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RESEARCH ARTICLE

Exploring the links among peripheral immunity, biomarkers, cognition, and neuroimaging in Alzheimer's disease

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Abstract

INTRODUCTION: We analyzed relationships among peripheral immunity markers, cognition, Alzheimer's disease (AD)-related biomarkers, and neuroimaging to understand peripheral immunity involvement in AD.

METHODS: Peripheral immunity markers were assessed in AD, non-AD neurodegenerative disorders, and controls, examining their connections with cognition, AD-related biomarkers, and neuroimaging using multiple regression models.

RESULTS: The study included 1579 participants. Higher levels of white blood cell, neutrophil, monocyte, neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), systemic immune-inflammation index (SII), and lower lymphocyte-to-monocyte ratio (LMR) were associated with cognitive decline and more severe anxiety and depression. The impact of lower LMR, lymphocyte count, and higher NLR on cognitive decline is mediated through cerebrospinal fluid amyloid beta (Aβ) levels. Additionally, increased PLR, NLR, and SII were associated with brain atrophy and hippocampal Aβ deposition (amyloid positron emission tomography).

DISCUSSION: Peripheral immunity markers offer a non-invasive and cost-effective means of studying AD-related pathophysiological changes, providing valuable insights into its pathogenesis and treatment.

KEYWORDS
Alzheimer's disease, amyloid beta, cerebrospinal fluid, cognition, neuroimage, peripheral immunity

Highlights

- Peripheral immunity markers linked to cognitive decline and anxiety/depression.
- Low LMR, LYM, and high NLR linked to reduced CSF Aβ, impacting cognition.
- High PLR, NLR, SII associated with brain atrophy and hippocampal Aβ deposition

1 | BACKGROUND

As life expectancy rises, there is a notable escalation in the global socioeconomic burden posed by neurodegenerative disorders such as Alzheimer's disease (AD), Parkinson's disease (PD), and frontotemporal dementia (FTD). Today, ≈ 6.2 million Americans aged ≥ 65 have AD, and without significant medical advancements, this number could rise to 13.8 million by 2060.[1] An increasing number of studies suggest that inflammation is a major contributor to the development of AD. While an inflammatory response in the brain can have beneficial effects in promoting tissue repair and removing cellular debris, sustained and excess responses can be detrimental and inhibit neuronal regeneration.[2]

Previously, it was believed that immune dysfunction in AD primarily affected the immune process within the central nervous system. However, accumulating evidence now suggests that the peripheral immune system also plays a crucial role in the development and progression of diseases. Communication between the brain and the peripheral immune system occurs through various pathways, including the blood–brain barrier, choroid plexus, and meninges.[3–5] This process leads to an augmentation in the population of peripheral blood cells engaged in inflammation and immune response. Nevertheless, there is limited understanding regarding the direction and timing of these pathways, as well as the potential for therapeutic interventions. The elevated neutrophil-to-lymphocyte ratio (NLR) has been recognized as a noteworthy independent predictor for AD,[6] PD,[7] and progressive supranuclear palsy (PSP).[8] Furthermore, it has the potential to forecast disease progression and survival rates in individuals with sporadic amyotrophic lateral sclerosis (ALS)[9,10] and multiple system atrophy (MSA).[11] Although previous studies have shown that platelet-to-lymphocyte ratio (PLR), monocyte-to-lymphocyte ratio (MLR), and systemic immune-inflammation index (SII) can also predict inflammatory status in various conditions, there is limited research on their involvement in neurogenerative diseases. Nonetheless, our understanding of the impact of peripheral immunity on AD is still in its early stages. Given the compelling recent findings, further research is needed to comprehensively explore the peripheral immune cells and their contribution to brain health and AD.

In this study, we aimed to investigate the associations among peripheral immunity markers, cognition, AD-related biomarkers, and neuroimaging data. The findings contribute to a better understanding of the involvement of peripheral immunity in AD, and suggest that peripheral immunity markers may serve as a non-invasive and cost-effective biomarker for AD-related pathophysiological change.

RESEARCH IN CONTEXT

1. **Systematic review**: We reviewed PubMed literature on peripheral immunity markers. Elevated neutrophil-to-lymphocyte ratio (NLR) predicts Alzheimer's disease (AD), Parkinson's disease, and progressive supranuclear palsy. It shows potential in predicting disease progression and survival rates in sporadic amyotrophic lateral sclerosis and multiple system atrophy. Limited research exists on platelet-to-lymphocyte ratio (PLR), monocyte-to-lymphocyte ratio (MLR), and systemic immune-inflammation index (SII) in neurodegenerative diseases.
2. **Interpretation**: Higher white blood cell, neutrophil, monocyte levels, NLR, PLR, SII, and lower lymphocyte-to-monocyte ratio (LMR) are associated with cognitive decline, severe anxiety, and depression. Lower LMR, lymphocyte count, higher NLR impact cognition via cerebrospinal fluid amyloid beta (Aβ) levels. Increased PLR, NLR, and SII relate to brain atrophy and hippocampal Aβ deposition (amyloid positron emission tomography).
3. **Future directions**: These findings enhance our understanding of peripheral immunity in AD and suggest that peripheral immunity markers may serve as non-invasive, cost-effective biomarkers for AD-related pathophysiological changes.

