease; • Weak KIR3DL1/HLA interactions associate with higher risk of rigidity and lower risk of resting tremor; • High-expressing KIR3DL1*002 is at higher frequency in patients who present with symptoms related to movement
7	Huang	• The fraction of resting NK cells in PD was significantly higher • NK cell mediated cytotoxic pathways were enriched in PD; • The mRNA level of prostaglandin D2 synthase (PTGDS) was positively correlated with the fraction of resting NK cells
8	Konstantin Nissen	An increase in CD16 median fluorescence intensity (MFI) on the mature NK cells(CD3-CD56^{dim}CD16+)during early PD (within 5 years of diagnosis);
9	Tian	In PBMC: • C32 (CD56+CD16+CD57-CD28-) cluster and C27 (CD56+CD16+CD57+CD28-) cluster increased in PD patients compared to those in HCs and both increased in patients with LOPD compared to those in EHCs; • C29 (CD56+CD16+CD57+CD28+) cluster was lower in patients with PD with no significant difference • Increase in C32 NK cells was associated with increased UPDRS and UPDRS-III scores, but decreased MMSE scores of PD patients;

of PD (Nalls et al., 2015). GWAS has implicated that SNCA, TMEM175, and GBA are associated with PD age at onset and are also implicated in α-syn aggregation pathways (Blauwendraat et al., 2019), which means that accumulation and clearance of α-syn are likely to be involved in the pathogenesis of age at onset. Besides, differences in the immune status exist in the patients with early- and late-onset PD (Tian et al., 2022). As for NK cells, functions alter with the differential expression of molecules on the cell surface in the relevant development stages and can be affected by various immune responses (Yu et al., 2013; Abel et al., 2018). It is possible that NK cells are related to age at onset in patients with PD.

Tian et al. analyzed peripheral mature NK cells marked by CD16 and CD56, and further distinguished cell maturity by expression of CD57 (a marker of terminal maturation) and CD28 (critical for co-stimulation of the cells to proliferate and produce IFN-γ) (Tian et al., 2022). Cytotoxicity of cells marked by CD57+CD28+ (CD29 clusters) is higher than those marked by CD57+CD28- (CD27 clusters) and CD57-CD28- (CD32 clusters). Interestingly, the researchers found higher CD27 and CD32 cell clusters and lower CD29 clusters in LOPD, while only higher CD32 clusters were noticed in EOPD, which suggested that age at onset might be associated with the development and toxicity alterations of NK cells in PD. Furthermore, considering that aging is one of the main risk factors for PD (Pagano et al., 2016; Poewe et al., 2017), it is crucial to control the variables such as age-matched healthy controls in the study design involving comparison between EOPD and LOPD.

NK Cells and Clinical Symptoms in PD

NK cells are essential effectors in innate immunity, since NK cells can infiltrate the CNS during PD progression (Earls et al., 2020). However, whether there are pathophysiological reflections on the clinical motor and non-motor symptoms is an important issue for us to understand the role of NK cells in PD. Tian et al. observed less toxic NK cells increased with more severe motor and cognitive dysfunctions based on the evaluation of UPDRS-III and MMSE scales (Tian et al., 2022). Anderson et al. found that high expression of inhibitory KIR on NK cells might protect against severe motor symptoms in patients (Anderson et al., 2020). Additionally, in the PFF α-syn model, increased motor symptoms, exacerbated motor deficits, and increased p-α-syn

inclusions were observed due to NK cell depletion (Earls et al., 2020). Therefore, we speculated that NK cells carry out killing and phagocytosis in a less toxic or non-hyperactivated way, which tend to play a protective role in the progression of PD.

However, previous studies have demonstrated that NK cells likely act as a double-edged sword in central nervous system disorders (Poli et al., 2013, 2018). On the one hand, they can induce the activation of neural cell death (Garofalo et al., 2020), while on the other hand, they have the ability to suppress CNS inflammation (Hao et al., 2010). In addition, immune dysfunction is quite complex in the development of PD. Pro-inflammatory or anti-inflammatory cytokines and cells, such as microglia, monocytes, T cells, B cells, and so on, are undoubtedly important participants in the pathological process and correlate with disease severity and disability in patients with PD (reviewed in detail in: Qin et al., 2016; Chen et al., 2018; Sabatino et al., 2019; Harms et al., 2021). However, only a few studies have explored their interactions with NK cells in PD till now, so the detrimental effect of NK cells is still uncertain and needs further investigation.

Confounding Factors Associated With the Function of NK Cells in PD

Drugs are the influential factors in the study design. Basu et al. reviewed that dopamine and its receptor agonists altered the function of NK cells, for example, administration of dopamine increased the killing ability of specific NK cells (Basu and Dasgupta, 2000). Thus, data collection may be better in newly diagnosed PD patients to reduce the effects of dopamine replacement therapies. Additionally, neurotransmitters and neuroendocrine factors, such as glucocorticoids, serotonin, and epinephrine, have been reported to affect the activities of NK cells through a complex communication between the immune system and the nervous system (Capellino et al., 2020).

LIMITATIONS

While we included the available studies on the relevant topic and systematically summarized the main results, there are some limitations in the article. First, heterogeneity of the inclusion criteria and experimental methods hindered a meta-analysis of the data, so we chose to report a descriptive analysis of our findings, rather than the quantitative conclusions. Second, a small sample size was found in most of the included studies, which reduced the reliability of the corresponding results. Third, the quality of some included studies was affected by several factors, such as lack of information on the basic characteristics of the patients, the absolute count of the measurement, and so on. Furthermore, publication bias toward studies with positive clinical outcomes cannot be ruled out.

FUTURE DIRECTIONS AND CONCLUSION

In the future, we hope that more promising translational therapeutic targets for PD will emerge with the conducting of extensive research on the essential pathological and physiological changes. It is known that NK cells can launch a rapid attack the next time they encounter the same antigen through antigen-mediated immune memory, and they can then present strong killing functions when perceiving a similar inflammatory environment. In addition, studies have shown that CNS-derived antigens can trigger neuroinflammation responses in PD. Therefore, there is a possibility that memory NK cells exist in the PD progression, and it is quite interesting to be explored in the future. In addition, NK cells have been reported to be associated with cognitive decline due to some complex mechanisms. Since non-motor symptoms, such as cognitive deficits, also have a certain impact on the life quality of patients with PD, looking for connections between NK cells and the non-motor symptoms is worthwhile in the basic experiments and clinical practice.

Overall, we reviewed the pathologic role of NK cells in Parkinson's disease systematically and summarized the characteristics of NK cell numbers, NKCC, and other surface molecules on the NK cells in patients with PD and animal models. Further, we analyzed the relationship between NK cells and the clinical manifestations in PD and speculated a probable neuroprotective role of NK cells. We hope that we have provided the necessary impetus for continued studies on the functions of NK cells in PD, and more potential biomarkers or targets will spring up during the diagnosis and treatment of PD in the future.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

LZ, YZ, and DF: conceptualization. LZ and YZ: methodology and data curation. LZ: writing—original draft preparation. YZ and DF: writing—review and editing. All authors have read and agreed to the published version of the manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnagi.2022.890816/full#supplementary-material

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ORIGINAL RESEARCH
published: 13 June 2022
doi: 10.3389/fnagi.2022.900773

Phosphorylated Tau 181 Serum Levels Predict Alzheimer's Disease in the Preclinical Stage

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Background: There is an urgent need for cost-effective, easy-to-measure biomarkers to identify subjects who will develop Alzheimer's disease (AD), especially at the pre-symptomatic stage. This stage can be determined in autosomal dominant AD (ADAD) which offers the opportunity to observe the dynamic biomarker changes during the life-course of AD stages. This study aimed to investigate serum biomarkers during different AD stages and potential novel protein biomarkers of presymptomatic AD.

Methods: In the first stage, 32 individuals [20 mutation carriers including 10 with AD, and 10 with mild cognitive impairment (MCI), and 12 healthy controls] from ADAD families were analyzed. All subjects underwent a complete clinical evaluation and a comprehensive neuropsychological battery. Serum samples were collected from all subjects, and antibody arrays were used to analyze 170 proteins in these samples. The most promising biomarkers were identified during this screening and were then measured in serum samples of 12 subjects with pre-MCI and 20 controls.

Results: The serum levels of 13 proteins were significantly different in patients with AD or MCI compared to controls. Of the 13 proteins, cathepsin D, immunoglobulin E, epidermal growth factor receptor (EGFR), matrix metalloproteinase-9 (MMP-9), von Willebrand factor (vWF), haptoglobin, and phosphorylated Tau-181 (p-Tau181) correlated with all cognitive measures (R^2 = −0.69–0.76). The areas under the receiver operating characteristic curve of these seven proteins were 0.71–0.93 for the classification of AD and 0.57–0.95 for the classification of MCI. Higher levels of p-Tau181 were found in the serum of pre-MCI subjects than in the serum of controls. The p-Tau181 serum level might detect AD before symptoms occur (area under the curve 0.85, sensitivity 75%, specificity 81.67%).

Conclusions: A total of 13 serum proteins showed significant differences between subjects with AD and MCI and healthy controls. The p-Tau181 serum level might be a broadly available and cost-effective biomarker to identify individuals with preclinical AD and assess the severity of AD.

Keywords: Alzheimer's disease, preclinical stage, phosphorylated tau 181, serum, biomarker

OPEN ACCESS

Edited by:
Chao Wang,
Chongqing Medical University, China

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Heng Du,
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Specialty section:
This article was submitted to Alzheimer's Disease and Related Dementias, a section of the journal Frontiers in Aging Neuroscience

Received: *21 March 2022*
Accepted: *10 May 2022*
Published: *13 June 2022*

Citation:
Qin W, Li F, Jia L, Wang Q, Li Y, Wei Y, Li Y, Jin H and Jia J (2022) Phosphorylated Tau 181 Serum Levels Predict Alzheimer's Disease in the Preclinical Stage. Front. Aging Neurosci. 14:900773. doi: 10.3389/fnagi.2022.900773

INTRODUCTION

Alzheimer's disease (AD) is the most common form of dementia among the elderly globally. Studies indicate that the brain pathology of AD starts to develop at least 10–20 years before the disease becomes clinically symptomatic (Bateman et al., 2012). This provides a window of opportunity to initiate preventive treatment. There is a need to identify widely available, easy-to-measure, and cost-effective biomarkers to identify AD in this pre-symptomatic stage (Molinuevo et al., 2018). Although autosomal dominant AD (ADAD) represents fewer than 1% of all AD cases, it provides a unique opportunity to investigate biomarker levels during this stage because the associated mutations are almost 100% fully penetrant, and symptom onset is relatively predictable in mutation carriers (Bateman et al., 2011; Sanchez-Valle et al., 2018).

Cerebrospinal fluid (CSF) biomarkers have shown strong correlations with clinical and cognitive measures in ADAD (Fagan et al., 2014). However, repeated CSF sampling is neither feasible nor cost-effective. The determination of serum biomarkers is less invasive, less costly, and can be performed more frequently than CSF investigations (Hampel et al., 2018; Zetterberg and Blennow, 2020).

Some studies have suggested that energy metabolism disorders, vascular alteration microenvironment hypoxia, oxidative stress, cell death, and chronic inflammation are also major contributors to the cognitive decline and neurodegenerative disorders associated with AD (Custodia et al., 2022; Yassine et al., 2022).

In this study, a custom protein chip was developed using 170 candidate biomarkers that have been implicated in AD. These proteins included synaptic proteins, inflammation factors, circulating cytokines, chemokines, and growth factors, et al. Then these protein levels were analyzed in serum samples from subjects in different stages of AD within the Chinese Familial Alzheimer's Disease Network. We also examined the relationship between potential candidate biomarkers and cognitive function measures such as MMSE scores. We aimed to establish the characteristic serum protein profiles in different stages of AD and potential novel protein biomarkers of presymptomatic AD.

MATERIALS AND METHODS

Study Design and Setting

All subjects of this study were selected from the Chinese Familial Alzheimer's Disease Network (CFAN), which is a multicenter, longitudinal cohort of familial AD (Jia et al., 2020, 2021). They were consanguineous members of families with mutations in the genes encoding the amyloid-beta precursor protein (*APP*), presenilin-1 (*PSEN1*), or presenilin-2 (*PSEN2*, Swardfager et al., 2010). Subjects who carried the mutations were identified, and family members that did not carry a mutation served as controls. This retrospective study consisted of two stages. In the first stage, we analyzed 170 protein levels using an antibody array in serums from 10 AD subjects, 10 MCI subjects, and 12 controls. In the second stage, we selected some proteins and assessed their abilities to distinguish patients with pre-MCI from controls using ELISA. The selection criterion is: the significantly different levels of proteins between control and AD/MCI, significant associations with all cognitive measures, and moderate or high accuracy in predicting MCI and AD (AUC > 0.8, sensitivity > 80%, and specificity > 80%). Twelve pre-MCI subjects and 20 controls were included.

The study protocol was approved by the Ethics Committee of Xuanwu Hospital, Capital Medical University, and all subjects gave written informed consent.

Clinical and Cognitive Assessment

The participants recruited in CFAN underwent a complete clinical evaluation, the tests of the known causative AD genes, and a comprehensive neuropsychological battery. The diagnosis of AD or MCI related to AD was made according to the National Institute on Aging and Alzheimer's Association (NIA-AA) criteria (Albert et al., 2011; McKhann et al., 2011). Subjects were classified as having pre-MCI if they carried one of the mutations, had no cognitive complaints, and a normal cognitive performance. All MCI patients had a "high likelihood" of developing AD according to the NIA-AA criteria (i.e., meeting the core clinical criteria for MCI plus carrying autosomal dominant mutations; Albert et al., 2011). All AD patients were diagnosed with "probable AD dementia (probable AD dementia in a carrier of an ADAD genetic mutation; McKhann et al., 2011). The controls were cognitively normal and had neither amnesia nor did they carry mutations in genes related to AD.

The neuropsychological battery assessed the cognitive domains of verbal ability, visuospatial construction, episodic memory, and executive functions. Cognitive progression was measured using the Mini-Mental State Examination (MMSE), Montreal Cognitive Assessment (MoCA), activities of daily living (Newman et al., 2021) and Clinical Dementia Rating (CDR). Both the MMSE and the MoCA are routine cognitive screening tests rated on a 30-point scale. An MMSE score or a MoCA score below the cutoff was used to classify patients as having a cognitive impairment (lower scores indicating greater impairment; Chen et al., 2016; Li et al., 2016). The cutoff varies with different education levels. The score on the ADL ranges from 0 to 80, with a lower score indicating a better functional independency (Mlinac and Feng, 2016). The algorithm-generated global CDR score produces a total possible score of 0–3, connoting a global level of functional status from no cognitive impairment (CDRglobal 0) to severe impairment (CDRglobal 3; Morris, 1993). The CDR sum of boxes score (CDRsob), by contrast, utilizes a summary of the individual domain box scores and yields a total score of 0–18 (higher scores indicating greater impairment), and is frequently used in dementia staging and tracking of progression over time (O'Bryant et al., 2008).

Genetic Screening

Serum samples analyzed in this study were obtained from this cohort CFAN. As a part of the routine assessment, genomic DNA was extracted from the peripheral blood samples as described previously (Qin et al., 2011). Exons 3–12 of the *PSEN1*, exons 1–12 of the *PSEN2*, and exons 16 and 17 of the *APP* genes were

amplified using polymerase chain reaction (PCR) and specific primers (see Additional file 1, **Supplementary Table S1**) and determined by Sanger sequencing. AD and MCI patients were screened for mutations in the *PSEN1*, *PSEN2*, and *APP* genes, whereas other family members were screened for the mutation segregating in their family using Sanger sequencing to identify their mutation status. *Apolipoprotein E* (*APOE*) genotypes were also determined by Sanger sequencing.

Antibody Arrays

We measured the relative concentrations of a total of 170 proteins (see Additional file 1, **Supplementary Table S2**) with antibody arrays (RayBiotech Inc., Peachtree Corners, GA, USA) according to the manufacturer's instructions. Briefly, a custom glass-based antibody array targeting the 170 proteins of interest was built, 100 μl of diluted serum sample was added to each well, incubated overnight at 4°C, and then extensively washed. We then incubated the wells with biotin-conjugated antibodies specific to the different proteins. Membranes were developed with Alexa Fluor® 555-conjugated streptavidin (Thermo Fisher Scientific, Waltham, MA, USA). The slides were scanned with 532 nm excitation and extracted using an InnoScan® 300 Scanner (Innopsys, Carbonne, France).

Enzyme-Linked Immunosorbent Assay

The selected proteins were further tested in samples of pre-MCI and control subjects using enzyme-linked immunosorbent assays in the second stage. The assay kit included Human Cathepsin D DuoSet (DY1014-05, R&D Systems, Inc., Minneapolis, MN, USA), von Willebrand factor (vWF; Human vWF-A2 DuoSet, DY2764-05, R&D Systems, Inc., Minneapolis, MN, USA), and (Human Tau pT181 ProQuantum, A46739, Invitrogen Corp., Carlsbad, CA, USA). All serum samples and kit components were equilibrated to room temperature before the assay, and the detection procedures were performed in accordance with the manufacturers' instructions. For cathepsin D and vWF detection, the serum samples were diluted, added to separate wells, and incubated with a sealed plate. After conjugation and washing with buffer, substrate solutions were added, and the wells were incubated for 30 min. Finally, a stop solution (Invitrogen Corp.) was added to stop the reaction, and the optical density was measured at 450 nm. For P-tau181 detection, the antibody-conjugate mixture and diluted samples were added to assay wells. After mixing thoroughly, they were incubated overnight at 4°C. Then quantitative PCR reactions were performed on the StepOnePlus™Real-Time PCR System (Thermo Fisher Scientific, Waltham, MA, USA). Concentrations were calculated according to standard curves. Standard samples containing the recombinant proteins, subjects' serum samples, and empty controls were all assayed in duplicates to reduce variation.

Functional Profiling of the Identified Proteins

Gene ontology (GO) enrichment analyses were conducted to assess the enrichment of these proteins in specific biological processes, molecular functions, and cell components. We used the Kyoto Encyclopedia of Genes and Genomes (KEGG) database to analyze the pathway enrichment of these proteins, and obtained an overview of their potential functional relevance affected by AD. Gene Ontology enrichment analysis, Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis, and the protein-protein interaction network were conducted in Metascape[1] (Zhou et al., 2019).

Statistical Analysis

Expression data from the two filters per sample were normalized to the median expression of all 170 proteins, followed by *Z*-score transformation (Ray et al., 2007).

Differences in categorical data between the groups, such as sex, and *APOE* ε4 carrier distributions, were analyzed using the χ^2 test. Differences in numerical data between the groups were evaluated using analysis of variance with Bonferroni *post-hoc* tests. Multiple linear regression analyses were used to assess potential associations between serum proteins and

[1]http://metascape.org/

TABLE 1 | Demographic and clinical characteristics of subjects within the Alzheimer's disease (AD), mild cognitive impairment (MCI), and control groups.

	Cohort 1				Cohort 2		
	Control	**MCI**	**AD**	***P* values**	**Control**	**pre-MCI**	***P* values**
N = 40	12	10	10	-	20	12	-
Age (years), mean (SD)	44.83 (12.95)	48.30 (5.14)	50.90 (5.67)	0.30	45.25 (11.51)	32.25 (5.26)	<0.01
Sex, M/F	6/6	9/1	4/6	0.05	12/8	4/8	0.27
MMSE, mean (SD)	29 (1.41)	24 (5.46)	12 (5.37)	<0.01	29 (1.36)	28 (2.56)	0.98
MoCA, mean (SD)	26 (3.01)	21 (4.10)	7 (3.92)	<0.01	27 (2.93)	26 (2.37)	0.37
CDR, mean (SD)	0	0.5 (0.24)	2 (0.94)	<0.01	0	0	-
CDR-SOB, mean (SD)	0	1.85 (1.83)	10.05 (5.25)	<0.01	0	0.06 (0.18)	0.17
APOE ε4 carrier	2 (16%)	2 (20%)	4 (40%)	0.41	3 (15%)	2 (17%)	0.90

MMSE, Mini-Mental State Examination; MoCA, Montreal cognitive assessment; ADL, activities of daily living; CDR, Clinical Dementia Rating; CDRsob, Clinical Dementia Rating sum of boxes; SD, standard deviation.

cognitive measures after adjusting for confounders. Receiver operating characteristic (ROC) curves were drawn by plotting the sensitivity against 1-specificity for different cut-off values. The area under the curve (AUC) was calculated for each. GraphPad Prism statistical software (version 8.1.1, GraphPad Software, San Diego, CA, USA) was used for analyses. Statistical significance was based on two-sided tests with an adjusted P-value < 0.05.

