Microglial Reactivity Correlates with Presynaptic Loss Independent of β-Amyloid and Tau

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Objective: Triggering receptor expressed on myeloid cells-2 (TREM2) and progranulin (PGRN) are critical regulators of microglia activation and can be detected in cerebrospinal fluid (CSF). However, whether microglial reactivity is detrimental or neuroprotective for Alzheimer disease (AD) is still debatable.

Methods: We identified 663 participants with baseline β-amyloid (Aβ) positron emission tomography (PET) and CSF biomarker data, including phosphorylated tau181 (p-Tau$_{181}$), soluble TREM2 (sTREM2), PGRN, and growth-associated protein-43 (GAP-43). Among them, 254 participants had concurrent longitudinal CSF biomarkers. We used multivariate regression analysis to study the associations of CSF microglial biomarkers with Aβ PET, CSF p-Tau$_{181}$, and CSF GAP-43 cross-sectionally and longitudinally. A Chinese aging cohort's independent CSF samples (n = 65) were analyzed as a validation.

Results: Higher baseline levels of CSF microglial biomarkers were related to faster rates of CSF sTREM2 increase and CSF PGRN decrease. Elevated CSF p-Tau$_{181}$ was associated with higher levels of CSF microglial biomarkers and faster rates of CSF sTREM2 increase and CSF PGRN decrease. In both cohorts, higher Aβ burden was associated with attenuated CSF p-Tau$_{181}$ effects on CSF microglial biomarker increases. Independent of Aβ PET and CSF p-Tau$_{181}$ pathologies, higher levels of CSF sTREM2 but not CSF PGRN were related to elevated CSF GAP-43 levels and faster rates of CSF GAP-43 increase.

Interpretation: These findings suggest that higher Aβ burden may attenuate the p-Tau-associated microglial responses, and TREM2-related microglial reactivity may independently correlate with GAP-43-related presynaptic loss. This study highlights the two-edged role of microglial reactivity in AD and other neurodegenerative diseases.

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Alzheimer disease (AD) is characterized by the accumulation of β-amyloid (Aβ) plaques and neurofibrillary tau tangles concurrent with progressive neuronal and synaptic loss strongly correlated with cognitive decline.[1–3] Although the underlying mechanism linked to synaptic loss is not well established, microglial-mediated phagocytosis and neuroinflammation in the brain, which are now acknowledged as central players in AD pathogenesis, may contribute to synaptic degeneration beyond the synaptotoxic effects of Aβ and tau.[1,4,5] Triggering receptor expressed on myeloid cells-2 (TREM2) and progranulin (PGRN) are critical regulators of microglial activation,

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phagocytosis, and proliferation.[6,7] *Trem2* deficiency prevented microglia-mediated synaptic removal by directly inhibiting microglial phagocytosis, and *Trem2* overexpression conversely exacerbated synaptic impairment.[8,9] *Pgrn* deficiency could also facilitate microglia-mediated synaptic elimination through complement activation.[6,10] Although the findings in animals are sufficiently investigated, knowledge of the involvement of microglia in the synaptic loss in humans of aging and AD is still limited.

Soluble TREM2 (sTREM2) and PGRN, primarily shedding from microglia, can be detected in cerebrospinal fluid (CSF). Thus, the CSF levels of sTREM2 and PGRN are considered markers of microglial TREM2 and PGRN signaling, reflecting the status of microglial reactivity.[11–13] This was supported by decreased CSF and plasma levels of sTREM2 and PGRN in patients with loss-of-function variants of *Trem2* and *Pgrn*.[14–16] In mice carrying the *Trem2* p.T66M missense mutation, the generation of brain sTREM2 also apparently decreased, accompanied by reduced activation and phagocytosis of microglia.[17] Previously, cross-sectional studies demonstrated that patients with symptomatic AD had elevated CSF sTREM2 and CSF PGRN in relation to CSF levels of Aβ and phosphorylated tau (p-Tau), indicating microglial immune responses to primary AD pathologies.[12,13,18] Accumulating evidence from human and animal models showed that higher CSF sTREM2 and microglial activation were associated with reduced Aβ and tau deposition.[19–21] Elevated CSF sTREM2 was also related to attenuated brain atrophy, glucose hypometabolism, and cognitive decline.[19,21–24] However, one recent study reported the accelerated effects of CSF sTREM2 and microglial activation on future tau deposition.[25] Supporting this, CSF sTREM2 has also been linked to Aβ-related tau aggregates.[24,26] Together, the roles of TREM2-dependent and PGRN-dependent microglial reactivity in the course of AD remain controversial.