2 | METHODS

2.1 | Participants

Participants with available blood count data were selectively recruited from the department of Neurology at Huashan Hospital between June 2019 and March 2023. The study population included individuals of Chinese Han ethnicity. We excluded patients without neurodegenerative diseases such as cerebrovascular diseases (stroke and intracranial hemorrhage), epilepsy, central nervous system infectious diseases, demyelinating diseases, and so forth. Additionally, participants with conditions such as malignant neoplasms, blood and blood-forming organ diseases, autoimmune diseases, and chronic inflammatory diseases, which could potentially affect leukocyte differential counts, were also excluded from the study. The diagnosis of neurodegenerative diseases, such as AD,[12] mild cognitive impairment (MCI),[13] FTD,[14] MSA,[15] PSP,[16] PD,[17] motor neuron disease (MND),[18] and dementia with Lewy bodies (DLB),[19] were established according to widely acknowledged consensus statements. Participants were diagnosed using Chinese Modified Mini-Mental State Examination (CM-MMSE) and the Montreal Cognitive Assessment (MoCA). The cut-off values after correcting for years of education used in the CM-MMSE were ≤24 for > 6 years of education, ≤20 for < 6 years of education, ≤17 for 0 years of education and for MoCA were <24 for >12 years of education, <22 for 7 to 12 education years, and <19 for <7 years of education. All procedures complied with the Declaration of Helsinki, and ethics approval was received from the institutional review board.

2.2 | Peripheral immunity markers

The complete blood count was processed immediately using the Mindray BC-6800 fully automated hematology analyzer. We gathered baseline count data for white blood cells (WBCs), neutrophils (NEs), monocytes, lymphocytes (LYMs), and platelets. From these data, we derived several inflammation indicators, including the NLR, lymphocyte-to-monocyte ratio (LMR), PLR, and SII. These indicators have been demonstrated in previous studies to be reliable predictors of inflammatory status across different conditions.[20]

2.3 | Plasma and cerebrospinal fluid biomarkers

Plasma levels of amyloid beta (Aβ)1-42 (Aβ1-42), phosphorylated tau181 (p-tau181) and neurofilament light chain (NfL) were quantified using single molecule arrays. Cerebrospinal fluid (CSF) levels of total tau (t-tau), p-tau181, and Aβ1-42 were measured using enzyme-linked immunosorbent assay kits. The assays used in this study demonstrated good precision, with inter- and intra-assay coefficients of variation < 20% and 10%, respectively. The lower limits of quantification for each biomarker, as provided by the manufacturers, were as follows: 0.378 pg/mL for Aβ1-42, 0.400 pg/mL for NfL, and 0.085 pg/mL for p-tau181. All plasma and CSF samples in this study were tested above these thresholds.

2.4 | Cognitive and neuropsychological assessments

Global cognition was evaluated using the Mini-Mental Status Examination (MMSE) and MoCA scales. Cognitive domain scales were assessed using the Digit Span Test (DST) and Boston Naming Test (BNT). Neuropsychological scales, including the Hamilton Anxiety Scale (HAM-A) and Hamilton Depression Scale (HAM-D), were used to evaluate anxiety and depression levels, respectively.

2.5 | Image acquisition and processing

A subgroup of 286 patients underwent structural magnetic resonance imaging (MRI) on a 3.0 T MRI scanner (Discovery 750, GE Healthcare). Among them, 199 patients received metabolic (fluorodeoxyglucose [18F-FDG]) positron emission tomography (PET) scans using a Biograph mCT Flow PET/computed tomography scanner (Siemens Healthcare). Additionally, 61 patients underwent tau (18F-Florzolotau [previously known as 18F-APN-1607]) PET imaging, while 113 patients underwent amyloid (18F-florbetapir [AV45]) PET imaging. The T1-weighted MRI scans were acquired with specific parameters, including sagittal slice orientation, 1.0 mm slice thickness, 184 slices per slab, 1.0 × 1.0 mm in-plane resolution, 256 × 256 matrix size, 3.2 ms echo time, 8.5 ms repetition time, 400 ms inversion time, and 12° flip angle. All scans passed a visual quality control check for artifacts before further processing. To analyze the MRI data, each participant's T1-weighted magnetization-prepared rapid acquisition gradient echo (MP-RAGE) image, acquired within 1 week, was segmented and parcellated using FreeSurfer, version 6.0 (Martinos Center for Biomedical Imaging), based on the Desikan–Killiany and Aseg atlases. This segmentation allowed for the extraction of brain region volumes. PET images were subsequently co-registered to the corresponding MP-RAGE scans using Statistical Parametric Mapping version 12. The co-registered PET images were used to extract relevant data for further analysis.

2.6 | Statistical analyses

Baseline demographic characteristics were compared using appropriate statistical tests. Continuous variables were described as mean (standard deviations), while categorical variables were presented as numbers (percentages). The comparison of peripheral immunity markers between the two diagnostic groups was conducted using analysis of variance (ANOVA), adjusting for age, sex, education, and body mass index (BMI). To explore the associations between peripheral immunity markers and cognitive function, as well as their relationships with plasma and CSF biomarkers and neuroimaging, multiple linear regressions were conducted, adjusting for age, sex, education, and BMI. Prior to conducting regression analysis, we standardized all variables using the *Z*-scale transformation for ease of comparison. This transformation was applied using the "scale" function in R software, where $z = (x - u)/s$, with u denoting the sample mean and s denoting the sample standard deviation.

Moreover, mediation analyses were conducted to examine whether the relationships between peripheral immunity markers and clinical phenotypes were mediated by plasma and CSF biomarkers, which serve as proxies for neuropathology. Four criteria were met to establish mediation: (1) significant associations between peripheral immunity markers and plasma/CSF biomarkers; (2) significant associations between peripheral immunity markers and clinical scales; (3) significant associations between plasma/CSF biomarkers and clinical scales; and (4) attenuation of the associations between peripheral immunity markers and clinical scales when including plasma/CSF biomarkers as mediators in the regression model. The "mediate" package in R was used to estimate the mediation or indirect effect, using 10,000 bootstrapped iterations for significance testing. Age, sex, education, and BMI were included as covariates in the mediation model.

All statistical analyses were performed using R version 4.1.2, and a two-tailed P value < 0.05 was considered statistically significant. In cases in which multiple comparisons were addressed, a more conservative significance level based on false discovery rate (FDR) correction was applied.

3 | RESULTS

3.1 | Demographics

A total of 1579 participants were included in this study. The demographic, clinical, and CSF/plasma biomarkers of the included participants are shown in Table 1. Based on different pathological

TABLE 1 Baseline clinical characteristics.