RESULTS

Study Participants

Table 1 shows the demographic and clinical characteristics of the enrolled individuals. Among the 20 symptomatic mutation carriers, 10 fulfilled the criteria of AD and 10 of MCI. The 20 mutation carriers showed 13 different mutations (number of subjects): F105I (n = 2), G378E (n = 1), H163R (n = 3), L282V (n = 1), L392V (n = 1), M139V (n = 1), G111V (n = 1), M139L (n = 2), and P433S (n = 1) mutations in the *PSEN1* gene, R62H (n = 1) and V214L (n = 1) mutations in the *PSEN2* gene, and V717I (n = 4) and I716T (n = 1) mutations in the *APP* gene (see Additional file 1, **Supplementary Table S3**). The second stage included 12 pre-MCI mutation carriers, and 20 controls. Twelve pre-MCI participants were mutation carriers with a known causative mutation of AD, including six carrying *PSEN1* mutation, four carrying *APP* mutation, and two carrying *PSEN2* mutation. Twenty controls were healthy non-carrier family members. The frequency of the *APOE* ε4 allele was higher in the AD and MCI groups than in the controls. The demographic data showed expected diagnosis-related cognitive characteristics with respect to MMSE, MoCA, CDRsob, and CDRglobal scores.

Serum Proteins in Different Diagnostic Groups

Among the 170 proteins analyzed, we identified 13 proteins that were differentially expressed in the three groups after adjusting for gender and age (**Figure 1**). Brain-derived neurotrophic factor (BDNF) levels were significantly downregulated in the serum of MCI and AD patients compared to those in the controls. Significant higher cathepsin D, immunoglobulin E (Chen et al., 2020), neuropilin-1, angiopoietin-2 (ANG-2), coagulation factor XI (FXI), epidermal growth factor receptor (EGFR), vascular endothelial growth factor A (VEGFA), intercellular adhesion molecule 1 (ICAM-1), matrix metalloproteinase-9 (MMP-9), von Willebrand factor (vWF), haptoglobin, and p-Tau181 levels were found in the serum samples of AD subjects than in the samples of controls (**Figure 2**). FXI, EGFR, VEGFA, ICAM-1, haptoglobin, and p-Tau181 levels were also significantly different in the MCI vs. control group comparison (**Figure 2**). No significant differences in these protein levels were observed between MCI and AD subjects.

Serum Proteins and Clinical Cognition

The correlations between serum proteins and cognitive measures are shown in **Figure 3**. Seven of the thirteen proteins were significantly correlated with all cognitive measures. Cathepsin D, IgE, EGFR, MMP-9, vWF, haptoglobin, and p-Tau181 showed a negative correlation with the Mini-Mental State Examination (MMSE; R^2 = −0.59–0.45) and the Montreal cognitive assessment (MoCA) scores (R^2 = −0.64–0.44), and positive correlations with the activities of daily living (Newman et al., 2021; R^2 = 0.50–0.65), CDRglobal (R^2 = 0.39–0.69) or the CDR sum of boxes (CDRsob) scores (R^2 = 0.36–0.61). The higher serum Cathepsin D, IgE, EGFR, MMP-9, vWF, haptoglobin, and p-Tau181 levels were associated with severity of memory impairment (as indicated by lower MMSE and MoCA scores, and higher ADL, CDRglobal, and CDRsob scores). These results suggest a possible link between these serum proteins and cognitive decline.

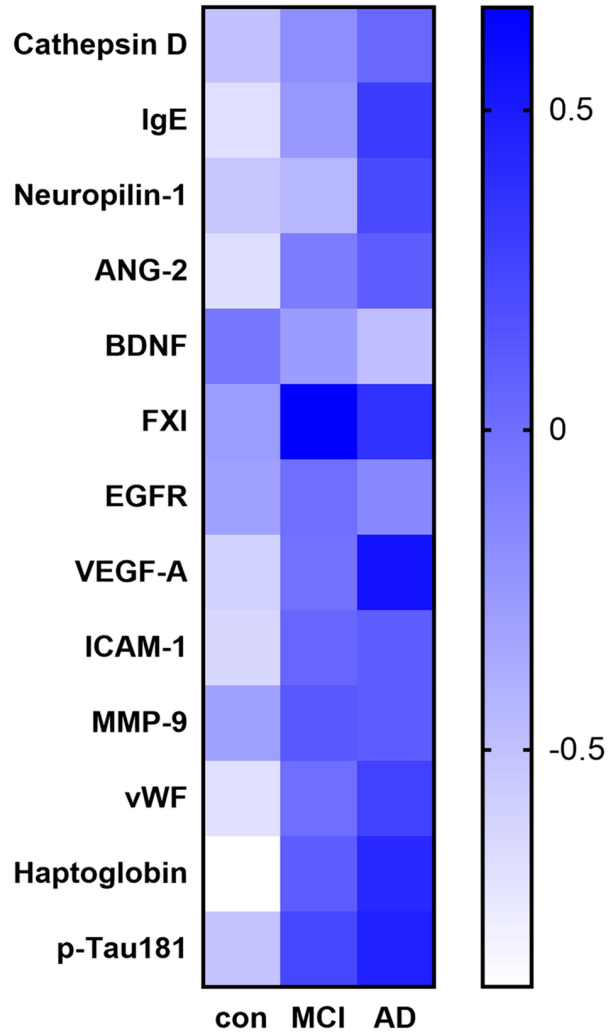

FIGURE 1 | Heat map of the identified potential biomarker proteins. The heatmap shows the overall expression of the 13 proteins with a significant difference in their serum levels (adjusted $P < 0.05$) between subjects with Alzheimer's disease (AD) or mild cognitive impairment (MCI) and the controls.

Functional Profiling of the Identified Serum Proteins

Gene ontology analyses indicated an involvement of the 13 proteins in the regulation of cell growth and the regulation

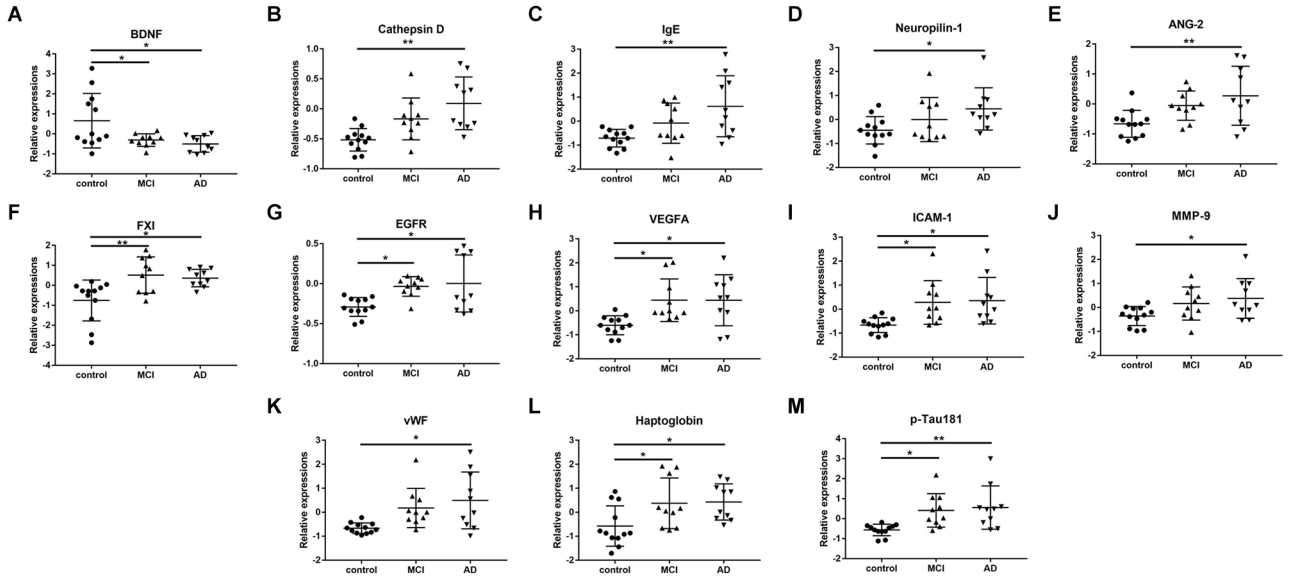

FIGURE 2 | The scatter plots showed the detail comparisons of serum levels of identified potential biomarker proteins between the control, mild cognitive impairment (MCI), and Alzheimer's disease (AD) groups. **(A)** Brain derived neurotrophic factor (BDNF); **(B)** Cathepsin D; **(C)** Immunoglobulin E (Chen et al., 2020); **(D)** Neuropilin-1; **(E)** Angiopoietin-2 (ANG-2); **(F)** Coagulation factor XI (FXI); **(G)** Epidermal growth factor receptor (EGFR); **(H)** Vascular endothelial growth factor A (VEGFA); **(I)** Intercellular adhesion molecule -1 (ICAM-1); **(J)** Matrix metalloproteinase-9 (MMP-9); **(K)** von Willebrand factor (vWF); **(L)** Haptoglobin; **(M)** Phosphorylated Tau-181 (p-Tau181). $^{*}P < 0.05$, $^{**}P < 0.01$.

	Cathepsin D	IgE	Neuropilin-1	ANG-2	BDNF	FXI	EGFR	VEGFA	ICAM-1	MMP-9	vWF	Haptoglobin	p-Tau181
MMSE	-0.59(0.00)	-0.49(0.004)	-0.32(0.07)	-0.49(0.005)	0.29(0.11)	-0.38(0.03)	-0.54(0.002)	-0.45(0.01)	-0.21(0.26)	-0.47(0.01)	-0.45(0.01)	-0.47(0.01)	-0.50(0.004)
MoCA	-0.64(0.00)	-0.50(0.004)	-0.34(0.06)	-0.47(0.009)	0.39(0.03)	-0.43(0.02)	-0.56(0.001)	-0.41(0.02)	-0.28(0.12)	-0.45(0.01)	-0.44(0.01)	-0.45(0.01)	-0.50(0.003)
ADL	0.64(0.001)	0.55(0.007)	0.27(0.22)	0.37(0.09)	-0.42(0.04)	0.46(0.03)	0.65(0.001)	0.52(0.01)	0.50(0.02)	0.65(0.001)	0.61(0.002)	0.57(0.01)	0.51(0.01)
CDRglobal	0.62(0.00)	0.54(0.001)	0.25(0.17)	0.37(0.038)	-0.27(0.13)	0.28(0.12)	0.69(0.00)	0.39(0.03)	0.13(0.47)	0.50(0.004)	0.39(0.03)	0.46(0.01)	0.61(0.00)
CDRsob	0.61(0.00)	0.49(0.005)	0.19(0.19)	0.35(0.05)	-0.23(0.21)	0.30(0.10)	0.68(0.0)	0.31(0.08)	0.05(0.78)	0.49(0.004)	0.36(0.04)	0.45(0.01)	0.61(0.00)

<-0.70 <-0.60 <-0.50 >0.40 >0.50 >0.60

FIGURE 3 | Correlations between serum levels of potential biomarker proteins and cognitive measures. The values presented are Spearman's rank coefficients r^2. Blue values indicate a $P < 0.05$. The color key indicates the strength of correlations based on the correlation coefficients. IgE, immunoglobulin E; ANG-2, angiopoietin-2; BDNF, brain-derived neurotrophic factor; EGFR, epidermal growth factor receptor; VEGF, vascular endothelial growth factor; ICAM-1, intercellular adhesion molecule -1; MMP-9, matrix metalloproteinase-9; vWF, von Willebrand factor; p-Tau, Phosphorylated Tau; MMSE, Mini-Mental State Examination; MoCA, Montreal cognitive assessment; ADL, activities of daily living; CDRglobal, Clinical Dementia Rating global; CDRsob, Clinical Dementia Rating sum of boxes.

of receptor binding (**Figure 4A**). The overall effect of up- or downregulation of the signaling proteins in the KEGG pathways predicted that the PI3K-Akt and NF-κB signaling pathway is involved in AD (**Figure 4A**). Network of GO and KEGG enriched terms colored according to clusters and *P*-values were also shown (**Figures 4B,C**).

Predictive Value of Serum Proteins

In the ROC analysis, the identified 13 serum proteins showed moderately high or high AUCs for distinguishing subjects with AD or MCI from the controls. Sensitivity, specificity, accuracy, and the 95% confidence intervals are shown in **Table 2**. Cathepsin D, VEGFA, ICAM-1, vWF, and p-Tau181 were predicted both in MCI and AD with AUC values, sensitivity, and specificity above 0.8. Among them, cathepsin D had the highest AUC value for distinguishing AD from control (AUC=0.93), and its performance for discriminating MCI from control was moderate (AUC=0.84). The AUCs of p-Tau181 in differentiating AD or MCI from control were 0.89 and 0.91, respectively.

Predictive Value of Serum Proteins for Pre-MCI

Cathepsin D, vWF, and p-Tau181 showed significant associations with cognitive measures and high accuracy in predicting MCI and AD. So we detected these three serum proteins in patients with pre-MCI and controls using ELISA. Among them, only serum p-Tau181 levels were statistically significantly higher in the pre-MCI subjects than in controls

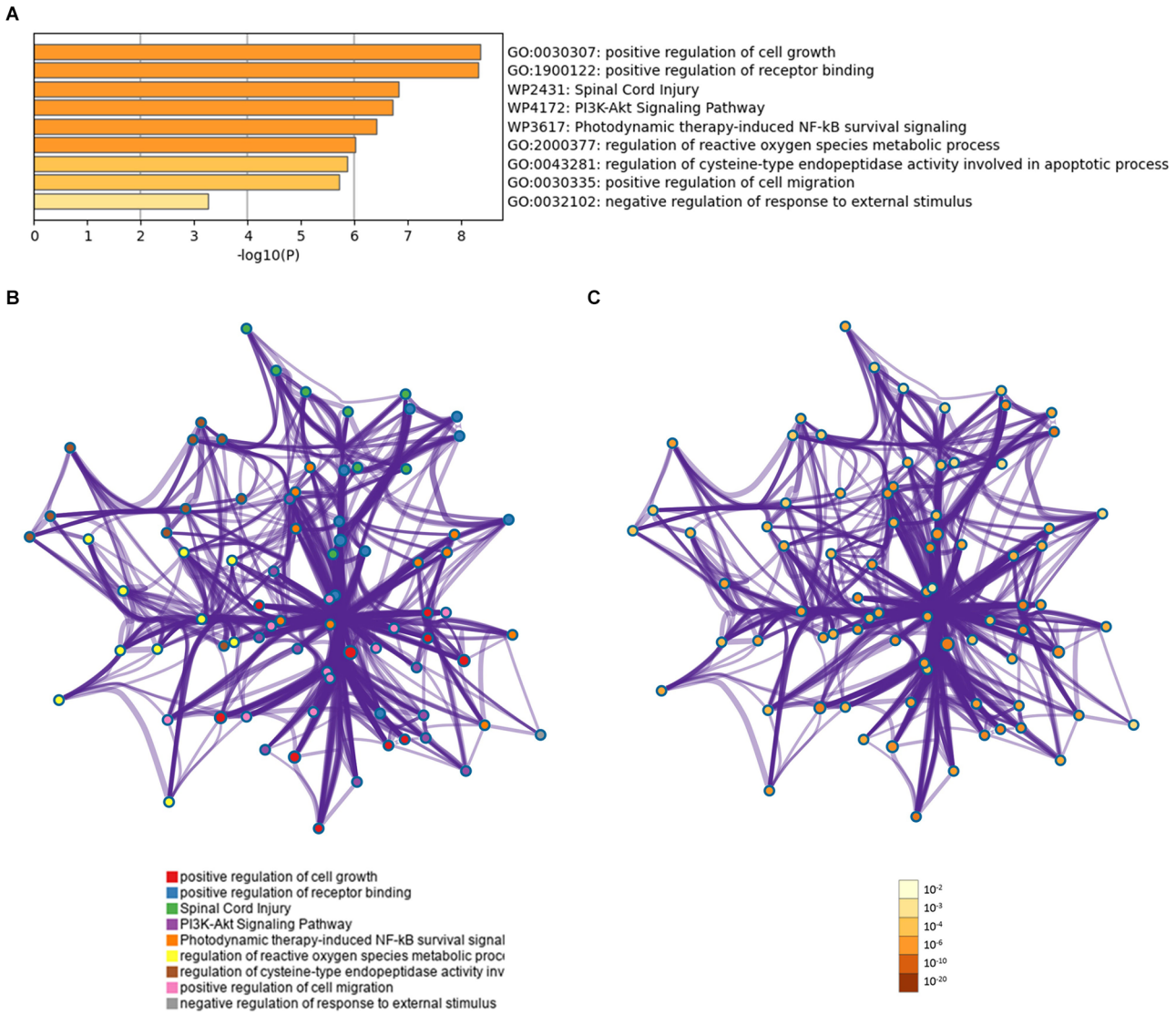

FIGURE 4 | Functional profiling of the identified proteins. **(A)** Shown are significantly enriched gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) terms. **(B)** Protein-protein interaction network of GO and KEGG enriched terms colored according to clusters. **(C)** Protein-protein interaction network of GO and KEGG enriched terms colored according to *P*-values.

(2.76 pg/ml vs. 4.04 pg/ml, $P < 0.01$; **Figure 5A**). The ROC analysis showed that p-Tau181 had a high AUC for distinguishing pre-MCI subjects from controls (AUC 0.83, sensitivity 83.33%, specificity 80%; **Figure 5B**). These results indicate that the serum p-Tau181 has value in discriminating early stages of AD from healthy subjects.

DISCUSSION

In this study on potential biomarkers for the development of AD, we found that the serum levels of 13 proteins were significantly different in MCI and AD subjects from those in the controls, and seven of these proteins were correlated with cognitive measures. Among these, only serum p-Tau levels were higher in pre-MCI than in control subjects and were able to distinguish pre-MCI.

Detecting AD as early as possible is vital to enable trials of disease-modifying agents that aim to prevent the development of symptoms in individuals who are still cognitively normal. ADAD makes it possible to identify presymptomatic individuals decades before they are destined to develop clinical symptoms (Dubois et al., 2016). The ability to detect multiple analytes in a serum sample has encouraged further research of this screening method that is less invasive than CSF sampling.

Therefore, this study examined a total of 170 candidate serum biomarkers using samples of ADAD family members in an attempt to identify a cost-effective, rapid, and reliable biomarker for early AD. We found that both MCI and AD subjects showed lower BDNF serum levels and higher cathepsin D, IgE, neuropilin-1, ANG-2, FXI, EGFR, VEGFA, ICAM-1, MMP-9,

TABLE 2 | Outcomes of the receiver operating characteristic curve analysis for the identified proteins.

	MCI vs. control					AD vs. control				
Proteins	**AUC**	**P value**	**Sensitivity**	**Specificity**	**95% CI**	**AUC**	**P value**	**Sensitivity**	**Specificity**	**95% CI**
Cathepsin D	0.84	0.007	80%	83.33%	0.66–1.02	0.93	0.0008	90%	83.33%	0.82–1.03
IgE	0.73	0.07	50%	100%	0.50–0.95	0.85	0.0056	70%	100%	0.68–1.03
Neuropilin-1	0.57	0.59	50%	66.67%	0.30–0.83	0.83	0.0084	80%	83.33%	0.66–1.00
ANG-2	0.82	0.01	80%	91.67%	0.62–1.01	0.78	0.025	70%	91.67%	0.58–0.99
BDNF	0.69	0.13	100%	41.67%	0.47–0.92	0.75	0.04	100%	41.67%	0.54–0.96
FXI	0.75	0.04	60%	100%	0.53–0.97	0.90	0.002	80%	91.67%	0.77–1.03
EGFR	0.94	0.0005	90%	100%	0.83–1.06	0.71	0.10	60%	83.33%	0.77–1.03
VEGF-A	0.93	0.0008	90%	83.33%	0.82–1.04	0.82	0.01	80%	91.67%	0.60–1.03
ICAM-1	0.88	0.002	80%	83.33%	0.74–1.03	0.92	0.001	80%	91.67%	0.80–1.03
MMP-9	0.73	0.06	60%	83.33%	0.50–0.96	0.77	0.03	60%	91.67%	0.56–0.98
vWF	0.92	0.001	90%	91.67%	0.79–1.04	0.82	0.01	70%	91.67%	0.60–1.02
Haptoglobin	0.80	0.02	100%	66.67%	0.61–0.99	0.83	0.01	100%	66.67%	0.66–1.00
p-Tau-181	0.91	0.001	80%	100%	0.78–1.04	0.89	0.002	80%	91.67%	0.75–1.03

AUC, area under the curve.