Here, we investigated the associations of CSF microglial biomarkers (sTREM2 and PGRN) with primary AD pathologies and subsequent presynaptic loss using extensive cross-sectional and longitudinal data from the Alzheimer's Disease Neuroimaging Initiative (ADNI) cohort and validated the primary analyses in an independent aging cohort from China. The specific aims of this study were to determine (1) how CSF microglial biomarkers correlate with each other and their association with primary AD pathologies and (2) how CSF microglial biomarkers correlate with presynaptic biomarker growth-associated protein-43 (GAP-43) in CSF. This study may provide novel insights into understanding the association of Aβ plaques, CSF p-Tau, and presynaptic loss with microglial reactivity and have critical clinical implications for the therapeutic strategies targeting microglia in neurodegenerative diseases.

Subjects and Methods

Participants

The data in this study were obtained from the ADNI database (ida.loni.usc.edu). The ADNI was established in 2003 known as a public–private partnership, led by principal investigator Michael W. Weiner, MD. The main objective of ADNI is to determine whether serial magnetic resonance imaging (MRI), positron emission tomography (PET), other biological markers, and clinical and neuropsychological assessments can be combined to track the progression of mild cognitive impairment (MCI) and early AD. The ADNI study was approved by institutional review boards of all participating centers, and written informed consent was obtained from all participants or their authorized representatives.

We identified 663 participants in this study, including 202 cognitively unimpaired (CU), 350 with MCI, and 110 with dementia. All participants had simultaneous (within 1 year) baseline ^{18}F-florbetapir (FBP) Aβ PET and CSF biomarker data, including p-Tau$_{181}$, sTREM2, PGRN, and GAP-43. Among them, 254 participants (77 CU, 166 MCI, and 11 dementia) had concurrent longitudinal CSF biomarker data with at least two measurements in a median (range) of 2.1 (1.3–6.0) years of follow-up. Following clinical cognitive status at baseline, all participants were divided into CU and cognitively impaired (CI; including MCI and dementia).

Additionally, we collected 65 participants (8 CU, 21 MCI, 36 dementia) with CSF samples from the Greater-Bay-Area Healthy Aging Brain Study (GHABS) in China (ClinicalTrials.gov ID: NCT06183658) as a validation cohort.[27]

CSF Biomarker Measurements

In the ADNI cohort, CSF p-Tau$_{181}$ was quantified using the fully automated Roche Elecsys at the University of Pennsylvania.[28] CSF microglial biomarkers were measured using a Meso Scale Discovery (MSD) platform-based assay (Haass group) at Ludwig Maximilian University of Munich.[13,18] CSF GAP-43 was measured by an in-house ELISA as previously described.[29] Linear mixed effect (LME) models were used to calculate slopes of CSF biomarkers for all the participants with longitudinal CSF data, controlling for the following independent variables: time, age, sex, and a random slope and intercept.

In the validation cohort, CSF Aβ$_{42}$ and CSF p-Tau$_{181}$ were quantified using the commercial Neurology 4-plex E kit (cat: 103670; Quanterix, Lexington, MA) and Advantage V2.1 kit (cat: 104111, Quanterix) by the Simoa HD-X. CSF sTREM2 was measured using an MSD platform-based assay developed by the Haass group.[13,18] CSF GAP-43 was detected using the commercial Human GAP-43 ELISA Kit (cat: abx250779, Abbexa, Cambridge, UK). All CSF biomarker measurements were conducted at the Shenzhen Bay Laboratory.