	Mean (SD)						
	CN ($n = 40$)	MCI ($n = 97$)	AD ($n = 440$)	SYN ($n = 474$)	Tau ($n = 165$)	TDP-43 ($n = 172$)	Other ($n = 190$)
Demographic characteristics							
Age	55.15 (12.05)	60.95 (11.31)	61.01 (9.05)	61.17 (9.86)	66.63 (7.54)	59.05 (11.53)	50.68 (15.48)
Sex = Male (%)	19 (47.5)	53 (54.6)	164 (37.3)	275 (58.0)	96 (58.2)	88 (51.2)	103 (54.2)
Education	11.02 (3.96)	9.97 (3.75)	8.82 (4.51)	8.19 (4.36)	8.08 (4.63)	9.18 (4.32)	8.89 (4.56)
BMI	23.97 (3.72)	23.28 (3.27)	22.26 (3.19)	23.96 (3.91)	24.84 (5.11)	23.13 (3.65)	22.36 (4.29)
Peripheral immunity markers							
WBC	5.79 (1.38)	5.47 (1.42)	5.52 (1.41)	5.56 (1.42)	5.97 (1.42)	5.60 (1.35)	5.57 (1.38)
NE	3.16 (0.93)	3.19 (1.06)	3.38 (1.23)	3.43 (1.12)	3.71 (1.18)	3.43 (1.21)	3.31 (1.14)
LYM	2.04 (0.68)	1.73 (0.53)	1.67 (0.50)	1.61 (0.51)	1.71 (0.51)	1.72 (0.56)	1.71 (0.56)
MNC	0.36 (0.12)	0.33 (0.10)	0.33 (0.11)	0.34 (0.10)	0.38 (0.10)	0.34 (0.10)	0.36 (0.12)
NLR	1.70 (0.70)	1.97 (0.76)	2.22 (1.20)	2.34 (1.17)	2.37 (1.13)	2.15 (0.92)	2.15 (1.03)
PLR	113.39 (50.15)	118.87 (38.04)	126.87 (47.95)	126.43 (49.33)	127.85 (48.25)	125.16 (44.46)	129.75 (51.91)
LMR	6.03 (2.12)	5.46 (1.89)	5.38 (1.79)	5.00 (1.70)	4.78 (1.67)	5.28 (1.91)	5.19 (2.11)
SII	349.56 (152.13)	375.02 (166.91)	441.71 (289.92)	437.75 (251.01)	480.69 (260.91)	426.88 (224.99)	439.45 (253.22)
Cognitive and neuropsychological assessments							
MMSE	27.11 (2.36)	24.34 (5.10)	14.28 (7.08)	23.06 (6.12)	18.47 (7.84)	18.20 (8.49)	23.56 (8.86)
MoCA	23.54 (3.83)	17.87 (5.73)	8.29 (5.66)	16.59 (6.68)	12.26 (6.49)	12.92 (7.60)	17.33 (7.22)
HAM-A	8.55 (5.37)	7.26 (5.03)	8.69 (6.41)	9.88 (7.35)	10.64 (6.53)	8.23 (6.94)	7.31 (6.04)
HAM-D	6.85 (5.42)	5.50 (4.09)	6.50 (5.24)	7.72 (5.86)	7.81 (4.61)	6.53 (5.77)	6.49 (5.46)
VFT	13.61 (5.34)	11.08 (4.69)	8.20 (7.42)	11.16 (5.67)	9.18 (8.61)	8.63 (5.00)	11.58 (5.52)
BNT	22.50 (4.68)	18.53 (5.26)	13.76 (6.53)	18.61 (5.85)	16.17 (6.11)	13.90 (8.27)	19.52 (5.95)
DST	12.93 (3.08)	10.65 (3.71)	9.15 (4.77)	10.41 (4.82)	10.09 (4.46)	10.99 (7.57)	11.23 (4.09)
AD-related biomarkers							
CSF Aβ1-42	1222.43 (631.89)	1029.57 (558.45)	595.98 (384.36)	922.33 (404.33)	2113.20 (254.84)	1236.72 (480.75)	748.04 (270.74)
CSF t-tau	183.82 (100.18)	281.70 (124.88)	569.70 (269.20)	325.01 (131.76)	473.26 (136.71)	381.37 (139.80)	231.21 (118.48)
CSF p-tau181	28.57 (15.02)	34.48 (17.09)	115.18 (53.93)	36.67 (18.98)	72.87 (13.17)	41.26 (24.57)	27.59 (20.84)
Plasma Aβ1-42	6.32 (1.08)	4.72 (1.30)	4.51 (1.21)	4.74 (1.50)	5.64 (1.65)	5.82 (1.75)	4.75 (1.36)
Plasma p-tau181	1.18 (0.38)	1.78 (0.74)	4.46 (1.94)	2.05 (1.48)	2.25 (1.88)	2.90 (2.17)	1.46 (0.52)
Plasma NfL	12.84 (4.80)	19.86 (12.12)	31.18 (16.40)	36.08 (35.48)	49.65 (32.03)	87.64 (83.44)	33.17 (24.75)

Abbreviations: Aβ, amyloid beta; AD, Alzheimer's disease; BMI, body mass index; BNT, Boston Naming Test; CN, cognitively normal; CSF, cerebrospinal fluid; DST, Digit Span Test; HAM-A, Hamilton Anxiety Rating Scale; HAM-D, Hamilton Depression Rating Scale; LMR, lymphocyte-to-monocyte ratio; LYM, lymphocytes; MCI, mild cognitive impairment; MMSE, Mini-Mental State Examination; MNC, mononuclear cells; MoCA, Montreal Cognitive Assessment; NE, neutrophils; NfL, neurofilament light chain; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; p-tau, phosphorylated tau; SD, standard deviation; SII, systemic immune-inflammation index; SYN, synucleinopathies, including Parkinson's disease, multiple system atrophy, and dementia with Lewy bodies; TDP-43, TAR DNA-binding protein 43; t-tau, total tau; VFT, verbal fluency test; WBC, white blood cell.