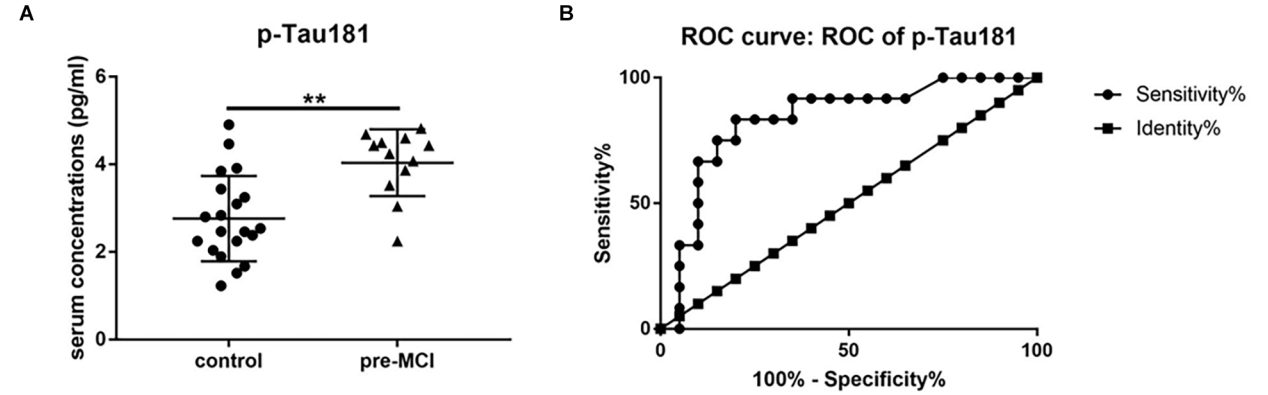

FIGURE 5 | The serum levels and receiver operating characteristics (ROC) analysis of p-Tau181. **(A)** Serum levels of p-Tau181 in pre-symptomatic mild cognitive impairment (pre-MCI), and controls with black horizontal lines indicating median values. *P*-values were determined by analysis of variance with Bonferroni *post-hoc* tests, $^{**}P < 0.01$. **(B)** ROC curve analyses of p-Tau-181 in pre-MCI vs. controls.

vWF, haptoglobin, and p-Tau181 serum levels than controls. Seven of them significantly correlated with the MMSE, MoCA, ADL, and/or CDR scores. Cathepsin D, vWF, and p-Tau181 showed significant associations with cognitive measures and high accuracy in predicting MCI and AD. In the second stage, these three serum proteins were detected in patients with pre-MCI and controls using ELISA. An antibody array can quantify 170 different biomarkers simultaneously, so we used a custom antibody array to screen many biomarkers in the first stage. After selecting three candidate biomarkers, we further verified them using ELISA, which can detect one biomarker at a time in more samples.

Our result is consistent with a random-effects meta-analysis that showed that patients with AD had significantly lower baseline peripheral blood serum levels of BDNF compared with healthy controls (Qin et al., 2017). BDNF single-nucleotide polymorphism modulates the association between beta-amyloid (Aβ) and hippocampal disconnection in AD and is an important factor in cognitive impairment in AD (Franzmeier et al., 2021). Elevating BDNF levels improved cognition in an AD mouse model (Choi et al., 2018).

Notably, our study identified high serum levels of the hemostasis factors FXI and vWF in AD subjects, and these have previously been reported as potential AD biomarkers (Loures et al., 2019; Begic et al., 2020). We also found that higher levels of FXI and vWF were associated with lower MMSE and MoCA scores, and associated with higher ADL scores. The vWF showed an AUC of 0.92 and 0.82 when it was used to distinguish MCI or AD from controls, respectively. Previous studies also showed significantly higher FXI and vWF levels in AD patients compared to control subjects (Laske et al., 2011; Begic et al., 2020). Further, an increase in FXI was associated with a reduction in cognitive function in individuals. Impaired clot initiation and formation rates were found in the plasma of AD patients (Suidan et al., 2018). Ryu and McLarnon (2009) have demonstrated abnormal immunostaining of vWF in the brains of AD patients. These data suggest that biological pathways involving coagulation and anticoagulation factors are related to AD.

We found high serum levels of neuropilin-1, ANG-2, and VEGFA in MCI and AD patients, and VEGFA showed a 90% sensitivity and 83.33% specificity in predicting MCI. These three proteins are regulators of angiogenesis. Both ANG-2 and VEGFA

were inversely correlated with the MMSE and MoCA scores. VEGFA is a pro-angiogenic factor that is essential during all stages of angiogenesis (Bosseboeuf and Raimondi, 2020). It can interact with the transmembrane protein neuropilin-1 to promote downstream signals, which are required for sprouting angiogenesis (Mamluk et al., 2002). Neuropilin-1 can also promote angiogenesis *via* VEGF-independent mechanisms and plays a role in regulating mitochondrial function and iron homeostasis, processes that are involved in the pathogenesis of AD (Kukreja et al., 2014; Peters et al., 2015). Muche et al. (2015) found up-regulation of VEGFA and neuropilin-1 in the entorhinal cortex with Aβ deposition in the Tg2576 mouse model. A clinical study suggested that neuropilin-1 modified the risk for poor cognitive scores based on APOE-ε4 status (Moore et al., 2020). ANG-2 has also been reported as upregulated in AD patients (Thirumangalakudi et al., 2006; Rocha de Paula et al., 2011).

An increasing number of studies reported that the brain in AD patients shows signs of inflammation (Janelidze et al., 2018; Park et al., 2020). We found a systemic inflammatory response in AD subjects, shown in the elevated serum levels of haptoglobin, IgE, and ICAM-1. Haptoglobin and IgE were negatively linked with MMSE and MoCA scores and positively linked with ADL and CDR scores. ICAM-1was able to distinguish AD patients from controls, with an AUC of 0.92, a sensitivity of 80%, and a specificity of 91.67%. Previous studies found increased plasma and brain haptoglobin levels in AD patients compared to controls (Song et al., 2015; Philbert et al., 2021) and an association between haptoglobin levels and the severity of cognitive impairment (Zhu et al., 2018). ICAM-1 level was higher in preclinical, prodromal, and dementia stages of AD (Janelidze et al., 2018) and linked with CDR-SB scores (Drake et al., 2021). However, Kester et al. (2011) did not find that ICAM-1 levels were significantly changed in AD. This conflicting result may be due to a different study population in terms of AD severity or a different course of AD or different kinds of test samples. In previous studies, haptoglobin suppressed amyloid fibril formation and prevented Aβ toxicity (Yerbury et al., 2009; Yerbury and Wilson, 2010). Haptoglobin and ICAM-1 levels have been suggested as useful markers of the progressive course of AD (Wang et al., 2015; Park et al., 2020). Allergy is a highly prevalent chronic inflammatory condition. Allergic mice with increased brain levels of IgE were found to have higher Tau phosphorylation in the brain (Sarlus et al., 2012). Our findings support the theory that inflammatory reactions underpin AD development and progression.

The MMP-9 was upregulated in the AD subjects in our study, which is consistent with other reports (Bruno et al., 2009; Gu et al., 2020). MMP-9 is a proteolytic enzyme that is critical for tissue formation, neuronal network remodeling, and blood-brain barrier integrity (Rempe et al., 2016; Ringland et al., 2020). A number of studies have shown that MMP-9 can influence AD pathogenesis and cognitive dysfunction through several mechanisms, including blood-brain barrier alterations, lipoprotein receptor shedding, inflammation, and neurodegeneration (Mroczko et al., 2013; Halliday et al., 2016; Shackleton et al., 2019).

Notably, we found altered levels of cathepsin D and EGFR in AD patients. Cathepsin D showed a sensitivity of 90% and specificity of 83.33% in identifying AD subjects. Both proteins are involved in autolysosomal functions, which contribute to the pathogenesis of AD (Uddin et al., 2019; Gadhave et al., 2021). In adult brains, pathological conditions such as AD activate EGFR in both neurons and astrocytes (Ceyzeriat et al., 2018). Polymorphisms in EGFR and cathepsin D genes have been associated with AD (Paz et al., 2015; Chen et al., 2018). Several studies have demonstrated that EGFR inhibitors may improve pathological and behavioral conditions in AD (Wang et al., 2013, 2017). They exert their therapeutic effects through the induction of autophagy and attenuation of reactive astrocytes (Tavassoly et al., 2021). Studies showed that the lysosome proteins are more sensitive to cellular metabolic alteration in AD compared to levels of Aβ or Tau proteins (Morena et al., 2017). Significantly higher levels of cathepsin D were found in patients with AD than in patients with frontotemporal dementia and healthy controls (Goetzl et al., 2015; Cheng et al., 2018). Previous findings support lysosomal enzymes as peripheral molecules that vary with the progression of AD, which makes them useful in recognizing preclinical AD (Goetzl et al., 2015; Morena et al., 2017).

Tau is a microtubule-binding protein that is increased and phosphorylated in AD and constitutes the main component in AD tangle and neurite pathology. Total Tau and p-Tau181 isoform levels were significantly increased in the CSF of AD patients (Dubois et al., 2014; Molinuevo et al., 2018; Zou et al., 2020). Karikari et al. (2020) found high p-Tau181 plasma levels in patients with AD and in MCI patients that developed AD. Studies (Barthelemy et al., 2020; Suarez-Calvet et al., 2020) showed that p-Tau181 levels were significantly increased in preclinical AD, when only subtle signs of Aβ pathology can be detected or as early as two decades before the development of aggregated tau pathology. Several studies indicated that p-Tau181 blood levels could accurately distinguish AD patients from other tauopathies in symptomatic AD (Janelidze et al., 2020; Thijssen et al., 2020). Consistent with these previous studies, we demonstrated that serum p-Tau181 levels were significantly higher in MCI and AD subjects than in controls and associated with all cognitive tests. Our data indicated that these serum proteins might be used to predict cognitive decline. Furthermore, we found that exclusively p-Tau181 serum levels were able to distinguish pre-MCI from controls. In Suárez-Calvet's study (Suarez-Calvet et al., 2020), they measured blood p-Tau181 changes in the preclinical stage of sporadic AD. The stage of AD is determined by the cutoff of CSF and PET biomarkers. The results may vary across different cutoffs. While in our study, the AD causative gene mutation carriers who had a normal cognitive performance were used as preclinical subjects. The certainty of disease and predictability of symptom onset of AD enables to accurately identify AD in the pre-symptomatic stage. Barthelemy et al. (2020) also quantified the phosphorylation state of the tau protein in dominantly inherited AD. But they detected p-Tau levels in CSF. To the best of our knowledge, our study is the first one to investigate serum p-Tau181 in the preclinical ADAD. In summary, serum p-Tau181 may help in the prediction of AD before the onset of cognitive impairment.

Limitations

This study had several limitations. First, the sample size was small. Because of the rarity of ADAD, the sample size limits the interpretation of our results that needs to be further explored in larger cohorts. Also, the association between serum proteins level and genetic mutations could not be analyzed because of the small sample size. Second, there are no ADAD data for all the proteins we analyzed, which precluded the comparison with published data. Third, this is a cross-sectional study. The shortness of the longitudinal evaluations of the individuals limits the interpretation of these results. We trust that future larger prospective studies investigating these serum biomarkers with a long-term follow-up will address these limitations.

CONCLUSIONS

In summary, a total of 13 serum proteins showed significant differences between subjects with AD and MCI and healthy controls. Furthermore, the serum levels of ANG-2, VEGFA, haptoglobin, and p-Tau181 were correlated with cognitive impairment. We highlight that the serum p-Tau 181 was found to distinguish pre-MCI subjects from normal controls. It will be helpful for early AD diagnosis and high-risk population screening of AD and initiate preventive treatment in asymptomatic people with AD.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/**Supplementary Material**.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of Xuanwu Hospital, Capital Medical University. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

WQ designed the project, performed the experiments, wrote and edited the manuscript. FL analyzed and interpreted data. LJ examined the patients. QW conducted genetic screening. YiL extracted DNA samples. YW and YaL helped to detect serum protein levels. HJ performed cognitive tests on participants. JJ designed the study and edited the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

This study was supported by the Key Project of the National Natural Science Foundation of China (81530036, U20A20354), Beijing Natural Science Foundation (7192077), National Key R&D Program of China (2017YFC1310100, 2016YFC1306305), the National Key Scientific Instrument and Equipment Development Project (31627803), and Beijing Brain Initiative from Beijing Municipal Science & Technology Commission (Z201100005520016, Z201100005520017).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnagi.2022.900773/full#supplementary-material.

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REVIEW
published: 01 July 2022
doi: 10.3389/fnagi.2022.890509

Insights Into the Role of Platelet-Derived Growth Factors: Implications for Parkinson's Disease Pathogenesis and Treatment

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OPEN ACCESS

Edited by:
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Reviewed by:
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Specialty section:
This article was submitted to Parkinson's Disease and Aging-related Movement Disorders, a section of the journal Frontiers in Aging Neuroscience

Received: *06 March 2022*
Accepted: *06 June 2022*
Published: *01 July 2022*

Citation:
Li D, Huang L-T, Zhang C-P, Li Q and Wang J-H (2022) Insights Into the Role of Platelet-Derived Growth Factors: Implications for Parkinson's Disease Pathogenesis and Treatment. Front. Aging Neurosci. 14:890509. doi: 10.3389/fnagi.2022.890509

Parkinson's disease (PD), the second most common neurodegenerative disease after Alzheimer's disease, commonly occurs in the elderly population, causing a significant medical and economic burden to the aging society worldwide. At present, there are few effective methods that achieve satisfactory clinical results in the treatment of PD. Platelet-derived growth factors (PDGFs) and platelet-derived growth factor receptors (PDGFRs) are important neurotrophic factors that are expressed in various cell types. Their unique structures allow for specific binding that can effectively regulate vital functions in the nervous system. In this review, we summarized the possible mechanisms by which PDGFs/PDGFRs regulate the occurrence and development of PD by affecting oxidative stress, mitochondrial function, protein folding and aggregation, Ca^{2+} homeostasis, and cell neuroinflammation. These modes of action mainly depend on the type and distribution of PDGFs in different nerve cells. We also summarized the possible clinical applications and prospects for PDGF in the treatment of PD, especially in genetic treatment. Recent advances have shown that PDGFs have contradictory roles within the central nervous system (CNS). Although they exert neuroprotective effects through multiple pathways, they are also associated with the disruption of the blood–brain barrier (BBB). Our recommendations based on our findings include further investigation of the contradictory neurotrophic and neurotoxic effects of the PDGFs acting on the CNS.

Keywords: platelet-derived growth factor, Parkinson's disease, dopaminergic neurons, oxidative stress, calcium homeostasis

INTRODUCTION

Second only to Alzheimer's disease (AD), Parkinson's disease (PD) is a common neurodegenerative disease worldwide (Ascherio and Schwarzschild, 2016). The main risk factor in PD is age, and its prevalence is estimated to be almost 0.3% in the general population of industrialized countries. In people over the age of 60, the prevalence of PD is 1%, and, in people over the age of 80, the prevalence is 3%. Based on prospective studies, the incidence of PD is approximately 8–18 per

100,000 people per year, the median age of the onset is 60 years, and the progression time from diagnosis to death is almost 15 years (Nussbaum and Ellis, 2003; Tysnes and Storstein, 2017). On average, the age of the onset in men is almost 2.1 years earlier than that in women, and the incidence is almost 1.5–2 times that in women (Martinez-Martin et al., 2012). This might be explained by studies that have shown that estrogen may have a protective effect on striatal dopaminergic neuron cells, indicating that the phenotype of PD in women is generally milder and the incidence of dyskinesia is lower (Gillies et al., 2014). Recently published data have indicated that the rising risk of PD is associated with global aging trends (Seljeseth et al., 2000). It is also speculated that the increased risk of PD may be related to changes in smoking behavior in the late 20th century and increased traffic-related air pollution (Lee et al., 2016b; Savica et al., 2016). Yang et al. (2020a) found that approximately 1 million people in the United States were diagnosed with PD in 2017, resulting in an economic burden of $51.9 billion. As such, in addition to posing a significant threat to human health, PD represents a serious economic burden; therefore, more research is urgently needed to find treatments that can prevent and/or reverse the progression of PD.

Cell death and atrophy in specific regions of the brain contribute to the basic pathological features of different neurodegenerative diseases. The pathophysiological mechanism of PD is mainly manifested *via* the selectivity of dopamine neurons in the midbrain substantia nigra (SN)-striatal pathway and the accumulation of Lewy bodies in the remaining neurons (Obeso et al., 2000; Chi et al., 2018). Studies have shown that "Parkinsonism" occurs when approximately 50–60% of SN neurons are lost, and 80–85% of dopamine in the striatum is depleted (Maraganore et al., 2004). All major signs of PD are associated with motor dysfunction, which includes resting tremors, bradykinesia, rigidity, and postural disturbances. Non-motor dysfunction manifestations include dysphrenia (e.g., anxiety and depression) and autonomic dysfunctions (e.g., hypotension, constipation, paresthesia, spasticity, olfactory dysfunction, and seborrheic dermatitis). In addition, disease progression may lead to cognitive decline in patients (Wird Ef Eldt et al., 2011). Selective degeneration of nigrostriatal dopaminergic neurons and their fibers in PD is a gradual process. Therefore, successful protection, regeneration, and functional recovery of the nigrostriatal dopamine pathway may delay PD progression. The pathogenesis of PD is mainly manifested *via* the degeneration of dopaminergic neurons, including defects in mitochondrial function, oxidative stress, impaired Ca^{2+} homeostasis, protein misfolding and aggregation, and neuroinflammation. Additionally, changes in genetic factors and glial cell proliferation are also intimately associated with the occurrence of PD (**Figure 1**).

In recent years, progress has been made in understanding the relationship between platelet-derived growth factors (PDGFs) and neurodegenerative diseases. PDGFs protect dopamine neurons, and this has been shown to play a role in PD. For example, Chen et al. (2021b) showed that the signaling pathways PI3K/Akt/GSK-3β and MEK/extracellular signal-regulated kinase (ERK) are involved in the process of MPP + toxicity after PDGF-BB treatment, which contribute to the phosphorylation and nuclear translocation of downstream effector cycle response element binding protein (CREB) (Chen et al., 2021b). Zheng et al. (2010) further confirmed that the nerve protective effect of PDGF-AA in this pathway is slightly weaker than that of PDGF-BB (Zheng et al., 2010). Recently, Chen et al. (2021a) have also confirmed that PDGF-BB promotes the generation of tyrosine hydroxylase (TH) by activating the transcription factor CREB, which transfers to the nucleus and is combined with the starting sub-region of TH genes in dopamine neurons (Chen et al., 2021a). Peng et al. (2012) verified that PDGF-CC can activate the PLC/PI3R pathway, thereby activating the transient receptor potential canonical (TRPC) channel and triggering Ca^{2+} elevation. This increase in Ca^{2+} inhibits the GSK3β signal in the PI3K/AKT pathway induced by PDGF, which further leads to β-catenin accumulation in the cytoplasm and, subsequently, induces gene expression related to cell survival (Peng et al., 2012). Chao et al. (2014) confirmed that PDGF-BB activates the P38 and Jun N-terminal kinases (JNK) mitogen-activated protein kinase (MAPK)/GSK-3β/β-catenin signaling cascade, thereby promoting the spread of neural progenitor cells (NPCs)(Chao et al., 2014). Furthermore, Yao et al. (2009) reported that PDGF-mediated PDGF-β receptors activate the PLC/IP3R pathway, which activates TRPC channels, increases Ca^{2+}, and activates the Pyk2/ERK pathway that leads to CREB activation, resulting in a protective effect on rat primary neurons (Yao et al., 2009). These signaling pathways linked to PDGFs are summarized in **Figure 2**.

In addition to the role of PDGFs in the signaling pathways associated with PD, we also summarized the effects of PDGFs on PD progression in this review. We further illustrated the possible applications and prospects of PDGFs for PD, with a focus on targeted gene therapy.

PLATELET-DERIVED GROWTH FACTORS

As a type of neurotrophic factors (NTFs), PDGFs are important mitogen and chemotactic agents. PDGFs can be expressed in mesenchymal cells, osteoblasts, and vascular smooth muscle cells (VSMCs) (Carl-Henrik and Bengt, 1999). NFTs are a class of proteins with a molecular weight of 10–35 kDa that play active roles in neuronal development, differentiation, survival, and plasticity (Huttunen and Saarma, 2019). Crucially, various NFTs have been shown to restore the dopaminergic nigrostriatal pathway, which is impaired in patients with PD.

Since Balk (1971) observed that normal chick embryo fibroblasts do not grow rapidly in low-calcium and platelet-free plasma while normal fibroblasts grow well after replacing plasma with serum, considerable research has followed on the growth-stimulating factors present in serum. It was found that platelets are the source of growth-stimulating activity, and their extracts can promote the growth and proliferation of fibroblasts, smooth muscle cells, and glial cells (Balk, 1971; Kohler and Lipton, 1974; Westermark and Wasteson, 1976; Aso et al., 1980). On this basis, PDGFs, also known as glioma-derived

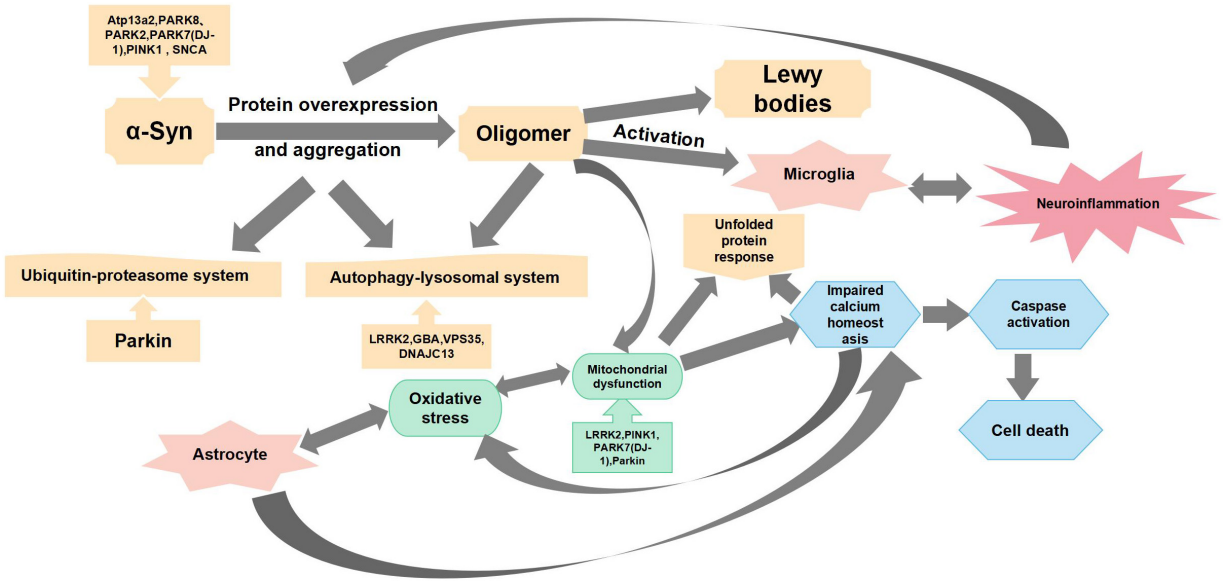

FIGURE 1 | Main mechanisms of Parkinson's disease pathogenesis. This figure summarizes the mechanisms of Parkinson's disease pathogenesis from oxidative stress, mitochondrial dysfunction, protein overexpression and aggregation, neuroinflammation, and impaired calcium homeostasis.