Amyloid PET and MRI

More details on FBP PET image and structural MRI acquisition can be found online (http://adni-info.org). FBP PET data were acquired in 4 × 5-minute frames from 50 to 70 minutes post-injection. PET images were motion-corrected, time-averaged, and summed into one static frame. Cortical FBP uptakes in 68 regions of interest defined by the Desikan–Killiany atlas[30] in FreeSurfer (V7.1.1) were extracted from each FBP PET scan that coregistered to individual corresponding structural MRI scan (closest in time to FBP PET scan). A composite standardized uptake value ratio (SUVR) was calculated by referring FBP uptake in AD summarized cortical regions (including frontal, cingulate, parietal, and temporal regions) to the mean uptake in the whole cerebellum.[31] The Aβ positivity was defined as AD summarized cortical regions with SUVR ≥ 1.11.[31]

Statistical Analysis

Statistical analyses were performed using the statistical program R (v4.1.1, R Foundation for Statistical Computing). Data in this study are summarized as the number (%) or median (range). We compared the demographics and clinical characteristics between CU and CI participants using either a 2-tailed Mann–Whitney *U* test or Fisher exact test with a significance threshold value of $p < 0.05$. LME models were used to investigate the longitudinal changes in CSF sTREM2 and CSF PGRN over time, and multivariable linear regression models were used to explore their association with each other cross-sectionally and longitudinally. Age, sex, education, and *APOE-ε4* status were included as covariates for all the models in this study.

To investigate how Aβ and p-Tau pathologies affect the TREM2-related and PGRN-related microglial reactivity, we used baseline Aβ PET or CSF p-Tau_{181} as dependent variables, and baseline or longitudinal CSF microglial biomarkers as independent variables in multivariate linear regression models. We also tested the interaction effect between Aβ PET and CSF p-Tau_{181} on CSF microglial biomarkers, as shown in the following equation:

$$CSF\ microglial\ biomarkers \sim A\beta\ PET \times CSF\ p-Tau_{181} + covariates \quad (1)$$

Subsequently, we performed mediation analyses (R; Lavaan package) to determine further the association of Aβ PET, CSF p-Tau_{181}, and CSF microglial biomarkers.

Another major aim of this study was to assess the direct effects of TREM2-related and PGRN-related microglial responses on presynaptic loss. To this end, we explored the association of CSF microglial biomarkers with CSF GAP-43 cross-sectionally and longitudinally. To investigate the independent effect of CSF microglial biomarkers, the main effects of Aβ PET and CSF p-Tau_{181} were further adjusted in our models. We also tested the interaction effect between Aβ PET and CSF microglial biomarkers on CSF GAP-43. The equation for the interaction is as follows:

$$CSF\ GAP-43 \sim CSF\ microglial\ biomarkers \times A\beta\ PET + CSF\ p-Tau_{181} + covariates \quad (2)$$

To determine whether our findings were driven by clinical impairment or the presence of abnormal amyloid plaques, we repeated all analyses when restricting the models to CU, CI, or Aβ+ participants only.

Results

Table 1 summarizes all participants' baseline demographics, CSF biomarker levels, and Aβ PET stratified by cognitive status. The CU and CI subgroups significantly differed in sex, duration of education, and prevalence of *APOE-ε4* carriers but not in age. Longitudinal data of CSF biomarkers are also displayed in Table 1. The demographics of the validation cohort are shown in Supplementary Table S1.

Longitudinal Changes in CSF Microglial Biomarkers

Overall, CSF sTREM2 showed significant increases (standard β [β_{std}] = 273, 95% confidence interval [ci] = 214–333, $p < 0.001$) over time, in contrast to the slight decreases in CSF PGRN ($\beta_{std} = -13.3$, 95% ci = −26.3 to −0.3, $p = 0.045$). Higher baseline levels of CSF microglial biomarkers were associated with faster rates of increase in CSF sTREM2 (sTREM2: $\beta_{std} = 0.81$, 95% ci = 0.73–0.90, $p < 0.001$; PGRN: $\beta_{std} = 0.24$, 95% ci = 0.12–0.37, $p < 0.001$) but with faster rates of decrease in CSF PGRN (sTREM2: $\beta_{std} = -0.25$, 95% ci = −0.37 to −0.12, $p < 0.001$; PGRN: $\beta_{std} = -0.93$, 95% ci = −0.98 to −0.89, $p < 0.001$). Furthermore, higher CSF sTREM2 was associated with elevated CSF PGRN at baseline ($\beta_{std} = 0.34$, 95% ci = 0.26–0.41, $p < 0.001$), but faster rates of increase in CSF sTREM2 were related to more rapid decrease in CSF PGRN over time ($\beta_{std} = -0.21$, 95% ci = −0.33 to −0.08, $p < 0.001$). Congruent results were obtained on testing in CU, CI, and Aβ+ participants (Supplementary Table S2).