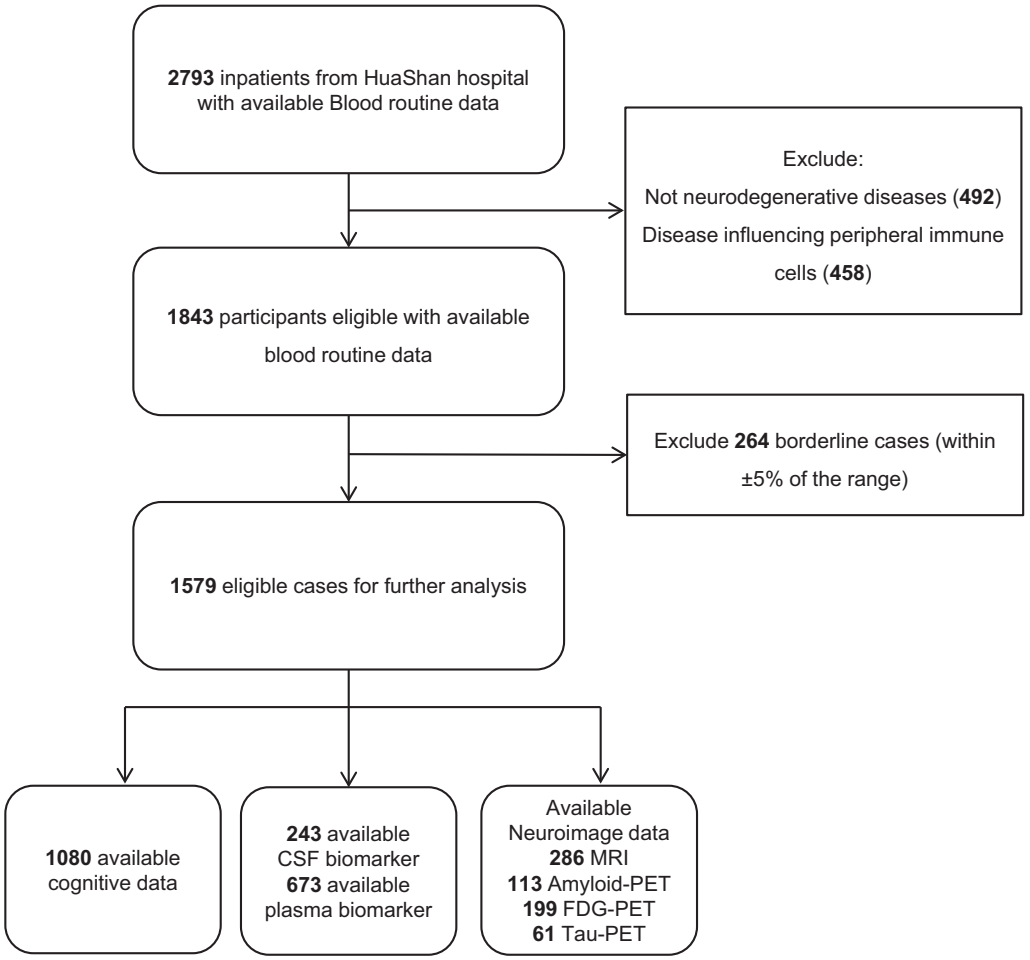

FIGURE 1 The flow diagram of the participants in this study. CSF, cerebrospinal fluid; FDG, 18F-fluorodeoxyglucose; MRI, magnetic resonance imaging; PET positron emission tomography.

subtypes, we categorized patients into: (1) AD, including logopenic variant primary progressive aphasia and posterior cortical atrophy; (2) tauopathies, including PSP and corticobasal degeneration; (3) TAR DNA-binding protein 43 (TDP-43) proteinopathies, including FTD and ALS; (4) synucleinopathies, including PD, MSA, and DLB; and (5) other, including essential tremor, Huntington's disease, hereditary spastic paraplegia, normal pressure hydrocephalus, and spinocerebellar ataxia. Overall, the mean age of participants was 60.03 (±11.35) years and 798 (50.54%) of them were males; 30.65% had more than 12 years of education. We added a flowchart to demonstrate the participant screening process (Figure 1). The levels of NEs, mononuclear cells (MNCs), and NLR showed positive associations with age (NE: $\beta = 0.08$, $P = 0.001$; MNC: $\beta = 0.001$, $P = 0.02$; NLR: $\beta = 0.01$, $P < 0.001$). In contrast, the levels of LYMs and LMR exhibited negative associations with age (LYM: $\beta = -0.006$, $P < 0.001$; LMR: $\beta = -0.03$, $P < 0.001$; Figure S1 in supporting information). The levels of WBCs, NEs, MNCs, NLR, PLR, and SII were higher in males compared to females. Conversely, the LMR levels were lower in males compared to females (Figure S2 in supporting information).

3.2 | Peripheral immunity marker levels in different diagnostic groups

The peripheral immunity markers across different diagnostic groups are illustrated in Figure 2, allowing for a visual examination of their distributions. Furthermore, a comparative analysis was conducted among the diagnostic groups, as shown in Figure S3 in supporting information, to investigate the differences in these indicators.