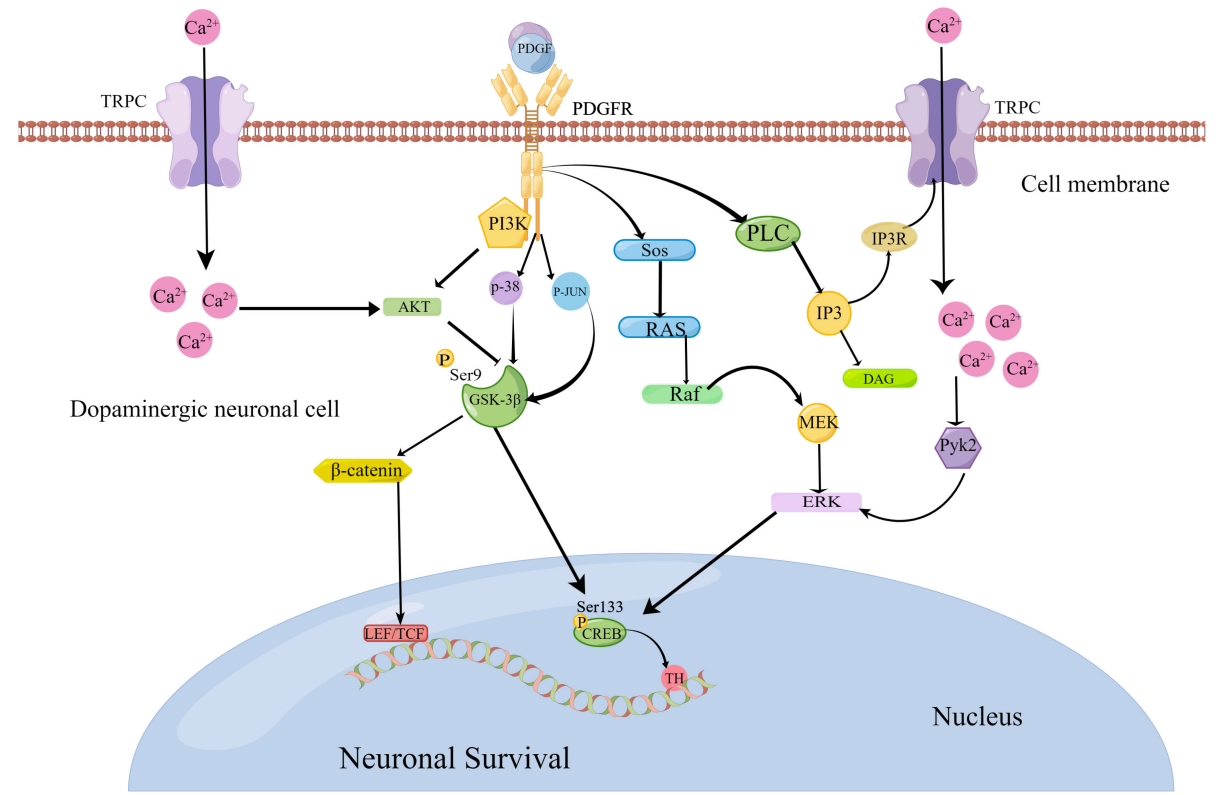

FIGURE 2 | Platelet-derived growth factor (PDGF)-mediated pathological signaling mechanisms in Parkinson's disease (www.figdraw.com). The binding of PDGF ligands and receptors can activate the PI3K/AKT/GSK-3β and MEK/ERK pathways to activate the transcription factor cycle response element binding protein (CREB) to promote the generation of tyrosine hydroxylase (TH). PDGFs can activate the P38 and JNK MAPK/GSK-3β/β-catenin signaling cascade. They can also activate the PLC/PI3R pathway to further activate the transient receptor potential canonical (TRPC) channel. As a result, Ca^{2+} elevation is triggered to suppress the GSK3β signal, which further leads to the accumulation of β-serial protein and gene expression related to cell survival. At the same time, elevated Ca^{2+} can activate the PYK2/ERK pathway, resulting in CREB activation.

growth factors and osteosarcoma-derived growth factors, were successfully isolated and purified.

Classification and Structure of Platelet-Derived Growth Factors

Platelet-derived growth factors are a family of cystine-knot-type growth factors composed of five functional subunits. Their structure is made up of highly homologous polypeptide chains (A, B, C, and D) that are formed by disulfide bonds (Kazlauskas, 2017; Huttunen and Saarma, 2019). These four types of polypeptide chains are encoded by four genes, among which PDGF-B was first identified *via* amino acid sequencing and presents a high homology with the simian sarcoma virus oncogene (Doolittle et al., 1983; Waterfield et al., 1983). The cDNA of PDGF-A was obtained by cloning, and its location was identified on Chromosome 7 (Betsholtz et al., 1986). The PDGF protein was discovered using biochemical methods; it is composed of PDGF-A and PDGF-B dimer proteins, as well as PDGF-AB heterodimers. In the early 2000s, genetic and biochemical methods were used to identify new ligands activated by PDGF-C and PDGF-D (Li et al., 2000; Bergsten et al., 2001; LaRochelle et al., 2001). As a receptor for PDGF, platelet-derived growth factor receptor (PDGFR) was also discovered in humans through cross-linking studies (Klinghoffer et al., 2002). Studies have also shown that PDGFR-α and PDGFR-β share common promoter proteins with c-Kit, c-Fms, and FLT (Kazlauskas and Cooper, 1989; Andrae et al., 2008). The five dimer isomers of PDGF have different affinities for the two PDGF tyrosine kinase receptors (TKRs) (Fredriksson et al., 2004; Janneth, 2015; Huttunen and Saarma, 2019); therefore, there are many possible interactions between PDGFs and PDGFRs (**Figure 3**). The phosphorylation of tyrosine residues in intracellular domains can be promoted by the polymerization of the associated subunit caused by the binding between the PDGF ligand and the receptor (Andrae et al., 2008; Janneth, 2015). Studies have shown that PDGFR-α and PDGFR-β share a common structure—five extracellular immunoglobulin (IG)-type domains and one intracellular tyrosine kinase domain (**Figure 3**). Various signaling pathways and mediators are activated by receptor phosphorylation, including MAPK, phosphoinositide 3-kinase (PI3K), the Wnt pathway, and phospholipase C (PLC). It has also been confirmed that the A-, B-, and C-polypeptide chains of PDGF can bind to PDGFR-α with high affinity, which means that PDGFR-α can be activated by homodimers PDGF-AA, PDGF-BB, and PDGF-CC, and the heterodimer PDGF-AB. The B- and D-polypeptide chains of PDGF can bind to PDGFR-β with high affinity, which means that PDGFR-β can

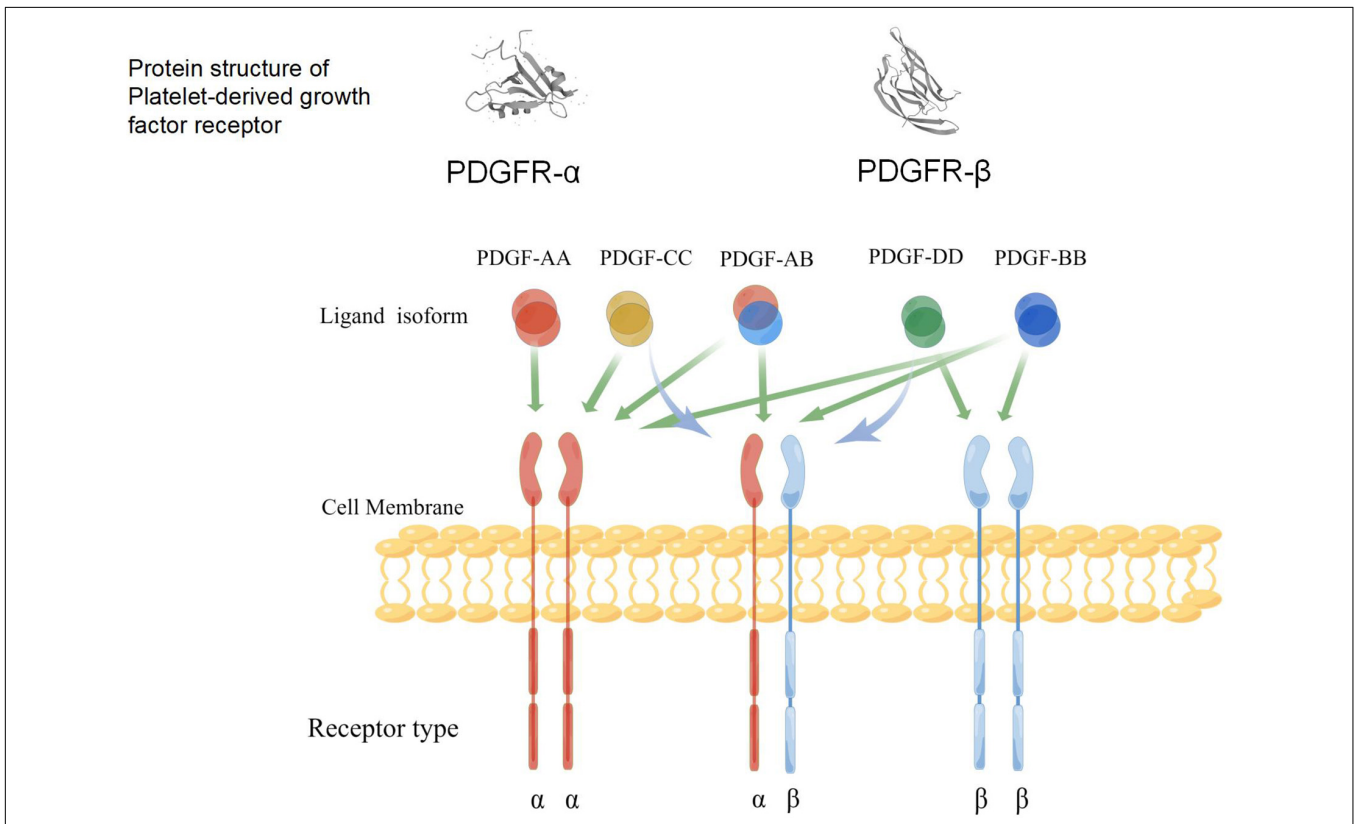

FIGURE 3 | A schematic diagram of the specific binding of platelet-derived growth factor (PDGF) ligand to the receptors, and the structure of its receptors (www.figdraw.com). Both PDGFR-α and PDGFR-β include an extracellular domain that combines with ligands, a single cross-membrane domain of extracellular signals to the intracellular regions, internal intracellular segments, kinase domain, and C-end acidic tail. Green arrows indicate the ability of different PDGF isoforms to bind and activate the dimeric receptor complex; blue arrows indicate the ability to activate receptor heterodimers.

only be activated by PDGF-BB and PDGF-DD (Carl-Henrik and Bengt, 1999). PDGFR-αβ heterodimers can be induced by PDGF-BB homodimers or PDGF-AB heterodimers. These interaction patterns suggest that PDGFR-α is more promiscuous than PDGFR-β, whereas PDGF-B is more promiscuous compared to other PDGFs. Smaller, less conformationally specific residues at the ligand–receptor interface account for the promiscuous nature of PDGFR-α, with fewer aromatic and hydrophobic residues than PDGFR-β, which has abundant aromatic residues on its ligand-binding surface. The presence of a large number of long-chain hydrophilic residues at the edge of the receptor-binding surface of PDGF-B explains its promiscuity (Lo Conte et al., 1999). PDGF-C is the only member of the PDGF family that has a propeptide-independent recombinant expression of growth factor domains (Shim et al., 2010), the receptor-binding surface, which may be more hydrophilic compared to that of other PDGFs. Thus, PDGF-C can adapt more easily to the ligand-binding surface of PDGFR-α. Finally, compared with other PDGFs, PDGF-CC is most similar to vascular endothelial growth factors (VEGFs), which implies that its underlying function is different from other PDGF members (Reigstad et al., 2005).

Distribution and Biological Functions of Platelet-Derived Growth Factors

Platelet-derived growth factors can be synthesized in various cells, including brain cells, such as neuronal progenitor cells, neuronal cells, astrocytes, and oligodendrocytes, and mainly function by either autocrine or paracrine stimulation. In the processes of wound repair, angiogenesis, and atherosclerosis, cell cycle and gene expression patterns can be regulated by PDGFs. The normal blood vessel wall expresses low levels of PDGF under physiological conditions, whereas, when the intima is damaged, a local increase in PDGF levels predicts adverse remodeling after vascular injury (Andrae et al., 2008). PDGFs can bind to PDGF receptors (PDGFR-α and PDGFR-β) through receptor tyrosine kinase (RTK) activity, which can bind to ligands and phosphorylate tyrosine residues of target proteins, stimulate receptor dimerization, and initiate intracellular signal transduction during biological functions (Chen et al., 2013). PDGF-A transcripts are expressed in the brain during late embryonic development of most neurons, which precedes the differentiation of most glial cells. While proteins containing PDGF-B chains have been found to localize in neurons throughout the CNS of adult non-human primates, such as *Macaca nemestrina* (Hart et al., 1989), its positive immunohistochemical staining reaction is limited to neuronal perinuclear regions and dendrites. The strength of this response varies with the location of the neuron; blood vessels stain weakly, while glial cells are not stained (Sasahara et al., 1991). PDGF-C and PDGF-A are co-expressed in heart, brain, liver, kidney, and testes, and PDGF-C is widely synthesized in various tissue cells of mouse embryos, including somites, craniofacial mesenchymal cells, cardiomyocytes, arterial smooth muscle cells, cartilage, mast cartilage cells, and the CNS. The expression of PDGF-C is also related to the formation of glandular ducts during embryonic development (Aase et al., 2002), and, in the adult nervous system, it is expressed in cerebellar neurons, anterior olfactory nucleus, pontine nuclei, cochlear neuronal cells, astrocytes, microglia, oligodendrocytes, and oligodendrocyte precursor cells (OPCs) (Tian et al., 2021). PDGF-D is widely synthetized in normal human tissues, and exhibits a high degree of expression in adrenal tissue; moderate expression in pancreas, adipose, heart, stomach, bladder, trachea, breast, ovary, and testis tissues; some degree of expression in brain, pituitary, liver, lung tissues; and low or no expression in small intestine, colon, skeletal muscle, thyroid, salivary gland, or thymus tissues (LaRochelle et al., 2001). PDGF-D can also be synthetized in VSMCs, endothelial cells, kidney epithelial cells, and fibroblasts (Borkham-Kamphorst et al., 2015).

Platelet-derived growth factor receptors, including PDGFR-α and PDGFR-β, are synthetized by various neuronal cell types during nervous system development, such as dopaminergic neurons in the SN, cortical neurons, striatal neurons, neurospheres, retinal ganglion cells, and neuronal cells in the inward and outer nuclear layers of the retina (Oumesmar et al., 1997).

PDGFR-α and PDGFR-β are class III RTKs (Lemmon and Schlessinger, 2010), although their expression patterns and physiological roles differ. For example, PDGFR-α signaling pathways regulate the development and formation of gastrula, neuroprotective tissues, and various organs, whereas PDGFR-β receptor expression is required for embryonic neural crest development, astrocyte development and differentiation, and dendritic spine morphogenesis and plasticity (Svitkina et al., 2010; Funa and Sasahara, 2014). In addition, the PDGFR-β signaling pathway plays an important role in the early stages of hematopoiesis and angiogenesis. Furthermore, a wide variety of mesenchymal cells are influenced in PDGFR-α-null embryos, while the deletion of embryonic PDGFR-β results in a lack of smooth muscle cells, particularly VSMCs and pericytes (Wu et al., 2008).

PDGF-BB is expressed in platelets, megakaryocytes, fibroblasts, smooth muscle cells, neurons, oligodendrocytes, and astrocytes (Krupinski et al., 1997; Yu et al., 2001, 2003; Board and Jayson, 2005; Kang, 2007; Trojanowska, 2008). As an important mitogenic factor, PDGF-BB is important for the induction of embryonic and vascular development, wound healing *in vivo*, chemotaxis regulation, and cell transformation *in vitro* (Yu et al., 2003; Andrae et al., 2008). PDGF-CC is widely expressed in different types of neuronal tissues, including the brain, eyes, and spinal cord (Hamada et al., 2000; Hao et al., 2000; Aase et al., 2002; Lei et al., 2007). Ding et al. (2004) also showed that a lack of PDGF-CC in mice leads to postnatal developmental defects and death, indicating that PDGF-CC is required for embryonic development (Ding et al., 2004).

PLATELET-DERIVED GROWTH FACTOR ROLES IN PARKINSON'S DISEASE

Platelet-derived growth factor regulates the functional activities of neurons by regenerating, stabilizing, and stimulating the synapses of neuronal axons, thereby regulating the synthesis

and release of neurotransmitters and affecting the expression of related transport proteins. The use of different PDGF isoforms in different research models demonstrated their potential in the protection and regeneration of specific neural cells (Iihara et al., 1997; Krupinski et al., 1997; Tang et al., 2010; Peng et al., 2012; Vasefi et al., 2012; Paul et al., 2015). This protective effect may be achieved by regulating mitochondrial function, oxidative stress, Ca^{2+} homeostasis, protein misfolding and aggregation, and neuroinflammation. PDGFs can also act on glial cells, neuroglobins (Ngb), and pericytes to affect the progression of PD (**Figure 4**).

Platelet-Derived Growth Factors Regulate Mitochondrial Function

Mitochondria balance cell death and survival, which is particularly important for maintaining aerobic balance in neurons in the brain, which consume 2% of the body's total oxygen. The energy required to maintain the ionic gradient across nerve elementary membranes drives this oxygen demand, which also generates action potential. This demand for energy reflects the importance of mitochondrial function in neuronal cells, and, therefore, mitochondrial dysfunction, mitochondrial DNA mutations, mitochondria-related DNA gene mutations, and the presence of mitochondria-related mutant proteins may be associated with PD. Mitochondria involved in the generation of adenosine triphosphate (ATP) through oxidative phosphorylation participate in the regulation of intracellular calcium ion levels and control membrane excitation and neurotransmission, and, thereby, regulate cell energy metabolism. Thus, mitochondrial damage results in blocked ATP production and elevated reactive oxygen species (ROS), which are one-electron reduction products of a class of oxygen *in vivo* that can modulate intracellular calcium levels and damage dopaminergic neurons (Rose et al., 2017; Norat et al., 2020). Furthermore, PD is associated with mitochondrial deletion and apoptosis activation (Simpkins et al., 2010). Importantly, mitochondria have different physiological properties in different tissues. For example, mitochondria isolated from the liver are unable to produce free radicals, which, in turn, causes mitochondria in the brain to produce high amounts of oxygen- and carbon-centered free radicals (Dykens, 2007). Mitochondrial damage also impedes the transport of long-distance organelles, rendering neurons with long axons and/or dendrites more vulnerable (Chang and Reynolds, 2006). In addition to proximal mitochondrial damage, the selective susceptibility of different mitochondrial defects in different brain regions may also be related to other factors, such as dopamine- and iron-rich SN, which can jointly exacerbate the oxidative stress caused by catecholamine autoxidation, as well as mitochondrial dysfunction (Hermida-Ameijeiras et al., 2004).