In terms of WBC and MNC levels, there were no statistically significant differences observed among the six groups in the intergroup comparisons. However, when examining NE levels, the tauopathies group showed a significant increase compared to the cognitively normal (CN) group, while no differences were observed among the other diagnostic groups. Furthermore, when considering LYM, NLR, and PLR levels, the CN group displayed lower levels compared to the other five diagnostic groups. The NLR levels in both the synucleinopathies group and tauopathies group were higher compared to the MCI group. Regarding the LMR, except for the MCI group, which showed no significant difference compared to the CN group, the levels in the other

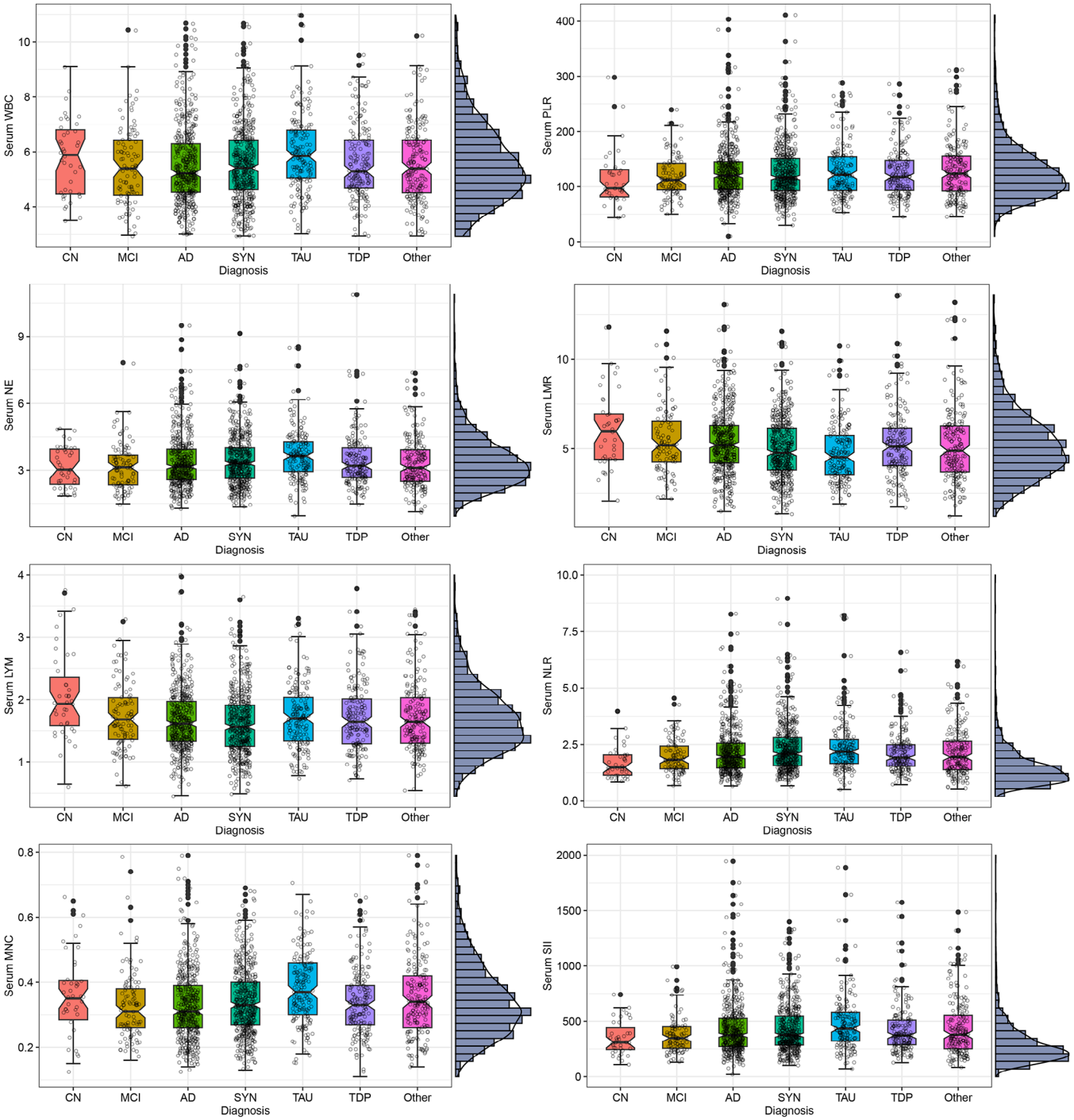

FIGURE 2 Distribution of peripheral immunity markers across diagnostic groups. AD, Alzheimer's disease (containing posterior cortical atrophy); CN, cognitively normal; MCI, mild cognitive impairment; SYN, synucleinopathies, including Parkinson's disease, multiple system atrophy, and dementia with Lewy bodies; TAU, tauopathies, including progressive supranuclear palsy and corticobasal degeneration; TDP, TAR DNA-binding protein 43 proteinopathies, including frontotemporal dementia and amyotrophic lateral sclerosis; Other, including essential tremor, Huntington's disease, hereditary spastic paraplegia, normal pressure hydrocephalus, and spinocerebellar ataxia.

diagnostic groups were lower than those in the CN group. Specifically, the LMR levels in the tauopathies group and SYN group were lower compared to the MCI and AD groups. Additionally, the LMR levels in the tauopathies group were lower than those in the TDP-43 proteinopathies group. In terms of SII, except for the MCI group, which showed no significant difference compared to the CN group, the levels in the other diagnostic groups were higher than those in the CN group. Specifically, the tauopathies group exhibited higher SII levels compared