Ceramide can inhibit mitochondrial respiratory chain activity and regulate the permeability of the mitochondrial inner membrane, although its production is not sufficient to induce apoptosis and necrosis in sphingomyelinase-deficient mutant cells or mitochondria (Martin et al., 2006). PDGFs promote mitochondrial glycosylation of ceramides to lactosylceramide (LacCer), which has been shown to potentially affect the function

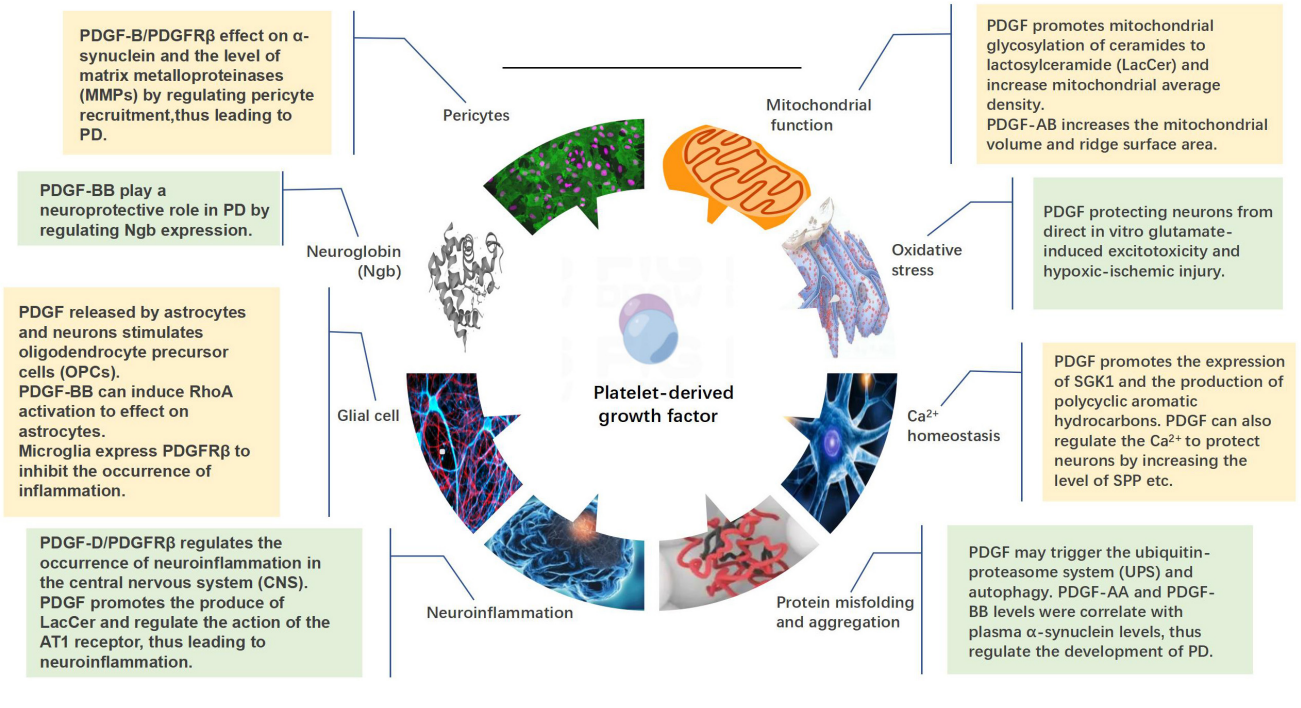

FIGURE 4 | Mechanisms by which platelet-derived growth factors (PDGFs) act on the development of Parkinson's disease, including mitochondrial function, oxidative stress, calcium homeostasis, protein misfolding and aggregation, neuroinflammation, glia, neuroglobin, and pericytes.

of mitochondria in nerve cells in various ways (Chatterjee et al., 2021). Novgorodov et al. (2016) also found that ceramide synthase activity is not proportional to ceramide levels, while LacCer levels are elevated. This confirmed the dominance of the glycosylation pathway in diabetic mice, showing that the synthesis of LacCer reduces ceramide levels. LacCer, one of the effectors of mitochondrial function, has also been shown to effectively inhibit State 3 respiration and reduce calcium retention capacity (CRC) in baseline mitochondria, resulting in mitochondrial dysfunction (Novgorodov et al., 2016).

The treatment of injured fibroblasts with PDGF-AB increases mitochondrial volume and the ridge surface area, reflecting its ability to induce ultrastructural changes associated with increased energy demand (Gosslau et al., 2001). Indeed, Gosslau et al. (2001) found that application of PDGF to NRK-49F fibroblasts led to a 57% increase in mitochondrial bulk density and a 65% increase in the cristae surface area compared to controls. At the same time, while the density of mitochondria in PDGF-treated cells decreased by 23%, their mean volume increased. These observations demonstrate the effect of PDGF on the mitochondria of nerve cells, which requires further research.

In conclusion, the existing research on the effects of the PDGF family on cell survival by affecting mitochondrial function remains limited and, in some cases, contradictory. As such, there are multiple promising avenues for future research on PDGFs.

Platelet-Derived Growth Factors Regulate Oxidative Stress

Regulate oxidative stress (ROS) generated by cells under oxidative stress in patients with PD can target and attack mitochondria. The long unmyelinated axons of the SN dopaminergic neurons increase energy consumption; this energy state leads to the damage and death of dopaminergic neurons, which leads to compensation by the residual neurons by accelerating the synthesis, metabolism, and renewal rate of dopamine. This further leads to the generation of more oxidative free radicals and further increases oxidative stress, thereby promoting PD progression (Jenner, 2003; Dias et al., 2013; Pissadaki and Bolam, 2013). High levels of ROS, nitric oxide (NO), interleukin 1β (IL-1β), interleukin 6 (IL-6), and tumor necrosis factor alpha (TNF-α) are neurotoxic, and further activation of the apoptotic pathway *via* the action of cytochrome C and caspase 3 affects the mitochondrial energy production in dopaminergic neurons (Menza et al., 2010). The cumulative effect of these processes can cause degenerative changes in the associated neurons (Wachter et al., 2010).

Many studies have demonstrated that PDGFs exert neuroprotective effects by protecting neurons and brain cells from direct *in vitro* glutamate-derived excitotoxicity and hypoxic-ischemic (H-I) injury. Neuronal excitotoxic death can result from overstimulation *via* elevated levels of extracellular glutamate acting on N-methyl-D-aspartate (NMDA) and alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors. The PDGF-B chain protects neurons by inhibiting NMDA-induced currents and transporting glutamate transporters to the cell membrane. It was found that the balance between N-methyl-D-aspartate receptor (NMDAR) and PDGF-B expression partly contributes to an individual's susceptibility to brain injury. Furthermore, enhancement of PDGF-B/receptor signaling may protect neonatal brains from H-I injury. Tseng and Dichter (2005) found that PDGF-BB preincubation weakens neuronal death caused by transient exposure to glutamate or NMDA. This protection is concentration- and time-dependent, and excitatory neurons were selectively protected (Egawa-Tsuzuki et al., 2004; Tseng and Dichter, 2005; Ishii et al., 2010). Other signaling mechanisms mediated by signal transducers and activators of transcription (STATs)and activated by PDGF ligands are important for cell proliferation, differentiation, survival, and transformation (Kang, 2007). For example, in vascular muscle cells, the activation of STAT 1, 3, and 6 by PDGF ligands occurs during airway remodeling in asthma *via* the activation of transmembrane NOX enzymes (nicotinamide adenine dihydrogen phosphate (NADPH) oxidase/dioxidase), leading to the production of hydrogen peroxide (H_2O_2) independent from ROS produced by the mitochondria (Simon et al., 2002). H_2O_2 acts as a secondary messenger in the STAT-activated signaling pathway and is important for the regulation of downstream phosphatase proteins. Therefore, H_2O_2 produced by PDGF ligands may have beneficial effects (Thannickal and Fanburg, 2000). This possible cellular protective effect of PDGF has not yet been verified in any PD model. It was shown that the transient increase in ROS levels following PDGF administration is likely attributable to the induction of membrane NOX enzymatic complexes. PDGF-promoted LacCer synthesis also activates NADPH oxidase to produce ROS, leading to a high oxidative stress environment that stimulates a range of signaling molecules and pathways, resulting in inflammation and atherosclerosis (Pagano et al., 1997). Whether different PDGF isoforms are involved in ROS generation in the nervous system has not yet been determined nor validated in PD models.

Microarray analysis of human umbilical vein endothelial cells (HUVECs) showed that oxidized low-density lipoprotein (oxLDL) induces high expression of PDGF-A and PDGFR-α after 6 h of pretreatment (Virgili et al., 2003). Bovine aorta endothelial cells (BAECs) driving firefly luciferase activity with a 643-bp PDGF-A promoter and exposed to oxLDL and native LDL (40 μg/ml) also showed increased PDGF-A promoter activity compared to vector-transformed cells. Increased expression of PDGF-A, PDGFR-α, and PDGFR-β has also been observed in response to oxLDL in VSMCs (Stiko-Rahm et al., 1992). PDGF-BB preconditioning for 24 h was shown to have significant protective effects against H_2O_2, glucose deprivation, and excitotoxic injury in cultured neurons, as demonstrated in most neuronal models (Cheng and Mattson, 1995; Tseng and Dichter, 2005; Zheng et al., 2010). Cabezas et al. (2015, 2016, 2018) found that pretreatment with PDGF-BB (200 ng/ml) can maintain mitochondrial membrane potential ($\Delta\psi m$) by decreasing the generation of superoxide and peroxide radicals. This, in turn, protects human astrocyte T98G cells from rotenone (50 μM), maintains mitochondrial ultrastructure, and boosts the activation of the PI3K/AKT signaling pathway (Cabezas et al., 2015, 2016, 2018). Northern blot analysis of BAECs exposed to H_2O_2 (25 and 50 μM) for 30 min also demonstrated a

significant increase in PDGF-B mRNA expression (Montisano et al., 1992). An early increase in PDGF-B mRNA levels was also observed by expression analysis of (total) RNA in rat lung cells extracted after oxidative injury (Fabisiak et al., 1989). Furthermore, oxLDL enhances PDGF-B expression in HUVECs in a dose-dependent fashion (Zhao and Xu, 2000). In contrast, in BAECs and human monocyte-originated macrophages, oxLDL reduces PDGF-B expression (Van Heek et al., 1998).

Based on this existing research, the protective effects of PDGF for cells under oxidative stress have been widely confirmed. However, the particular responses of different subtypes and in certain cells still require further research.

Platelet-Derived Growth Factors Regulate Ca^{2+} Homeostasis

Previous works have confirmed that dopaminergic neuron activity is affected by an imbalance in Ca^{2+} homeostasis and the modulation of Ca^{2+} carriers (Cieri et al., 2017). Changes in mitochondrial dynamics are also associated with the formation of contact sites in the endoplasmic reticulum. Effective endoplasmic reticulum–mitochondrial communication is required for the maintenance of mitochondrial bioenergetics, Ca^{2+} homeostasis, and cell survival (Paillusson et al., 2016). Thus, disruption of Ca^{2+} signaling molecules and other components may trigger excess Ca^{2+} influx, leading to the degeneration of dopaminergic neurons. Neuroinflammation can also lead to the abnormal expression and aggregation of α-synuclein (Kilpatrick, 2016). Mutual aggravation of α-synuclein and mitochondrial dysfunction, as well as mutual promotion of mitochondrial dysfunction and oxidative stress, further leads to impaired Ca^{2+} homeostasis, creating positive feedback. Such damaging effects are, therefore, continuously enhanced until caspases are activated, thereby inducing cell death (Tran et al., 2020). It has been reported that PDGF can participate in the production of polycyclic aromatic hydrocarbons, and, upon binding to its specific RTK, PLC is activated through a G protein-dependent or independent process. This results in an increase in diacylglycerol (DAG) and inositol triphosphate (IP3) levels, which results in the activation of PLC and the mobilization of intracellular Ca^{2+} (Clark et al., 1995; Yamamura et al., 2021). Cell proliferation, differentiation, and migration are subsequently induced. Serum and glucocorticoid-inducible kinase 1 (SGK1) expression can be stimulated by PDGF in megakaryocytes and circulating platelets. In megakaryocytes, SGK1 activates NF-κB, which causes the expression of calcium release-activated calcium channel 1 (Orai1). Orai1 is a Ca^{2+} channel protein that promotes Ca^{2+} entry, termed store-operated calcium entry (SOCE). SOCE and several Ca^{2+}-sensitive platelet functions can be enhanced by SGK1, including degranulation, activation of integrin αIIbβ3, phosphatidylserine exposure, aggregation, and thrombosis (Lang et al., 2015). The level of sphingosine 1-phosphate (SPP), a phosphorylated derivative of sphingosine that acts as an intracellular secondary messenger, can rapidly and transiently increase under the action of PDGF to release Ca^{2+} from internal sources. This occurs independently of inositol trisphosphate receptor (InsP3R), and SPP may link sphingolipid signaling to cellular Ras-mediated signaling by increasing phosphatidic acid levels (Spiegel et al., 1994).

Numerous studies have indicated that, under normal and pathological conditions, intracellular calcium concentration can modulate PDGF-BB signaling (Ridefelt et al., 1995; Pinzani, 2002; Peng et al., 2012; Paul et al., 2015). In this regard, PLC can be activated by PDGF-BB, leading to the formation of IP3 and DAG, which increase the mobilization of Ca^{2+} from the intracellular compartment and lead to the activation of protein kinase C (Powis et al., 1990; Ridefelt et al., 1995). PDGFR-β activates calcium channels, suggesting that PDGF-BB promotes calcium influx and mitosis (Hammerman et al., 1993; Johnson et al., 1993; Prncpadscussan, 1993; Ling et al., 1995). These findings suggest that PDGF-BB has important neuroprotective properties. Indeed, the signaling mechanism of PDGF-BB involved in neuronal protection also includes the activation of anti-apoptotic and survival pathways, such as MAPK, PI3K-AKT, c-JNK, and NF-κB pathways (Romashkova and Makarov, 1999; Wang et al., 2010; Zheng et al., 2010). Calcium influx into cells modulates the activation of these signaling pathways, further affecting the phosphorylation of glycogen synthase kinase 3β (GSK3β) and β-catenin (Zhu et al., 2009; Peng et al., 2012). During cell growth, differentiation, apoptosis, and stress response, PDGF-BB can activate NF-κB by regulating the PI3K/AKT pathway while reducing the production of matrix metalloproteinase 9 (MMP-9) and VEGF (Romashkova and Makarov, 1999; Wang et al., 2010). In other words, the inactivation of GSK3β can be caused by the activation of PI3K/AKT. Furthermore, the inactive form of GSK3β prevents the degradation of β-catenin, thereby promoting its accumulation in the cytoplasm and translocation to the nucleus. This contributes to the activation of genes involved in cell survival (Peng et al., 2012). Additionally, β-catenin is involved in mitochondrial homeostasis, regulation of ATP production, and lipid oxidation (Lehwald et al., 2012; Arrázola et al., 2015). PI3K/AKT also negatively regulates the transcription factor forkhead (FOXO), which contributes to cell survival, oxidative stress regulation, and mitochondrial membrane polarization (Burgering and Medema, 2003; Kato et al., 2006). The importance of the PI3K/AKT pathway in these cellular metabolic processes is self-evident, reflecting the potential value of PDGF-BB.

Platelet-Derived Growth Factors Regulate Protein Misfolding and Aggregation

Insoluble α-synuclein fibers are made of proteins composed of oligomers formed from soluble α-synuclein monomers, and, as these proteins aggregate, they form Lewy bodies (Melki, 2015). In the substantia nigra pars compacta (SNpc), the generation of Lewy bodies is one of the main pathological characteristics of primary PD. Oligomers formed by α-synuclein monomers can induce neuroinflammation by activating microglia, thereby promoting PD. The main pathological features of PD have been replicated in an α-synuclein mouse model (Hasegawa et al., 2017),

and oxidation, nitration, the ubiquitin-proteasome system, and the lysosomal autophagy system were all found to be closely related to the degradation of α-synuclein (Wong and Krainc, 2017). The ubiquitin-proteasome system can degrade short-lived soluble proteins by regulating the gene encoding of the protein parkin (PRKN). The lysosomal autophagy system mainly degrades long-lived macromolecular proteins and affects the occurrence and development of PD by regulating the genes encoding the proteins leucine-rich repeat kinase 2 (LRRK2), β-glucocerebrosidase (GBA), vacuolar protein sorting-associated protein 35 (VPS35), and DNAJC13 (Maraganore et al., 2004). The unfolded protein response (UPR) is also involved in PD (Hashida et al., 2012). Elevated levels of ROS and calcium homeostasis imbalance during episodes of PD lead to changes in proteins containing α-synuclein, which stimulate the activation of UPR-regulated proteins, such as PERK kinase, inositol 1α-dependent enzyme (Ire1α), transcription factor 6α (ATF6α), and GRP78/Bip (Wang et al., 2009; Gorbatyuk et al., 2012; Sen, 2015). UPR is a pro-survival response as it reduces protein biosynthesis and enhances the degradation function of the endoplasmic reticulum, thereby reducing the endoplasmic reticulum burden and maintaining intracellular homeostasis (Walter and Ron, 2011). For the first time, Ishimura et al. (2014) demonstrated that PDGF-BB can induce UPR activation in the proliferation of coronary artery smooth muscle cells (CASMC) (Ishimura et al., 2014). Dihazi et al. (2013) also demonstrated that PDGF-stimulated renal fibroblasts or tubular cell lines generate ER stress and activate the UPR. This means that rapidly growing fibroblasts stimulated by cytokines can induce ER stress, leading to the protective UPR and increased expression of folding partners as a protective response (Dihazi et al., 2013).

PDGF-AA and PDGF-BB levels are associated with plasma α-synuclein levels in patients with PD ($p < 0.0003$), indicating their potential as PD biomarkers. Lue et al. (2016) biochemically validated that levels of thymus, activation-regulated chemokine (TARC), and PDGF-AA are significantly different between the patients with PD with and without dementia (Lue et al., 2016). Furthermore, under the regulation of PDGF promoter, transgenic mice overexpressing human α-Synuclein were generated by Masliah et al. (2000); 12 months later, these mice were found to have motion defects and a loss of dopamine (Masliah et al., 2000). Subsequently, Rockenstein et al. (2002) verified that, in the neocortex and limbic system, the PDGF promoter promotes the expression of human α-synuclein (Rockenstein et al., 2002). Şengül et al. (2021) further verified that overexpression of α-synuclein upregulates the secretion levels of PDGF-AA and PDGF-BB; while the expression of PDGFR-B in cells was shown to increase, PDGFR-B was predicted to interact with α-synuclein based on the Fp-Class PPI prediction tool. Based on Reactome pathway analysis, these authors found that PDGF may trigger the ubiquitin-proteasome system (UPS) and autophagy *via* the intracellular PI3K/Akt or MAPK pathway (Şengül et al., 2021). These studies demonstrate that PDGF may regulate the degradation of α-synuclein through UPR and autophagy.

Platelet-Derived Growth Factors Regulate Neuroinflammation

The occurrence of PD caused by neuroinflammation is closely related to a variety of genes, including LRRK2. Studies have found that neuroinflammation can activate innate and adaptive immunity in PD by promoting the misfolding and aggregation of α-synuclein (Gao et al., 2008). Inflammatory responses in the olfactory system and gut tissue can also trigger high levels of α-synuclein misfolding, allowing α-synuclein aggregates to escape normal degradation mechanisms (Tomé et al., 2013). Sampson et al. (2016) demonstrated that inflammatory responses in the gut microbiota can promote microglial activation as well as α-synuclein pathological states and motor deficit states (Sampson et al., 2016).

The pathological process of inflammation is related to leukocyte extravasation and migration and involves cell-derived mediators (e.g., cytokines and adhesion molecules) (McCurley et al., 2013). Accumulating evidence suggests that neuroinflammation may play an important role in the pathological changes in patients with PD. PDGF-D can selectively agonize PDGFR-β isoforms, while, on the other hand, PDGFR-β affects macrophage activation, cell infiltration, and cell migration in CNS inflammation (Yang et al., 2016). Transactivation of PDGFR can be induced through the action of the AT1 receptor in VSMCs and tissues, which may support cell growth and migration (Heeneman et al., 2000; Schellings et al., 2006; Suzuki and Eguchi, 2006). PDGFR transactivation also mediates angiotensin II (Ang II)-induced ERK activation in mesangial cells. Ang II, however, depends on the AT1 receptor and acts through RTKs (PDGFR and endothelial growth factor receptor, EGFR) and non-RTKs [proto-oncogene tyrosine-protein kinase (Src), non-receptor protein-tyrosine kinase (Pyk2), and JAK/STAT]. AT1R-mediated activation of NADPH oxidase leads to the production of ROS, thereby promoting neuroinflammation. Simultaneously, these signaling cascades lead to the development and progression of glutamate excitotoxicity, apoptosis, cerebral infarction, astrocyte proliferation, nociception, neuroinflammation, and other neurological disease processes (Mondorf et al., 2000). LacCer synthase/LacCer has also been shown to play a role in cell proliferation, inflammation, and cancer (Asada et al., 1997). Using primary rat astrocytes, Pannu et al. (2004) demonstrated the potential role of LacCer synthase/LacCer in TNF-α-induced inflammation (Pannu et al., 2004). Astrocyte proliferation and LacCer synthase activity increase after stimulation with TNF-α; this was alleviated using D-threo-1-phenyl-2-decanoylamino-3-morpholino-1-propanol (D-PDMP), along with a 20-mer antisense oligonucleotide in rats. This led to reduced PI3K, Ras, and ERK1/2 expression, and the inhibition of astrocyte proliferation. LacCer also activates inducible nitric oxide synthase (iNOS), thereby promoting NO generation. NO is a neuronal messenger that becomes toxic at high concentrations and exacerbates the progression of several neurodegenerative diseases (Won et al., 2007).