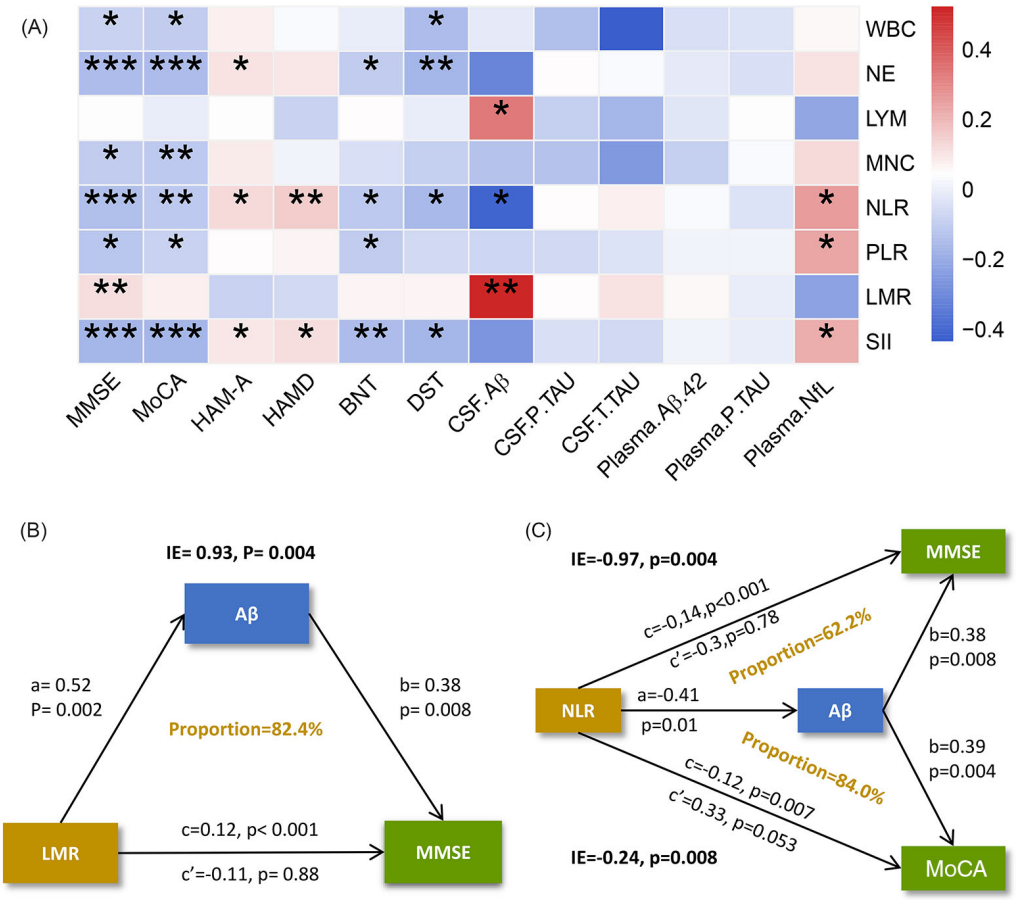

FIGURE 3 Associations of peripheral immunity markers with cognition and CSF/plasma biomarkers. A, Heat map shows associations of blood inflammation indicators with cognition and CSF/plasma biomarkers, with colors representing the association coefficients (β) of multiple linear regressions. The color bar represents the range of β values. Models were adjusted for age, sex, education, and BMI. Controlling for multiple comparisons was performed with the false discovery rate method of Benjamini and Hochberg. Significance:*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, -$P \geq 0.05$. B, Results of mediation analyses. Aβ, amyloid beta; BMI, body mass index; BNT, Boston Naming Test; CSF, cerebrospinal fluid; DST, Digit Symbol Test; HAM-A, Hamilton Anxiety Scale; HAM-D, Hamilton Depression Scale; LMR, lymphocyte-to-monocyte ratio; LYM, lymphocytes; MMSE, Mini-Mental State Examination; MoCA, Montreal Cognitive Assessment; MNC, monocytes; NfL, neurofilament light chain; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; SII, systemic immune-inflammation index; WBC, white blood cells.

to the other diagnostic groups. Furthermore, the synucleinopathies group showed higher SII levels compared to the MCI group.

3.3 | Associations of peripheral immunity markers with CSF/plasma biomarkers

We investigated the associations between amyloid and tau PET scans and the plasma and CSF biomarkers of AD. The results are presented in the Table S1 in supporting information. The levels of blood LYMs ($\beta = 0.33$, $P = 0.03$) and LMR ($\beta = 0.52$, $P = 0.002$) were positively associated with high CSF Aβ levels. Conversely, the levels of NLR ($\beta = -0.41$, $P = 0.01$) were negatively associated with high CSF Aβ levels. Additionally, NLR ($\beta = 0.26$, $P = 0.02$), PLR ($\beta = 0.23$, $P = 0.04$) and SII ($\beta = 0.22$, $P = 0.03$) were positively associated with high plasma NfL levels (Figure 3A, Table S2 in supporting information).

3.4 | Associations of peripheral immunity markers with cognitive/neuropsychological assessments

The blood levels of WBC were negatively associated with MMSE ($\beta = -0.09$, $P = 0.04$), MoCA ($\beta = -0.10$, $P = 0.02$) and DST scores ($\beta = -0.16$, $P = 0.04$). NE levels were negatively associated with MMSE ($\beta = -0.15$, $P < 0.001$), MoCA ($\beta = -0.15$, $P < 0.001$), BNT ($\beta = -0.10$, $P = 0.03$), and DST scores ($\beta = -0.17$, $P = 0.009$), while positively associated with HAM-A ($\beta = 0.10$, $P = 0.04$). MNC levels were negatively associated with MMSE ($\beta = -0.11$, $P = 0.02$) and MoCA scores ($\beta = -0.25$, $P = 0.007$). NLR levels were negatively associated with MMSE ($\beta = -0.14$, $P < 0.001$), MoCA ($\beta = -0.12$, $P = 0.006$), BNT ($\beta = -0.12$, $P = 0.02$), and DST scores ($\beta = -0.16$, $P = 0.03$), while positively associated with HAM-A ($\beta = 0.12$, $P = 0.01$) and HAM-D scores ($\beta = 0.15$, $P = 0.004$). PLR levels were negatively associated with MMSE ($\beta = -0.12$, $P = 0.04$), MoCA ($\beta = -0.08$, $P = 0.049$), and BNT scores

($\beta = -0.10$, $P = 0.04$). LMR levels were positively associated with MMSE scores ($\beta = 0.12$, $P = 0.009$). SII levels were negatively associated with MMSE ($\beta = -0.17$, $P < 0.001$), MoCA ($\beta = -0.17$, $P < 0.001$), BNT ($\beta = -0.15$, $P = 0.002$), and DST scores ($\beta = -0.17$, $P = 0.01$), while positively associated with HAM-A ($\beta = 0.10$, $P = 0.048$) and HAM-D scores ($\beta = 0.11$, $P = 0.03$; Figure 3A, Table S3 in supporting information).

3.5 | Associations of peripheral immunity markers with neuroimaging markers

High NLR levels were associated with atrophy in several brain regions, including the left insula, right fusiform gyrus, thalamus, lateral orbitofrontal cortex, superior temporal gyrus, as well as bilateral putamen. Elevated PLR levels were linked to atrophy in the left caudal middle frontal gyrus, inferior temporal gyrus, posterior cingulate cortex, precuneus, rostral anterior cingulate cortex, superior frontal gyrus, as well as the bilateral hippocampus, pallidum, and putamen. Moreover, elevated SII levels were associated with atrophy in the left caudal middle frontal gyrus, lateral orbitofrontal cortex, rostral middle frontal gyrus, superior frontal gyrus, and insula, as well as the right fusiform gyrus, lateral orbitofrontal cortex, precentral gyrus, and rostral middle frontal gyrus. Bilateral atrophy was also observed in the hippocampus, putamen, pallidum, and right amygdala, along with the right thalamus. Furthermore, plasma NLR showed significant positive associations with the uptake of the amyloid tracer 18F-AV45 in the left hippocampus, while SII demonstrated strong positive associations with the uptake of 18F-AV45 in the right hippocampus. However, we did not observe any associations between peripheral immunity markers and FDG-PET or TAU-PET (Figure 4, Figures S4-S7 in supporting information).

3.6 | Mediation analysis

Based on the aforementioned associations, we further observed that the relationship between LMR and MMSE scores was totally mediated by Aβ, with a mediation proportion of 82.4% (Figure 3B). Similarly, the association between NLR and MMSE scores was totally mediated by Aβ, with a mediation proportion of 62.2%. Additionally, the association between NLR and MoCA scores was totally mediated by Aβ, with a mediation proportion of 84.0% (Figure 3C).

4 | DISCUSSION

In this study, we thoroughly investigated the connections among eight peripheral immunity markers and various biomarkers, cognitive function, and neuroimaging markers in a large cross-sectional cohort of the Chinese population. Our findings revealed compelling evidence of connections between WBCs, NEs, MNCs, NLR, PLR, LMR, and SII with cognitive function decline. Moreover, we observed significant relationships among NEs, NLR, and SII with the severity of anxiety and depression, while LYMs, NLR, and LMR exhibited connections with CSF Aβ levels. Furthermore, NLR, PLR, and SII demonstrated significant links with plasma NfL levels and brain atrophy. Notably, NLR and SII were also associated with the uptake of 18F-AV45 in the hippocampus, indicating their potential involvement in AD.

In neurodegenerative diseases such as AD, PD, and MSA, neuroinflammation within the brain is characterized by the reactive morphological changes observed in glial cells. This cellular and molecular response is similar across different diseases and even resembles the reaction seen in conditions like stroke or traumatic injury. Due to methodological limitations, there have been challenges in conducting widespread in vivo assessments of immune dysfunction within the central nervous system (CNS) in humans. Nonetheless, a growing body of evidence suggests that neurodegenerative diseases are accompanied by an inflammatory response in the CNS, which is reflected, to some extent, by the increased expression of pro-inflammatory markers in the CSF. A study demonstrated a negative association between CSF MLR and executive function in individuals with aging and dementia. This finding highlights the value of CSF inflammation indicators in identifying connections between innate immune dysfunction and neurodegenerative processes.[21] This immune dysfunction was previously thought to be restricted to the CNS, but accumulating data indicate pivotal contributions of the peripheral immune system as well.

Consistent with most previous studies, we found the levels of peripheral immunity markers differed between sexes and changed with age.[22,23] This could be explained by estradiol, which has been found to prolong the survival of neutrophils by delaying their apoptosis and decreasing the production of LYMs in the bone marrow.[24,25] Research has indicated that as individuals age, their immune system changes both in composition and function. These changes lead to increased levels of chronic inflammation and a decreased capacity to mount effective immune responses against pathogens. In aging mice, there is a shift in hematopoietic stem cells toward producing more myeloid cells at the expense of lymphoid cells, and similar patterns have been observed in elderly humans.[26,27]

Our study revealed that LYM levels were significantly lower in all disease groups compared to the CN group, consistent with previous research.[28,29] However, there were no differences in WBC and monocyte levels among the groups. In terms of NE levels, we observed a difference only between the CN group and the tauopathies pathology group. The variations in the ratio of inflammatory cells between the groups can largely be attributed to variations in LYM levels. A decrease in LYM count indicates a significant reduction in the body's ability to defend against infections. The decrease in peripheral LYM cells in AD can be attributed to three reasons. First, changes in the thymic environment may hinder LYM differentiation. Second, CD8+ LYMs may be trapped in the brain of AD patients. Third, degenerative processes and the presence of immunostimulatory molecules may attract T cells to specific sites. The role of these invading T cells in AD is still unclear, as they can both contribute to inflammation and offer protective functions. Additionally, genetic instabilities may shorten the lifespan of LYMs in AD. These findings suggest that LYMs and NEs may