Other Mechanisms by Which Platelet-Derived Growth Factors Regulate Parkinson's Disease Progression

Platelet-Derived Growth Factors Regulate Glial Cell Changes

Dopaminergic neurons play a major role in the progression of PD; however, there are reports that glial cells are also involved in its inflammatory and degenerative processes (McGeer and McGeer, 2008; Mena and Garcia de Yebenes, 2008; Ahmed et al., 2013; More et al., 2013). As the major cell type in the mammalian brain, astrocytes constitute glial cells along with oligodendrocytes and microglia (Chen and Swanson, 2003), and are part of a syncytial network, including pericytes, endothelial cells, and neurons (Bushong et al., 2002). Astrocytes play a significant role in the development and maintenance of the BBB, promoting neurovascular coupling, releasing chemokines and glial transmitters to recruit cells, regulating calcium release, and transporting glutamate. Astrocytes can send signals through the glutamate aspartate transporter and the excitatory amino acid transporter, thereby maintaining brain metabolism, regulating brain pH and specific transporter uptake of γ-aminobutyric acid, as well as producing antioxidant enzymes (Volterra and Meldolesi, 2005; Hamby and Sofroniew, 2010; Parpura et al., 2011). During brain injury (e.g., oxidative stress), these processes are affected to varying degrees, and their effects on neuronal cells can result in pathological conditions and neurodegenerative diseases (Kimelberg and Nedergaard, 2010). Neurons have a lower antioxidant capacity than astrocytes and, therefore, are more susceptible to damage, requiring stronger metabolic coupling to antagonism oxidative stress both under normal conditions and in cases of brain damage (Hamby and Sofroniew, 2010). Antioxidative protection of neurons, NTFs, and substrates required for neuronal metabolism, and reuptake of glutamate can be supported by astrocytes (Greve and Zink, 2009; Barreto et al., 2011b). When astrocytes are severely damaged, neurons die; postmortems of the brains of patients with PD have demonstrated increased astrocyte reactivity, interferon-gamma and neurotrophic factor release, glutathione peroxidase (GPx) levels, and the endocytosis of α-synuclein by glial cells (Chung et al., 2010).

Astrocytes respond to brain injury, including oxidative stress in neurodegeneration through the process of "reactive astrogliosis" (Barreto et al., 2011a,c, 2012). This involves changes at the molecular level, including the increased expression of glial fibrillary acid protein (GFAP), vimentin, nestin, and the Ras homologous protein (RhoA). Oxidative stress is prevented by increasing glutamate uptake to produce glutathione, releasing adenosine to protect neurons, degrading β-amyloid peptides, regulating the BBB, and forming glial inclusions. In addition, reactive astrocytes can release inflammatory cytokines (including TNFα) and ROS (Sugaya et al., 1998; Fitch and Silver, 2008; Duffy et al., 2009; Hamby and Sofroniew, 2010; Kang and Hebert, 2011). Astrocyte proliferation may play conflicting roles during PD episodes. For example, it has been reported that an increase in the number of reactive astrocytes plays a role in dopaminergic neuron repair (Chen et al., 2005; Francesca et al., 2013), yet reactive astrocyte content is reduced in the pathological tissue of patients with PD after death. This implies that the excessive accumulation of α-synuclein can inhibit astrogliosis and exert neuroprotective effects (Song et al., 2009; Tong et al., 2015). Irreversible changes in the cytoskeleton of astrocytes can be caused by the inactivation of RhoA by the botulinum C3 toxin, resulting in astrocytic morphological stellation associated with actin and intermediate filament disassembly (Ramakers and Moolenaar, 1998). It has also been reported that increases in peroxide and NO levels induce the activation of Rho/Rho-associated protein kinase (ROCK) based on a vascular model (Noma et al., 2007; Kagiyama et al., 2010). The activation of RhoA in endothelial cells during angiogenesis could be caused by PDGF-BB (Nobes et al., 1995; Amerongen et al., 2003). In this regard, Fujimura and Usuki (2012) confirmed that exposure to 100-nM rotenone or inorganic mercury suppressed the expression of cell division control protein 42 (CDC42) and Rac1 without affecting the expression of RhoA, and this process was accompanied by axonal degeneration and cortical brain cell death (Masatake and Fusako, 2012). Similarly, the pharmacological inhibition of ROCK reduces ERK1/2 phosphorylation even after stimulation of glioblastoma cells with PDGF-BB (Zohrabian et al., 2009). A seminal study by Richardson et al. (1988) demonstrated that PDGFs stimulated growth of cultured OPCs from rat optic nerves (Richardson et al., 1988). Subsequent studies using *in vivo* experiments in mice confirmed that OPCs can express PDGFR-α, and PDGF-AA was found to promote OPC proliferation (Fruttiger et al., 1996, 1999; Calver et al., 1998). *In vivo* astrocytes and neurons are able to stimulate OPCs through the paracrine release of PDGF (Fruttiger et al., 2000), with PDGF-AA appearing to be a determinant of OPC proliferation rates (Woodruff et al., 2004). Additionally, Huang et al. (2014) found that matrix-derived PDGF-C is a key factor in the recruitment and activation of oligodendrocyte progenitors (Huang et al., 2014).

As the primary immune cells of the CNS, microglia are important in host defense against invading microorganisms and tumor cells. Microglia may also play a dual role in immune responses, protecting the CNS by amplifying inflammatory responses and mediating cellular degeneration (Gonzalez-Scarano and Baltuch, 1999). Importantly, the pathogenesis of neuroinflammation and neurodegenerative diseases including PD may be related to the activation of microglia. It was also found that microglial cells highly express PDGFRβ, the receptor of αVβ3 integrin molecules, the binding of which contributes to tissue regeneration, angiogenesis, and tumor metastasis (Schneller et al., 1997). This was confirmed in a subsequent study in mouse microglial cells, where inflammation was suppressed by the interaction between the microglial PDGFRβ receptor and the αVβ3 integrin complex of the MBP-primed Th2 cells (Roy and Pahan, 2013).

Platelet-Derived Growth Factors Regulate Neuroglobin Expression

Ngb has been shown to perform neuroprotective functions by targeting neurons and astrocytes during pathological processes, such as focal ischemia, AD, strokes, and traumatic brain injury

(Emara et al., 2009; Chen et al., 2015; Avila-Rodriguez et al., 2016; Xie and Yang, 2016). Furthermore, studies have shown that PDGF-BB can upregulate cytoglobin expression in hepatic stellate cells as well as the expression of GPx1 and Ngb in an astrocyte model during rotenone injury. This indicates that PDGF-BB may play a neuroprotective role in PD by regulating Ngb expression (Cabezas et al., 2018).

Platelet-Derived Growth Factors Regulate Pericyte Abundance

Pericytes are important for regulating blood pressure and the structural integrity of the blood vessel wall. Pericyte dysfunction triggers the breakdown of the BBB, leading to the accumulation of toxic proteins in the brain and a reduction in cerebral blood flow, which, in turn, reduces the delivery of nutrients and oxygen to the brain, leading to secondary neurodegeneration (Sagare et al., 2013).

Matrix metalloproteinases (MMPs) are zinc-dependent endopeptidases involved in the breakdown of the extracellular matrix in normal physiological processes and disease processes, especially in PD (Yong, 2005; Cauwe and Opdenakker, 2010). As an important member of MMPs, MMP-9 is upregulated in PD (He et al., 2013). Among the BBB-constituting cells, brain pericytes were found to be the most MMP-9-releasing cells in response to thrombin stimulation (Machida et al., 2015). Furthermore, the overexpression of α-synuclein stimulates MMP-9 activity (Lee et al., 2010), while neuronal cell death caused by the dopaminergic neurotoxins 6-OHDA (6-hydroxydopamine) and MPP (+) can be ameliorated by the administration of MMP-9 inhibitors (Joo et al., 2010). Machida et al. (2017) found that thrombin activates two independent signaling pathways by acting on PAR-1 expressed by brain pericytes (the PKCθ-Akt and PKCδ-ERK1/2 pathways) and causes brain pericytes to release MMP-9, which leads to BBB dysfunction (Machida et al., 2017). Dohgu et al. (2019) confirmed that pericytes are also capable of releasing various inflammatory cytokines/chemokines in response to monomeric α-synuclein to induce BBB dysfunction, thus contributing to the progression of PD (Dohgu et al., 2019). In pericytes, tunneling nanotubes (TNTs) function as F-actin-based membranous channels, which connect cells and contribute to cell-to-cell transmission of α-synuclein (Dieriks et al., 2017).

PDGF-B plays a crucial role in the recruitment of pericytes to various vascular beds, including the brain, kidneys, heart, lungs, and adipose tissue (Brownlee, 2001), and recruited pericytes are reduced in number in the absence of the PDGF-B allele (Benjamin et al., 1998). Tallquist et al. (2003) found that the number of pericytes in heterozygous PDGFR-β mice was reduced, which also suggests that the number of pericytes may be related to PDGFR-β expression (Tallquist et al., 2003). PDGFR-β has also been found to act as a non-specific diagnostic marker for pericytes (Armulik et al., 2011). Gene ablation of PDGF-B or PDGFR-β in mice was also found to result in almost identical phenotypes, namely, perinatal death following extensive microvascular leakage and hemorrhage (Leveen et al., 1994). Severe pericyte deficiency also causes microvascular dysfunction in these mice, and while pericytes appear to be induced in the absence of PDGF-B or PDGFR-β, subsequent selection (expansion and spread) of pericyte populations fails due to reduced pericyte proliferation. The migration of pericytes along new vessels may also be impaired when PDGF-B/PDGFR-β signaling is disrupted. In angiogenesis, PDGF-B is expressed by sprouting endothelial cells, whereas PDGFR-β is expressed by pericytes/VSMC precursor cells (Lindahl et al., 1997; Hellström et al., 1999). This suggests that the interaction between these two cell types is a paracrine stimulation. In chimeras consisting of PDGFR-β-positive and PDGFR-β-negative cells, only PDGFR-β-positive cells were found to aggregate between VSMCs/pericytes, suggesting that the development of these cells is directly dependent upon on PDGFR-β. In addition, Enge et al. (2003) found that the knockout of the endothelial-specific PDGF-B gene results in VSMC/pericytic defects (Enge et al., 2003). Gene ablation of PDGF-B in hematopoietic cells or neurons (the other two major sources of PDGF-B) has no apparent effect on the vasculature, and the available evidence confirms that endothelial PDGF-B signaling controls pericyte recruitment during angiogenesis. The number of pericytes (or progenitors) that can be recruited may depend on the amount of PDGF-B available (van Heyningen et al., 2001). Notably, the effect of PDGF-B on mature vasculature is dose dependent (Ejaz et al., 2008). Padel et al. (2016) found that a 2-week treatment with PDGF-BB promoted the recovery of behavioral function and partially restored the nigrostriatal pathway. At the same time, pericytes in the striatum of PD model mice were activated, and this change could be reversed by PDGF-BB treatment. This demonstrates that brain pericytes may play a role in the pathogenesis of PD and may be a target for PDGF-BB treatment of PD neural recovery mechanisms (Padel et al., 2016). Subsequently, using an *in vitro* model of dopaminergic injury, Gaceb et al. (2020) demonstrated that PDGF-BB/PDGFRβ-mediated brain pericyte secretion affects the expression of dopamine markers, which may shed light on the mechanism by which PDGF-BB promotes neural recovery in PD (Gaceb et al., 2020). These studies provide inspiration for future related research in this field.

PLATELET-DERIVED GROWTH FACTORS FOR THE TREATMENT OF PARKINSON'S DISEASE

The treatment of PD is currently mainly limited to symptomatic treatments, such as the use of drugs levodopa, carbidopa, and dopamine receptor agonists, as well as catechol-O-methyltransferase (COMT), monoamine oxidase (MAO-B) inhibitors, and deep brain stimulation. The aim of treatment is primarily to maintain or prolong the patient's daily activities without slowing or reversing the progression of PD. However, these treatments tend to lose their efficacy after 2–5 years (Hauser et al., 2006; Bronstein et al., 2011; Post et al., 2011; Chmielarz and Saarma, 2020). Therefore, there is an urgent need to identify effective and long-lasting neuroprotective agents to avoid further degeneration of nigrostriatal neurons and axons, thereby slowing the development of disease. Decreased levels of

NTFs and knockdowns of their receptors have been reported to trigger neuronal loss and other outcomes related to disease progression (Rinne et al., 1989; Lorigados Pedre et al., 2002). As an endogenous growth factor that boosts neuronal survival and differentiation, the therapeutic potential of PDGF has been validated in various neurodegenerative diseases, owing to its neuroprotective and neurorestorative properties. Upregulated expression of PDGF-A and PDGF-B mRNA, and PDGF-AA, PDGF-BB, and PDGF-AB proteins, as well as PDGFR-α and PDGFR-β receptors, has been observed in neuronal samples from patients with ischemia. This suggests that these ligands and their receptors may be important for neuronal survival in damaged brain regions. Indeed, the neuroprotective effects of exogenous PDGF-BB during focal ischemia have been confirmed in multiple studies (Krupinski et al., 1997; Sakata et al., 1998), and glutamate- and NMDA-induced neuronal death in the hippocampus may also be blocked. Lesions caused by NMDA hyperstimulation in neonatal rats can be decreased by intracerebral administration of PDGF-BB (Egawa-Tsuzuki et al., 2004; Tseng and Dichter, 2005), and pretreatment with PDGF-BB at doses of 120–240 ng/ml has been demonstrated to reduce pyramidal neuron death during ischemic injury in rats (Iihara et al., 1997). Damage to the striatal dopaminergic system *in vivo* also provides evidence of elevated PDGF-B levels, which may reflect the endogenous neuroprotective effects of PDGF-B (Funa et al., 1996). Based on a rodent model of PD, Zachrisson et al. (2011) demonstrated that intracerebroventricular administration of PDGF-BB provides an alternative strategy for restoring function in PD. According to their animal model of nigrostriatal injury, the administration of PDGF-BB treatment for 2 weeks resulted in the increased expression of striatal dopamine transporter binding sites and SN TH, normalizing amphetamine-derived rotational behavior in 6-OHDA-injured rats. The same authors showed that PDGF-BB can also promote the proliferation of NPCs in the subventricular zone, and, when co-infused with a proliferation inhibitor, can block the recovery of dopaminergic neuron function. This work suggests that PDGF-BB has a restorative effect on 6-OHDA- and MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine)-injured rat dopaminergic neurons, and, further, application in human clinical trials did not produce adverse effects (Zachrisson et al., 2011).

The effects of PDGF-BB on dopaminergic neurons are the result of persistence rather than direct pharmacological effects. In a PD mouse model with partial 6-OHDA medial forebrain tract lesions, along with the restoration of the nigrostriatal pathway and the inhibition of pericyte activation, PDGF-BB was found to guard against behavioral disorders (Padel et al., 2016). Chao et al. (2014) also confirmed that PDGF-BB ameliorates human immunodeficiency virus-1 (HIV-1) transcriptional transactivator (Tat) levels by activating p38 and N-terminal kinase/mitogen-activated protein kinase (JNK/MAPK) pathways, thereby impairing the proliferation of NPCs, which are specialized cells with the potential to develop into neurons during neurogenesis. The researchers also reported that the novel GSK-3β/β-catenin pathway is involved in neurogenesis mediated by PDGF-BB; as the main substrate of GSK-3β, the level of nuclear β-catenin increased in the presence of PDGF-BB, indicating its potential stimulating effect on NPC proliferation (Chao et al., 2014). In rat hippocampal neurons, PDGF-BB regulates Arc/Arg3.1 gene expression by activating the MAPK/ERK pathway and, therefore, affects synaptic plasticity and long-term potentiation (Peng et al., 2008). Recently, it has been reported that neuroprotection in PD animal models can be induced by PDGF-CC levels and their signaling (Tang et al., 2010). Oxidative stress, neurotoxin production, and apoptosis can be inhibited, modulating GSK-3β activity *in vivo* and *in vitro via* PDGF-C, thus acting on various neuronal cell types. In contrast, mice lacking PDGF-CC expression show an increased rate of neuronal death (Tang et al., 2010). Peng et al. (2012) demonstrated that PDGF-CC can protect mouse neuronal cells from apoptosis caused by different types of toxins containing 6-OHDA and HIV TaT, and can also activate TRPC Channel 1 to prevent the production of HIV TaT toxin, which regulates downstream protein pathways, such as the GSK3β pathway in the SH-SY5Y neuroblastoma cell line (Peng et al., 2012). Additionally, serotonin receptor agonists have also been shown to block NMDA-induced cell death by increasing PDGFR-β expression in primary hippocampal neurons (Vasefi et al., 2012, 2013). Studies have shown that Notch3-/- mice have reduced PDGFR-β levels, which indicates that the PDGFR-β signaling pathway may interact with Notch signaling, thereby jointly affecting neurodegenerative diseases (Jin et al., 2008; Nadeem et al., 2020).

The safety and the tolerability of intraventricular recombinant human PDGF-BB (rhPDGF-BB) administration in patients with PD were evaluated in a double-blind, randomized controlled trial by Paul et al. (2015). The results demonstrated that all doses of rhPDGF-BB were well-tolerated by the human body; RhPDGF-BB had no unresolved adverse effects on the patients with PD and a positive effect on the binding of dopamine transporters in the right putamen (Paul et al., 2015). At present, the therapeutic effect of PDGFs on PD has been verified in cell and animal experiments, but relevant clinical trials are still lacking, and the application of PDGFs in PD still faces ethical and immuneresponse-related questions. **Table 1** summarizes existing trials on the safety and efficacy of PDGFs for the treatment of certain diseases, including PD. Nevertheless, their application in clinic settings still faces many problems. First, in terms of performance, the clinical benefits of PDGFs are presently still low. Second, the dose of PDGFs delivered to the brain is uncertain (Bartus and Johnson, 2017); PDGFs also have a short half-life *in vivo* and poor pharmacokinetic properties, and the permeability of PDGFs through the BBB is very low. It is, therefore, necessary to identify intracranial delivery routes.

The degeneration of nigrostriatal dopaminergic neurons is the main pathogenic characteristic of PD. A feasible treatment may be the local delivery of therapeutic proteins with neuroprotective and restorative properties into the neuronal pathways projecting to the dorsal striatum *via* axonal transport through dopaminergic neuron cell bodies in the SNpc. Additionally, the effects of PDGFs on neuronal therapeutic targets may be limited by other factors, such as the degradation of the PDGF protein itself, the role of clearance mechanisms *in vivo* (such as liver and kidney metabolism), and the possible combination

TABLE 1 | Clinical trials of platelet-derived growth factors (PDGFs) for disease treatment.

PDGF	Clinical trials	References
PDGF	It was confirmed that the PDGF purified from human placenta (EAP) can induce tritiated thymidine incorporation in Chinese hamster lung fibroblasts (CCL39).	(Marez et al., 1987)
PDGF	Autologous adipose tissue grafts for human immunodeficiency virus facial lipoatrophy achieved better results without the addition of PDGFs.	(Fontdevila et al., 2014)
PDGF	This clinical experiment confirmed that PDGF/IGF-1 can promote periodontal regeneration.	(Giannobile et al., 1994)
PDGF	This clinical trial demonstrated that PDGF can promote periodontal regeneration in localized bone defects.	(Nevins et al., 2013)
PDGF	PDGF can be used as adjunctive treatment for pressure ulcers; preoperative treatment with rhPDGF-BB showed a greater ability to heal wounds than surgery alone.	(Kallianinen et al., 2000)
rhPDGF	This clinical trial evaluated the effect of calcium hydroxide as a matrix carrier for recombinant human PDGF on pulp tissue healing after pulp capping.	(Al-Hezaimi et al., 2020)
PDGF inhibitor	The combined intravitreal injection of ranibizumab (a vascular endothelial growth factor inhibitor) and E10030 (a PDGF inhibitor) was preliminarily shown to be safe, but its therapeutic efficacy remains limited.	(Hwang et al., 2021)
PDGF inhibitor	In this phase IIb clinical trial, PDGF antagonist E10030 was administered in combination with the anti-vascular endothelial growth factor drug ranibizumab (Lucentis) in the treatment of neovascular age-related macular degeneration, showing a favorable safety and efficacy profile.	(Jaffe et al., 2017)
PDGF-B	In this phase I trial, the safety of H5.020cmv.pdgf-B was evaluated for the treatment of diabetic insensitive foot ulcers.	(Margolis et al., 2000)
PDGF-BB	Intra-arterial injection of bone marrow mononuclear cell in patients with subacute stroke induced changes in serum levels of PDGF-BB, which may be related to prognosis.	(Moniche et al., 2014)
PDGF-BB	This clinical trial demonstrated that PM coverage of periodontal defects was associated with the upregulation of initial gingival crevicular fluid growth factors, which could improve surgical outcomes.	(Gamal et al., 2016)
PDGF-BB	This clinical trial demonstrated that purified recombinant human platelet-derived growth factor-BB/beta-tricalcium phosphatecan be used as an effective autograft substitute.	(DiGiovanni et al., 2013)
PDGF-BB	rhPDGF-BB + β-TCP is safe and effective in the treatment of periodontal defects, increasing bone formation and soft tissue healing.	(Jayakumar et al., 2011)
PDGF-BB	This clinical trial validated the safety and efficacy of rhPDGF-BB for the treatment of periodontal bone defects.	(Nevins et al., 2005)
PDGF-BB	Topical application of rhPDGF-BB and (rh)insulin-like growth factor-I to periodontal lesions was found to be safe and promote bone regeneration.	(Howell et al., 1997)
PDGFR-α	This clinical trial demonstrated that patients with high pretreatment anti-PDGFRA antibody levels raise the risk-to-benefit ratio of nilotinib.	(Chen et al., 2018a)

with various components of peripheral tissues (Thorne and Frey, 2001). Invasive treatments are considered unethical for patients in the early stages of PD, and PDGFs can only be delivered directly to the patient's brain through intracranial surgery. For this reason, it is particularly important to develop an efficient peripheral PDGF delivery system for alternative therapies (Carl-Henrik and Bengt, 1999).

Treatment of Parkinson's Disease Based on the Interaction of Stem Cells and Platelet-Derived Growth Factors

In recent years, research on stem cells in the treatment of neurodegenerative diseases, including PD, has made great progress. Preclinical studies have shown the potential of mesenchymal stem cells (MSCs) for neural transplantation, as this subtype of stem cells is able to migrate to sites of damaged neural tissue, following bone marrow-derived MSCs or amniotic fluid-derived stem cells by intravenous and intracranial transplantation (Li et al., 2001; Chopp and Li, 2002; Hellmann et al., 2006; Cipriani et al., 2007). As a type of MSCs, human endometrial-derived stem-like cells (HEDSCs) have been characterized, which can transdifferentiate into cartilage, bone, fat, and muscle *in vitro* (Schwab and Gargett, 2007; Gargett et al., 2009). It has also been confirmed that HEDSCs are able to differentiate into dopamine-producing neurons and have the ability to migrate. *In vivo*, HEDSCs can be transplanted, they migrate to the diseased site, and differentiate spontaneously. The therapeutic benefits of increasing dopamine concentrations in a mouse model of immunocompetent PD have been also demonstrated based on flow cytometry and HEDSC characterization, which were strongly positive for both PDGF-Rβ and CD146 (Wolff et al., 2011).

Ebrahimi-Barough et al. (2013) exposed endometrial stromal cells to growth factors and mitogen PDGF-AA and obtained oligodendrocyte progenitor cells. Their RT-qPCR results verified that these cells expressed OPC markers, including PDGFRα. Furthermore, mir-338 was successfully used to promote oligodendrocyte differentiation of human endometrial-derived stromal cell (hEnSC)-derived OPC, and the data suggest that these cells can be differentiated into the pre-oligodendrocyte phenotype *in vitro* (Ebrahimi-Barough et al., 2013).

Pelegri et al. (2019) found that 100-ng/ml VEGF/PDGF had the greatest effect on the proliferation of hippocampal neural stem cells (HNSC), and the differentiation pathway induction in this treatment group showed the most significant oligodendrocyte and neuronal markers and morphological characteristics (Pelegri et al., 2019). These promising findings should be verified in further studies on brain and spinal cord injuries, including PD. Additional studies showing that PDGF

interacts with stem cells to affect cell function are summarized in **Table 2**.

The application of embryonic stem cells (ESCs) for PD therapy has been validated by multiple studies, yet the use of ESCs faces difficult ethical issues due to access and application methods. The discovery of the induced pluripotent stem cell (iPSC) technology has greatly helped address these problems. Specifically, numerous studies have demonstrated that iPSCs and ESCs are molecularly and functionally equivalent, avoiding the ethical issues and the constraints of traditional methods. This provides a meaningful solution for a sustainable source of pluripotent stem cells without the need for immunosuppressive agents to combat immune rejection after implantation therapy (Yefroyev and Jin, 2022). Similar to ESCs, iPSCs show self-renewal ability and multi-directional differentiation potential. In recent years, iPSCs and various neurons derived from them have been used in the treatment of neurodegenerative diseases, including PD. Rhee et al. (2011) demonstrated that the behavioral deficits in a rodent model of PD were significantly ameliorated by dopamine neurons derived from protein-based hiPSCs (human-induced pluripotent stem cells) (Rhee et al., 2011). Hartfield et al. (2014) also confirmed that hiPSC-induced dopaminergic neurons can synthesize, secrete, and reabsorb dopamine (Hartfield et al., 2014). Song et al. (2020) have recently developed a more efficient method for generating clinical-grade iPSCs by combining metabolic-regulating microRNAs with reprograming factors. The

TABLE 2 | A summary of the interaction between platelet-derived growth factors (PDGFs) and stem cells.

PDGF	Effect on stem/Progenitor cells	References
PDGF	Stem cells (human adipose-derived stem cells) incorporating PDGF and organisms (biological mineral coated fibers) can be used to successfully regenerate vascularized bones.	(Lee et al., 2020)
PDGF	PDGF can regulate extracellular vesicles of adipose-derived mesenchymal stem cellsto regulate protein expression and their functions.	(Lopatina et al., 2018)
PDGF	Hematopoietic stem cells overexpressing PDGF for regenerative therapy are beneficial for the improvement of myocardial function in rats, while the level of tissue connexin 43 and proangiogenic molecules increased after infarction.	(Das et al., 2009)
PDGF	The differential activation of phospholipases probably is significant for neurotrophic PDGF in HiB5 neuronal hippocampal stem cells. Neuronal differentiation by neurogenic PDGF in the HiB5 cells may be regulated by the activation of phospholipase C and D.	(Sung et al., 2001)
PDGFs	Platelet-rich plasma (PRP) immobilized on gelatin microspheres (GMs) by a mussel-inspired polydopamine (GM-pDA-PRP) was used for creating a microenvironment for the proliferation of adipose-derived stem cells. PDGF prolonged and localized production was induced by enhanced PRP adhesion.	(Zhou et al., 2017)
PDGF-AA	PDGF-AA and expression of exosome CD81 and CD9 can be secreted by cell-free stem cell-derived extract (CCM) formulated from human progenitor endothelial stem cells (hPESCs). CCM promoted cell proliferation and induced stem cell migration.	(Gupta et al., 2020)
PDGF-AA	Tppp3 + PDGFRA + cells are equivalent to tendon stem cells. Tppp3-PDGFRA + fibro-adipogenic progenitors coexist in the tendon stem cell niche and promote the production of fibrotic cells.	(Harvey et al., 2019)
PDGF-AA/PDGFRα	In mesenchymal stem cells (MSCs), PDGF-AA was found to activate the BMP-Smad1/5/8 pathway, which requires BMPRIA and PDGFRα together to promote MSC osteogenic differentiation and MSC migration.	(Li et al., 2014)
PDGFR-α	In zebrafish, trunk neural crest migration to the dorsal aorta is required for hematopoietic stem cell specification, which is regulated by PDGF signaling.	(Damm and Clements, 2017)
PDGF-AB PDGF-BB	The effectiveness of human serum on human adipose-derived stem cellproliferation depends on the concentrations of endogenous PDGFs.	(Damm and Clements, 2017)
PDGF-BB	Co-overexpression of PDGF-BB and IL-4 was found in co-infected MSCs, which promote cell proliferation and viability, as well as osteogenesis.	(Zhang et al., 2021)
PDGF-B	Gene embedded (pDNA-platelet-derived growth factor, PDGF-B) porcine acellular urinary bladder matrix with transfected mesenchymal stem cells can release PDGF-B, which promotes neovascularization and new tissue formation. The secretion of other growth factors was promoted by the expression of PDGF, leading to PDGF-mediatedregenerative activity.	(Paramasivam et al., 2021)
PDGF-BB	PDGF-BB-treated cells were associated with the endothelial network and expressed markers of perivascular cells while also promoting satellite cell self-renewal. The treated cells obtained the ability to migrate across the endothelium.	(Gerli et al., 2019)
PDGF-BB	The proliferation of mesenchymal stem cells in human periodontal ligament was promoted by PDGF-BB.	(Mihaylova et al., 2018)
PDGF-BB	PDGF-BB promoted fibroblast growth in factor 2 mouse embryonic stem cell conditioned medium (mESC-CM), which is important for the antisenescence effect of mESC-CM.	(Bae et al., 2016)
PDGF-BB	PDGF-BB promoted 3D-encapsulated mesenchymal stem cellsdose-dependent proliferation, spreading, and migration.	(Lienemann et al., 2015)
PDGF-BB	Treatment with PDGF-BB activated Akt phosphorylation, decreased p53 expression, and reduced radiation-induced apoptosis in mouse intestinal progenitor/stem cell.	(Liu et al., 2014)
PDGF-BB	PDGF-BB promoted the proliferation of human mesenchymal stem cells.	(Dong et al., 2015)
PDGF-BB	Membrane sections with higher PDGF-BB concentrations created a better environment for human adipose-derived stem celltenogenesis.	(Min et al., 2014)
PDGFR-β	The activation of PDGFR-β contributed to vascular smooth muscle cell differentiation.	(Shimizu et al., 2008)
PDGFR-β	PDGFR-β signaling pathways are involved in the differentiation of embryonic stem cells into smooth muscle cells.	(Xiao et al., 2007)

induced cells exhibit the electrophysiological characteristics of dopamine neurons, and these researchers reported that their transplantation into a PD rodent model potently restores motor function and reactivates the host brain, helping progress toward human personalized autologous cell therapy for PD (Song et al., 2020). The study by Kikuchi et al. (2017) demonstrated that human iPSC-derived dopaminergic progenitor cells survive and function as midbrain dopaminergic neurons in a PD primate model (a cynomolgus monkey) treated with the neurotoxin MPTP (Kikuchi et al., 2017). In addition, secondary changes in the GABAergic nervous system can also directly or indirectly affect the pathogenesis of PD (Mochizuki et al., 2008). Studies have also demonstrated that human-derived iPSCs can be directly induced into GABAergic neurons, thus providing candidate cells for PD therapy (Liu et al., 2013). However, while iPSCs can replace patient-specific diseased cells, this approach is not without limitations. For example, genomic instability and epigenetic aberrations must be considered, which may be caused by reprograming (Weissbein et al., 2014). Furthermore, due to the low degree of differentiation of iPSCs, once out of control, they may form difficult-to-treat tumors. At present, there is a lack of clinical research on the relationship between iPSCs and PDGFs, and their possible combined role in PD generation is unknown.

Parkinson's Disease Treatment Based on the Interaction of Genes and Platelet-Derived Growth Factors

Several studies have shown that PDGFs can be combined with other cells, carriers, cytokines, and biological materials to deliver effects to the injury site (Johnson et al., 2010). In terms of genes, Liu et al. (2011) constructed the hS100B transgenic vector by inserting the human *S100B* gene downstream to the PDGF promoter, which was then microinjected to generate transgenic mice. They confirmed that S100B overexpression in the brain leads to a motor coordination disorder, which may be related to the downregulation of D2DR and GRK2 expression, increased dopamine anabolism, and reduced 5-HT levels (Liu et al., 2011).

As a rapidly developing technology, the efficient delivery of genetic material in neurons using viral vectors is a key approach of gene therapy, thus modulating the expression of one or more specific genes. Gene therapy for PD has undergone three significant changes in the past two decades (Bjorklund and Davidsson, 2021), from *ex vivo* gene transfer using retroviral vectors to *in vivo* gene expression using adenovirus, herpes simplex virus (HSV), or lentiviral vectors. Recently, a major breakthrough has been made in the application of adeno-associated virus (AAV) vectors in CNS gene therapy, involving the repair of dopamine synthesis, strengthening of trophic factor production and lysosomal function, or alteration of the interactions between different functional nodes of the basal ganglia, including clinical trials for PD (Merola et al., 2020).

In the case of PD gene therapy, AAV capsid engineering shows some promise. For example, an AAV-2 vector containing a hybrid CMV E/PDGF promoter was developed and found to be superior in driving gene expression in SN dopaminergic neurons (Wang et al., 2005). However, not every cell surface can be targeted for AAV-engineered therapy, making precise and specific regulation of each target difficult. Although gene therapy is generally irreversible, in some cases, it can be modulated by modulating the inflammatory state and the cascade of apoptosis. Earlier approaches to inserting cell-type-specific promoters into the AAV genome to drive its transgene expression led to disappointing results with unsatisfactory specificity (Mudannayake et al., 2016). Current sequencing technologies allow the addition of short enhancer sequences upstream of minimal promoters, which helps accurately identify short enhancer elements with specificity. This approach was successfully used to identify enhancer elements associated with PD (Graybuck et al., 2021). Relevant dopamine neuron subtypes and disease states can be identified by the AAV vector, enabling precise-targeted expression of therapeutic transgenes, avoiding immune responses, and reducing the dose required. Engineered AAV vectors can be combined with cell therapy from exogenous cell sources, such as ESC or IPS cells, to facilitate dopamine engraftment for enhanced therapeutic efficacy. Therefore, AAV engineering has the potential to radically improve target specificity (Xiong et al., 2021).

In a future where autologous cell transplantation (including reprograming) is commonplace, any disease-causing genetic changes in host cells may be treated by viral vector engineering (e.g., alpha-synuclein overexpression) (Stoddard-Bennett and Reijo Pera, 2019). Such therapies can target macroautophagy, slow neuronal aging, and rejuvenate dopamine neurons, even in presymptomatic PD or in people at high risk of developing PD (Benito-Cuesta et al., 2017; Lu et al., 2020; Minhas et al., 2021). Therefore, genetically targeted therapy could be used for neuroprotection and the prevention of neurodegenerative diseases in the wider population. In addition, delivery strategies based on nanoparticles (NPs) can improve the stability of NTFs and, thus, have potential for clinical applications. NPs have been confirmed to function as non-viral vehicles to deliver drugs to different cells and organs (Oberdörster et al., 2007). The advantage of this approach is that they can passively accumulate in specific cells by improving the stability and physicochemical properties of active drugs. However, most of the current approved nanomedicines are cancer drugs; the application of NPs in PDGF-replacement support therapy for PD may become an important clinical strategy to slow down or even reverse disease progression in the future (Bondarenko and Saarma, 2021).

Parkinson's Disease Treatment Based on the Interaction of Epigenetic Modifications and Platelet-Derived Growth Factors

Epigenetics refers to changes in gene expression or function that occur without changes to the DNA sequence. This plays an important role in regulating neural development, neural stem cells, and the physiological functioning of the nervous system. In recent years, epigenetic modification, as the link between heredity and the environment, has gradually attracted attention for its relationship with the

pathogenesis of PD (Jasiulionis, 2018).Epigenetic modifications include DNA methylation, DNA hydroxymethylation, histone modifications, and non-coding RNA (ncRNA)-mediated changes in gene expression.

It has been confirmed that DNA methylation can regulate the PD pathogenic gene *SNCA* (Matsumoto et al., 2010). Based on Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-deactivated Cas9 (dCas9) fused with the catalytic domain of DNA-methyltransferase 3A (DNMT3A), Kantor et al. (2018) targeted methylation of *SNCA* intron 1 using a lentiviral vector, which resulted in the downregulation of SNCA mRNA and its protein in PD patient-derived neurons, thereby alleviating associated cellular phenotypic features, including mitochondrial ROS production and cellular activity (Kantor et al., 2018). In addition, the *MAPT* gene is associated with the pathogenesis of PD because it encodes the microtubule-associated protein tau. Different *MAPT* methylation levels have been observed in frontal cortex and peripheral blood leukocytes between patients with PD and the controls (Masliah et al., 2013). Marshall et al. (2020) found that more than 70% of 1,799 differentially methylated sites of enhancers in neurons of patients with PD had elevated methylation levels. Among the 2,885 genes involved, these researchers identified many risk genes related to the pathogenesis of PD, including *DCTN1*, *PRKN*, and *DJ-1*, and showed that the inactivation of the *TET2* gene can have a therapeutic effect on PD by reducing neuroinflammation (Marshall et al., 2020). Han et al. (2014) demonstrated that VSMC proliferation can be caused by the epigenetic regulation of PDGFs; they found that homocysteine induces the hypomethylation of the PDGF gene promoter region and upregulates its mRNA and protein expression (Han et al., 2014). However, the specific roles of PDGF methylation in PD need to be explored in the future.

In recent years, the DNA hydroxymethylation epigenetic modification has gradually attracted attention, as its correlation with neurodegenerative diseases has been verified (Sherwani and Khan, 2015). Studies have shown that, in patients with PD, the median level of the hydroxylated form of 5-methylcytosine, 5-hydromethylcytosine (5hmC), almost doubles that in controls (Stöger et al., 2017). This suggests that DNA hydroxymethylation is associated with the pathogenesis of PD, although there is still a lack of research in this area. Thus far, no research has focused on the DNA hydroxymethylation of PDGF genes.

Histones are octamers composed of H2A, H2B, H3, and H4, with tail residues presenting various types of post-transcriptional modifications, including methylation, acetylation, phosphorylation, ubiquitination, ADP ribosylation, deamination, proline isomerization, and lysine threoninylation. Studies have shown that histone acetylation levels are significantly elevated in midbrain dopaminergic neurons of patients with PD (Park et al., 2016). Curcumin, a histone deacetylase (HDAC) inhibitor, was found to reduce apoptosis and improve motor deficits in a DJ-1 knockout rat model of PD (Chiu et al., 2013). HDAC inhibitors are also able to reduce neuroinflammation by reducing microglial activation, thereby protecting dopaminergic neurons. On the other hand, as the main cause of autosomal dominant familial PD, the pathogenic effects of *LRRK2* mutations are closely related to histone modification. *LRRK2* can bind to the ser424 site of HDAC3 and directly phosphorylate HDAC, thereby stimulating its activity and promoting histone H4 deacetylation, resulting in gene transcriptional repression. In addition, increased nuclear DNA damage and abnormal histone methylation were observed in striatal neurons of *Lrrk2*-/-aged mice (Chen et al., 2020). Histone modifications are also present in certain neural factors promoting dopaminergic neuron growth. These include modifications in the promoter regions of glial cell line-derived neurotrophic factor (GDNF) and brain-derived neurotrophic factor (BDNF). A variety of HDAC inhibitors can also cause upregulation of GDNF and BDNF gene expression (Yue et al., 2014). Owens (2007) showed that phenotypic switching of smooth muscle cells (SMCs) in response to PDGF-BB *in vitro* is ended, owing to the loss of a subset of activating histone modifications at gene loci encoding SMC marker genes (Owens, 2007). This may be enlightening for the research related to PDGF histone modifications.

m^6A methylation is the most prevalent modification of eukaryotic mRNA. Foo et al. (2017) analyzed m^6A-modified genes in 1,647 sporadic patients with PD and 1,372 controls. These authors found 214 rare mutations in all m^6A-modified genes, and 16 common mutations were found in seven genes, implying that the pathogenesis of PD is related to m^6A methylation (Foo et al., 2017). Studies have also shown that m^6A reduction can induce the expression of NMDA receptor 1, increase oxidative stress and Ca^{2+} influx, and cause dopaminergic neuron apoptosis (Chen et al., 2019). At present, research on the role of m^6A methylation in the pathogenesis of PD remains very limited. Zeng et al. (2021) showed that, compared with that in the controls, m^6A methylation was increased in the lung tissues of a rat model with pulmonary arterial hypertension, and the up-methylated genes coding for the PDGF signaling pathway were primarily enriched (Zeng et al., 2021). Further research exploring the relationship between PDGFs and m^6A methylation is needed.

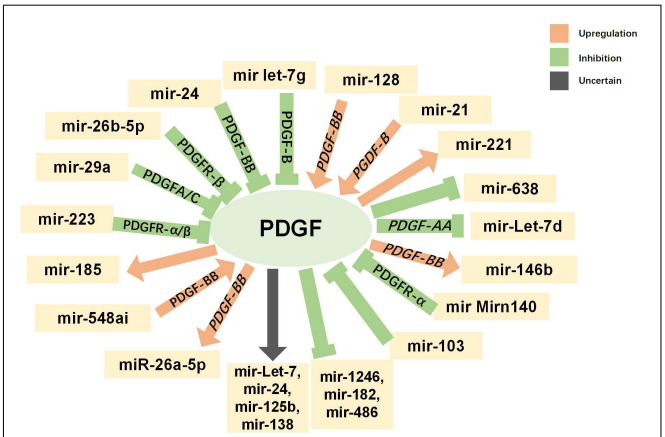

FIGURE 5 | Interaction between platelet-derived growth factors (PDGFs) and microRNAs. Green indicates inhibitory regulation; orange indicates facilitative regulation; and gray indicates unclear regulation.

Non-coding RNAs, including microRNAs (miRNAs) and long non-coding RNAs (lncRNAs), have been found to have varying expression levels in PD patients, and take part in regulating the pathogenetic mechanisms of PD (Roy et al., 2022). For example, miR-7 and miR-153 were proved to lower SNCA (synuclein α) expression levels (Junn et al., 2009; Doxakis, 2010), which is closely related to PD. Negatively controlled by LRRK2, miR-let-7 and miR-184 are involved in the reduction of dopamine levels in dopamine-producing cells (Gehrke et al., 2010). Both miRNAs and lncRNAs have been found to be diagnostic biomarkers for PD (Zhang et al., 2022). The interaction between PDGF and non-coding RNAs has been explored, especially in SMCs. MiR-24 can inhibit the proliferation and angiogenesis of VSMCs by suppressing the PDGF-BB signaling pathway (Yang et al., 2018). In PDGF-BB-stimulated airway smooth muscle cells (ASMCs), the expression of lncRNA PINT was reduced, while miR-26a-5p expression was increased (Gao et al., 2021). The current understanding of the interactions between non-coding RNAs (miRNA and lncRNA) and PDGF is summarized in **Figures 5,6** and more details are provided in **Tables 3**, **4**. As the association between PDGFs and non-coding RNAs in PD treatment has not been explored, the information in these tables may help provide ideas for future research on PD-related treatments.