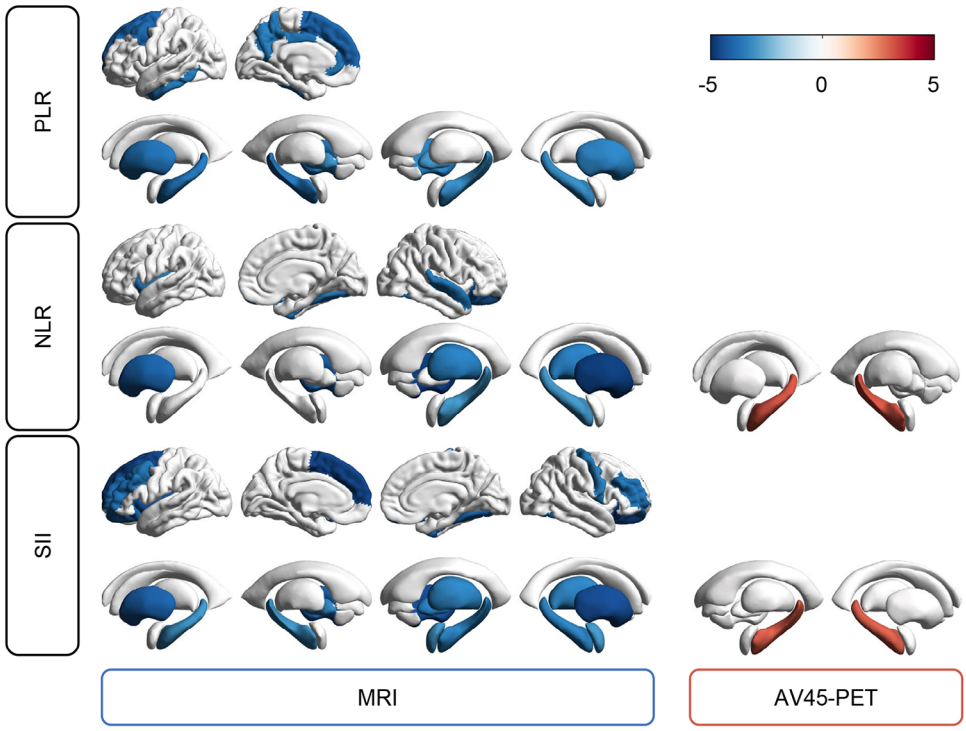

FIGURE 4 Associations of peripheral immunity markers with volumes of the cortical and subcortical regions. The *t* value was generated from multivariable regression analyses after adjusting for age, sex, education, and BMI. Controlling for multiple comparisons was performed with the false discovery rate method of Benjamini and Hochberg. The color bar represents the range of *t* values. AV45 PET, 18F-florbetapir positron emission tomography; BMI, body mass index; MRI, magnetic resonance imaging; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; SII, systemic immune-inflammation index.

be involved in the pathogenesis of neurodegenerative diseases through certain mechanisms, necessitating further research.

LYMs, NLR, and LMR are highly correlated with the levels of Aβ in CSF. Furthermore, through mediation analysis, we found that the impact of NLR and LMR on cognitive function is fully mediated by Aβ. The prevailing consensus from numerous prior studies suggests an overall increase in functional T cells in the blood of AD patients.[30,31] However, these previous studies generally lack a comprehensive analysis of AD biomarkers, warranting a more detailed investigation. Aβ is a characteristic pathological feature of AD, and our research findings suggest that LYMs may be involved in the pathophysiological processes of AD, potentially occurring in the early stages of the disease. APP is cleaved by β-secretase and γ-secretase to produce Aβ, which forms amyloid plaques.[32,33] Autoantibodies to Aβ have been found to be elevated in AD patients and mice, indicating Aβ can act as a self-antigen.[34] Aβ-reactive T cells were also detected in the blood of AD patients.[35] Unexpectedly, recent research has revealed that CD4+ T cells exhibit high levels of beta-secretase (BACE1) expression. BACE1 can cleave the extracellular portion of amyloid precursor protein, resulting in the production of Aβ. Moreover, BACE1 has been found to play a role in activating T cells in both the experimental autoimmune encephalomyelitis and AD mouse models.[36] A recent study convincingly demonstrated that elevations in antigen-experienced adaptive immune cells in the bloodstream are strongly correlated with the status of cerebral Aβ and its dynamic changes over time.[37]

In addition, we found significant associations among peripheral immunity markers and cognitive function, anxiety and depression, blood NfL levels, and brain atrophy. These markers are classified under the "N" category in the ATN (amyloid/tau/neurodegeneration) framework, representing neuronal death. Numerous studies have consistently reported a connection between peripheral markers of inflammation and cognitive function.[38–40] Regarding WBCs, there is substantial evidence suggesting a link among various types of leukocytes and endothelial dysfunction, thrombosis formation, and the generation of superoxide radicals. Moreover, leukocytes have been implicated in the development of vascular complications.[41,42] A prospective follow-up study conducted by Vinkers et al. demonstrated that generalized atherosclerosis contributes to cognitive decline in older adults.[43] Taking these findings into account, it can be inferred that elevated WBC counts are associated with the progression of vascular damage in the human brain. Studies have revealed that neutrophils play a role in AD models. In the APP/PS1 mouse model, removing neutrophils through antibody injection resulted in improved blood flow to the brain and better performance in the object replacement task.[44] Similarly, in the 5XFAD and 3XTg-AD models, sustained neutrophil depletion during the early stages of the disease led to reduced glial activation in the brain, enhanced performance in contextual fear conditioning and Y-maze tasks, and prolonged cognitive benefits.[45] The main reason behind these findings is that neutrophils enter the brain of these mice and gather near Aβ plaques,

where they release neutrophil extracellular traps, leading to increased oxidative stress, brain tissue damage, glial activation, and sustained brain inflammation. It is possible that neutrophils have an impact on cognitive function in AD, and regulating their activity may be a viable therapeutic option to alleviate related impairments.

This study is constrained by the sample size, and as a result, future research could further explore the utility of the p-tau181/Aβ42 ratio as a biological marker with a larger and more diverse sample population.

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CONFLICT OF INTEREST STATEMENT

The authors declare no competing interests. Author disclosures are available in the supporting information.

CONSENT STATEMENT

All participants or legal guardians gave their written informed consent. All procedures complied with the Declaration of Helsinki, and ethics approval was received from the institutional review boards.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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