A variety of epigenetically related drugs is currently under investigation. For example, DNMT inhibitors can simultaneously upregulate the transcription of neuroprotective genes, including TH genes and PD causative genes, such as *SNCA* and *UCHL1* (Wang et al., 2013). Although studies have confirmed that HDAC inhibitors can exert neuroprotective effects *in vitro* and *in vivo*, their mechanism of action in PD still needs further research (Hegarty et al., 2016). The study of epigenetic modifications in PD is of great significance for discovering potential biomarkers for early diagnosis of PD and its treatment; however, epigenetic modification itself is complex and affected by various factors, such as diet and environment. Therefore, the treatment of PD *via* epigenetic modification targets requires further basic and clinical research.

Parkinson's Disease Treatment Based on the Interaction of Exosomes and Platelet-Derived Growth Factors

Exosomes are extracellular vesicles secreted by various kinds of cells. These can be loaded with different cargos, including proteins, lipids, non-coding RNAs, and drugs. Exosomes are ideal carriers as the cargos can be uptaken by target cells without provoking an immune response and avoiding the impediment of the BBB (Wang et al., 2020). Exosomes have been found to play important roles in neurodegenerative diseases, including PD. There is growing evidence that exosomal miRNAs play a key role in PD progression (Cardo et al., 2013; Hoss et al., 2016; Wang et al., 2020). Meanwhile, feasibility of a miRNA exosome-based delivery system has been confirmed in an animal model. Mao et al. found that exosomes carrying miR-34a, released from astrocytes, can enhance the sensitivity of dopaminergic neurons to neurotoxins in a PD model by targeting Bcl-2 (Mao et al., 2015). Moreover, delivery of the loaded drugs to target neuronal cells may increase therapeutic efficacy. For example, Haney et al. developed an exosome-based delivery system for a potent antioxidant, catalase, to treat PD; the catalase-loaded exosomes provided significant neuroprotective effects both in *in vivo* and *in vitro* models of PD (Haney et al., 2015). Yang et al., 2021 demonstrated that exosome-mediated antisense oligonucleotide delivery significantly improved locomotor functions in α-syn A53T mice after exosome–drug injection.

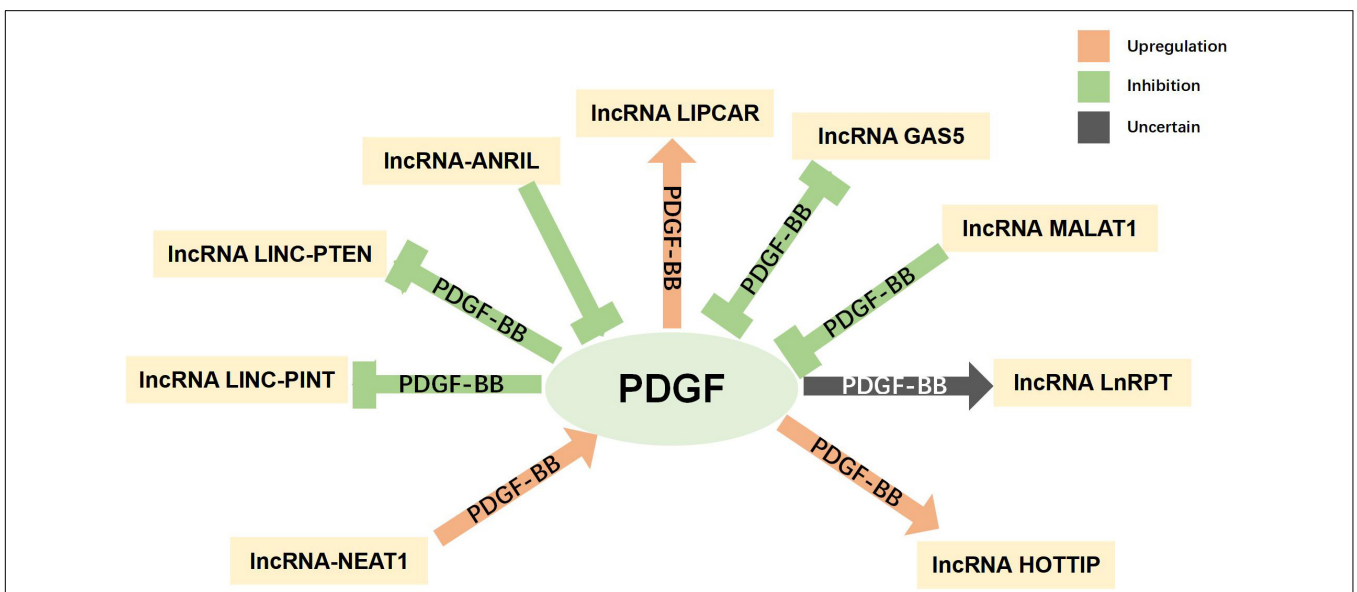

FIGURE 6 | Interaction between platelet-derived growth factors (PDGFs) and long non-coding RNA. Green indicates inhibitory regulation; orange indicates facilitative regulation; and gray indicates unclear regulation.

TABLE 3 | Interaction between platelet-derived growth factors (PDGFs) and microRNA.

MicroRNA	Mutual effects of microRNAS and PDGF	References
miR-548ai	MiR-548ai inhibitor mitigates endothelial cell dysfunction induced by exosomes (PDGF-BB, TGFβ1, TNFα, and IL1β) derived from dysfunctional smooth muscle cells.	(Xie et al., 2021)
miR-185	The level of extracellular miR-185 increased in PDGF-stimulated vascular smooth muscle cells.	(Si et al., 2021)
miR-223	Overexpression of miR-223 alleviates GLI family zinc finger 2and platelet-derived growth factor receptor α/βexpression in hepatic stellate cells (HSCs), thus inhibiting the activation and proliferation of HSC.	(Wang et al., 2021)
miR-29a	At the transcriptional and translational levels miR-29a decrease the expressions of PDGFC and PDGFA.	(Yang et al., 2019b)
miR-26b-5p	MiR-26b-5p produces a negative adjustment of PDGFR-β and interacts with non-coding RNA maternally expressed gene (lncMEG3).	(Yang et al., 2019a)
miR-24	MiR-24 can reduce the expression of AP-1 by suppressing the PDGF-BB signaling pathway, thereby inhibiting the proliferation and angiogenesis of vascular smooth muscle cells.	(Yang et al., 2018)
miR let-7g	By targeting PDGF-B genes, miRlet-7g suppresses the phenotypic switching of vascular smooth muscle cells.	(Wang et al., 2017)
miR-21	Via miRNA-21-mediated PDCD4 downregulation, PDGF-BB stimulates cell proliferation and promotes the development of thyroid-associated ophthalmopathy.	(Lee et al., 2016a)
miR-221	By inducing miR-221, PDGFs affect both cell proliferation and the epithelial-mesenchymal transition phenotype, thus leading to the downregulation of p27Kip1 and TRPS1.	(Su et al., 2013)
miR-638	miR-638 expression was decreased in proliferative human VSMCs and its expression inhibited both SMC proliferation and migration in response to PDGF stimulation.	(Li et al., 2013)
miR-Let-7d, miR-146b, miR-638	In proliferative human VSMCs, miR-638 expression was decreased and both SMC proliferation and migration in response to PDGF stimulation were suppressed. PDGF-AA inhibited miRlet-7d, while PDGF-BB induced miR-146b in cancer cells. The induction of miR-146b by PDGF-BB is regulated via MAPK-dependent induction of c-fos.	(Shao et al., 2011)
miR-Let-7, miR-24, miR-125b, and miR-138	The basal characteristics of multipotent mesenchymal stromal cell differentiation into osteoblasts is PDGF mediation of microRNA regulation, which may promote differentiation.	(Goff et al., 2008)
mirn140	mirn140 downregulates Pdgf signaling during palatal development. mirn140 function deficiency increases PDGFRA protein levels.	(Eberhart et al., 2008)
mir-103	MiR-103 was increased after baicalintreatment. PDGF-BB-induced abnormal proliferation of smooth muscle cells was inhibited by BA, whereas miR-103 knockdown suppressed proliferation.	(Zhai and Wang, 2022)
miR-26a-5p	In PDGF-BB-stimulated airway smooth muscle cells, the expression of long non-coding RNA LINC-PINT and PTEN was reduced, while miR-26a-5p expression was increased.	(Gao et al., 2021)
miR-128	The signaling pathway of NF-κB can be activated by lncRNA-NEAT1 competitively binding to miR-128, which promotes PDGF-BB-induced inflammatory response and the phenotype transformation of airway smooth muscle cells.	(Song et al., 2022)
miR-1246, miR-182,miR-486	PDGFs regulate exosomal miRNA release from vascular smooth muscle cells (VSMCs). miRNA (miR-1246, miR-182, and miR-486) expression decreased in exosomes derived from PDGF-stimulated VSMCs, which is related with an increase in endothelial cell migration.	(Heo et al., 2020)

PDGF-loaded exosomes have also been explored. Exosomes, including PDGF-AA derived from differentiating human skeletal myoblasts (HSkM), can induce *in vitro* myogenesis of human adipose-derived stem cells (HASCs) and improve *in vivo* muscle regeneration (Choi et al., 2016). Adipose tissue, bone marrow, and umbilical cord MSCs secrete exosomes holding PDGF-BB, which can promote keratinocyte and dermal fibroblast proliferation and migration (Hoang et al., 2020). The interaction between exosomes and PDGFs is summarized in **Table 5**.

Parkinson's Disease Treatment Based on Single-Cell Transcriptomics and Platelet-Derived Growth Factors

As an emerging technology, single-cell transcriptomics supports the direct analysis of gene expression at the single-cell level as well as the analysis of intracellular population heterogeneity and the definition of cell types, cell states, and dynamic transitions of cells. In addition to identifying novel cell subtypes and rare cell populations, single-cell sequencing techniques also contribute to understanding transcriptional dynamics and gene regulatory relationships. For example, Hook et al. (2018) characterized dopaminergic neuronal populations in the mouse brain at embryonic and early postnatal time points using single-cell RNA-seq. Their scRNA-Seq results confirmed 110 marker genes associated with PD in postnatal dopaminergic neurons, demonstrating how this approach opens up a new era in PD gene research (Hook et al., 2018). Lang et al. (2019) mimicked a disease model of PD in iPSC-dopamine neurons and further identified HDAC4 as a regulator of the PD cell phenotype. Their work demonstrates that single-cell transcriptomics can exploit cellular heterogeneity to clarify disease mechanisms and identify therapeutic targets (Lang et al., 2019). In an *in vitro* model of human PD, dopamine neuron-specific stress responses were revealed using single-cell

TABLE 4 | Interaction between platelet-derived growth factors (PDGFs) and long non-coding RNA.

Long non-coding RNA	Mutual effects of long non-coding RNA and PDGF	References
lncRNA-NEAT1	The signaling pathway of NF-κB can be activated by lncRNA-NEAT1 competitively binding to miR-128, which promotes PDGF-BB-induced inflammatory response and the phenotype transformation of airway smooth musclecells.	(Song et al., 2022)
lncRNA LINC-PINT lncRNA LINC-PTEN	In PDGF-BB-stimulated airway smooth muscle cells, the expression of long non-coding RNA LINC-PINT and PTEN was reduced, while miR-26a-5p expression was increased.	(Gao et al., 2021)
lncRNA-ANRIL	The phenotypic switchover of vascular smooth muscle cells (VSMCs) to the synthesis of phenotype derived by PDGF was suppressed by the overexpression of lncRNA-ANRIL, which also reversed the decrease in AMPK activity in PDGF-treated VSMCs.	(Hu et al., 2020)
lncRNA LIPCAR	In PDGF-BB- and ox-LDL-treated human VSMCs, the expression of lncRNA LIPCAR was significantly increased.	(Wang et al., 2019)
lncRNA GAS5	In PDGF-BB-treated vascular smooth muscle cells (VSMCs), growth arrest-specific transcript 5 (GAS5) was decreased. PDGF-BB-induced VSMC proliferation and migration was suppressed by overexpression of GAS5.	(Liu et al., 2019)
LncRNA MALAT1	The downregulation of MALAT1 decreased PDGF-BB-induced proliferation and migration by suppressing autophagy.	(Song et al., 2018)
LncRNA HOTTIP	The expression level of long non-coding RNA (lncRNA) HOTTIP was increased in proliferating endothelial cells induced by PDGF-BB.	(Liao et al., 2018)
LncRNA LnRPT	PDGF-BB regulates the expression of lncRNA in pulmonary artery smooth muscle cells.	(Chen et al., 2018b)

TABLE 5 | Interaction between platelet-derived growth factors (PDGFs) and exosomes.

PDGF	Role of exosomes	References
PDGF	Exosomal circ-ATP10A regulates PDGF expression and promotes multiple myelomaangiogenesis.	(Yu et al., 2022)
PDGF	Skin tissues in adipose-derived stem cell exosomes had higher levels of PDGFs than controls.	(Wu et al., 2021)
PDGF	Enrichment of extracellular vesicles (EVs) with activated receptor tyrosine kinases was reduced by inhibiting mTOR signaling in EV donor cells based on PDGF stimulation.	(Gao et al., 2020)
PDGF	PDGF regulates exosomal miRNA release from vascular smooth muscle cells (VSMCs). miRNA (miR-1246, miR-182, and miR-486) expression decreased in exosomes derived from PDGF-stimulated VSMCs, which was related to an increase in endothelial cell migration.	(Heo et al., 2020)
PDGF-A	The change of extracellular matrix composition was simulated by PDGF-A, which is important for the formation of aortic aneurysms through the induction of pathological phenotype switching of SMCs.	(Aschacher et al., 2021)
PDGF-AA	Exosomes including PDGF-AA derived from differentiating human skeletal myoblasts (HSkM) can induce *in vitro* myogenesis of human adipose-derived stem cells (HASCs) and improves *in vivo* muscle regeneration.	(Choi et al., 2016)
PDGF-AA,PDGF-BB	The levels of plasma endothelial cell-derived exosome and platelet-derived exosomeproteins related to atherosclerosis are different in patients with cerebrovascular disease.	(Goetzl et al., 2017)
PDGF-BB	Exosomal miR-301a-3p derived from adipose-derived mesenchymal stem cellscan lighten remodeling and inflammation of airway smooth muscle cells stimulated by PDGF-BB.	(Feng et al., 2022)
PDGF-BB	Adipose tissue mesenchymal stem cells, bone marrow mesenchymal stem cells, and umbilical cord mesenchymal stem cells secreted exosomes containing PDGF-BB, which can promote keratinocyte and dermal fibroblast proliferation and migration.	(Hoang et al., 2020)
PDGF-B	The exosomes released from astrocytes exerted neurotoxic effects on neurons, which was related to increased miR-29b and decreased PDGF-B expression.	(Hu et al., 2012)
PDGFRβ	Activated platelet-derived exosomes including miR-223, miR-339, and miR-21 can be transferred into smooth muscle cells and decrease PDGFR-β expression.	(Tan et al., 2016)

transcriptomics, with implications for cell replacement therapy. The progression of PD involving multiple cell types, such as astrocytes, oligodendrocytes, and microglia, has also been validated with single-cell transcriptomics (Tiklová et al., 2020). Current research on single-cell omics in PD still needs to be developed to improve our understanding of the etiology of PD at the level of different cell types and has an irreplaceable role in targeted therapy.

Single-cell transcriptomics has been used to determine the roles of PDGFs in certain physiological and pathological mechanisms. Based on single-cell transcriptional profiling, Gan et al. found that the PDGF cascade is an important cue in the nucleus pulposus microenvironment of healthy human intervertebral disks (Gan et al., 2021). With the help of single-cell transcriptome profiling of fetal osterix-expressing cells, Bohm et al. identified PDGF-PDGFRβ

signaling as a critical functional mediator of skeletal stem and progenitor cell expansion, migration, and angiotropism during bone repair (Böhm et al., 2019). No studies, however, have used single-cell transcriptomics to explore the roles of PDGFs in the pathophysiological mechanisms or treatment of PD.

Parkinson's Disease Treatment Based on the Interaction of Clustered Regularly Interspaced Short Palindromic Repeats and Platelet-Derived Growth Factors

The CRISPR-Cas system, a series of RNA-guided endonucleases, is an adaptive immune defense against viruses or foreign gene integration formed by bacteria during long-term evolution. The type II CRISPR system includes four genes (*Cas9*, *Cas1*, *Cas2*, and *Csn1*) and two non-coding RNAs (pre-crRNA and tracrRNA) that can target and degrade foreign DNA in a sequence-specific manner (Garneau et al., 2010). Soldner et al. (2016) used the CRISPR/Cas9 gene-editing method to introduce two mutations into isogenic human pluripotent stem cells, changing only the genes on one chromosome and leaving the other chromosome unchanged. Although one of these two mutations had no effect, the other mutation (from A to G) increased SNCA expression (Soldner et al., 2016). This approach could potentially be used to identify other causative genes in sporadic PD. Wang et al. (2016) also used the CRISPR/Cas9 system to inject Cas9 mRNA and a variety of sgRNAs into Bamaxiang pig prokaryotic embryos, targeting three different loci—DJ-1, Parkin, and Pink1. The piglets appeared healthy and behaved normally, with no manifestations of mutations being identified. This shows that the CRISPR/Cas9 system has great potential in the treatment of PD (Wang et al., 2016). Guhathakurta et al. (2021) further developed a CRISPR/dCas9-based site-specific H3K4me3 demethylation system that recruited the demethylase JARID1A catalytic domain to the SNCA promoter, significantly reducing the level of H3K4me3 modification at the SNCA promoter. At the same time, the level of α-synuclein was significantly reduced (Guhathakurta et al., 2021). The gene-editing technology mediated by the CRISPR system can not only accurately introduce disease-causing genes into specific cells to construct human disease model cells but also achieve repair through homologous recombination mediated by the CRISPR/Cas technology. This provides new opportunities for treatment at the gene level for genetic variant-related neurodegenerative diseases, including PD.

The CRISPR/Cas9 system, as well as PDGFR-B siRNA and pCMV3-PDGFR-B plasmid, was used to identify PDGFR-β as a potential therapeutic target for pterygium (Mai et al., 2019). Lin et al. established a thyroid cancer cell line with 95.6% PDGFRA gene insertion and deletions through CRISPR/Cas9, and demonstrated that xenograft distant lung metastasis was completely eliminated by PDGFRA gene editing; therefore, PDGFRA could be an effective target to inhibit distant metastasis in advanced thyroid carcinoma (Lin et al., 2021). CRISPR/Cas9-mediated depletion of PDGFRβ in cell lines derived from epiretinal membranes of patients with proliferative vitreoretinopathy attenuated vitreous-induced Akt activation and cell proliferation, migration, and contraction (Yang et al., 2020b). Therefore, the CRISPR/Cas9 system is a promising method to better understand the association between PD and PDGFs, and to treat PD with PDGF-derived drugs.

CURRENT CHALLENGES AND FUTURE DIRECTIONS

As the global population continues to age, the incidence of PD is increasing in a substantial portion of the population. For this reason, the pathogenesis and clinical treatment of PD remain a primary focus of research. Based on our review of the current literature, as a member of the NT family, PDGFs are known to play a significant role in neurodegenerative diseases, and their effects on the course of PD are increasingly considered in the context of CNS cells, including mitochondrial function, oxidative stress, regulation of Ca^{2+} homeostasis, protein folding and aggregation, and neuroinflammation. We have also summarized several possible approaches and opportunities for clinical application.

Although there is still a general lack of research on PDGFs, we believe that the application of PDGFs to the emerging approaches, such as gene precision therapy, stem cell-based therapy, and the exosome-based delivery system, will significantly impact the diagnosis and treatment of PD. As we mentioned before, the study of epigenetic modifications in PD is the key to discovering potential biomarkers for early diagnosis and treatment; since PDGFs have been found to have crucial roles in PD, the potential for PDGF epigenetically related drugs is huge. Moreover, with the help of exosome delivery systems, recipient neuronal cells can be targeted without provoking an immune response and without the impediment of the BBB. In addition, delivery strategies based on NPs can improve the stability of PDGFs and have potential clinical applications. Furthermore, with the advancement of relevant studies, CRISPR/Cas9-mediated alteration of PDGF-related genes in neuronal cells will also be an interesting option to explore.

In the current age of precision gene therapy, it is particularly important to find the right target to treat PD. Single-cell transcriptomics of different neuronal cell types has an irreplaceable role in providing precise targets for this disease. Previous studies have found that PDGFs can not only be epigenetically regulated but also delivered precisely *via* exosomes. However, more cellular and animal studies are needed to prove the roles of PDGFs in these new strategies against PD. The unknown confounding factors, especially in the intricate human microenvironment, present an enormous challenge. As abioscaffold containing PDGFs and other chemokines has been used in the field of bone regeneration (Hao et al., 2021; Ngah et al., 2021), three-dimensional printing, which allows for the creation

of suitable biomechanical microenvironments that include PDGFs, may be a valuable strategy to explore in the future.

AUTHOR CONTRIBUTIONS

DL, L-TH, QL, and J-HW collected, analyzed, and interpreted current literature, and drafted the manuscript. C-pZ, QL, and J-HW helped revise the manuscript. All of the authors have read and approved the final manuscript.

FUNDING

This study was supported by National Key R&D Program of China, Nos. 2018YFC2002100, 2018YFC2002104.

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