Aβ Plaques Attenuate CSF p-Tau_{181}-Associated CSF Microglial Biomarker Increases

Across all participants, elevated CSF p-Tau_{181} was associated with higher baseline levels of CSF sTREM2 ($\beta_{std} = 0.42$, 95% ci = 0.35–0.50, $p < 0.001$) and CSF PGRN ($\beta_{std} = 0.25$, 95% ci = 0.17–0.33, $p < 0.001$) as well as faster rates of increase in CSF sTREM2 ($\beta_{std} = 0.41$, 95% ci = 0.29–0.53, $p < 0.001$) and decrease in CSF PGRN ($\beta_{std} = -0.17$, 95% ci = −0.30 to −0.04, $p = 0.012$; Fig 1). The associations with CSF

TABLE 1. Demographics and Characteristics of Participants

Characteristics at Baseline	All, n = 663	CU, n = 202	CI, n = 461
Age, yr	72.4 (55.2–91.5)	72.6 (56.4–86.0)	72.4 (55.2–91.5)
Female	299 (45%)	106 (52%)	193 (42%)
APOE-ε4 carrier	308 (46%)	57 (28%)	251 (54%)
Education, yr	16 (8–20)	16 (8–20)	16 (9–20)
Aβ PET, SUVR	1.15 (0.84–2.00)	1.05 (0.84–2.00)	1.26 (0.84–2.00)
CSF biomarkers			
p-Tau$_{181}$, pg/ml	23.3 (8.0–97.0)	19.1 (8.0–60.1)	25.4 (8.2–97.0)
sTREM2, pg/ml	3,421 (504–12,012)	3,514 (504–12,012)	3,391 (518–11,714)
PGRN, pg/ml	1,524 (538–3,664)	1,519 (538–2,806)	1,534 (654–3,664)
GAP-43, pg/ml	4,614 (1,088–19,971)	4,306 (1,167–17,927)	4,724 (1,088–19,971)
Longitudinal CSF biomarkers	n = 254	n = 77	n = 177
Visits of microglial biomarkers	2 (2–3)	2 (2–3)	2 (2–3)
Duration of microglial biomarkers, yr	2.1 (1.4–5.1)	2.1 (1.4–4.4)	2.0 (1.7–5.1)
Visits of GAP-43	2 (2–4)	2 (2–3)	2 (2–4)
Duration of GAP-43, yr	2.1 (1.3–6.0)	2.2 (1.3–5.0)	2.1 (1.7–6.0)

Data are presented as median (range) or n (%).
APOE = apolipoprotein E; Aβ = β-amyloid; CI = cognitively impaired; CSF = cerebrospinal fluid; CU = cognitively unimpaired; GAP-43 = growth-associated protein-43; PET = positron emission tomography; PGRN = progranulin; p-Tau$_{181}$ = phosphorylated tau181; sTREM2 = soluble triggering receptor expressed on myeloid cells-2; SUVR = standard uptake value ratio.

p-Tau$_{181}$ remained significant even controlling for the main effect of Aβ PET. In contrast, augmented Aβ PET was associated with lower baseline levels of CSF sTREM2 ($\beta_{std} = -0.20$, 95% ci = −0.28 to −0.11, $p < 0.001$) and CSF PGRN ($\beta_{std} = -0.16$, 95% ci = −0.26 to −0.07, $p < 0.001$) but not with slopes of CSF microglial biomarkers after accounting for CSF p-Tau$_{181}$. When the models were restricted to CU, CI, or Aβ+ participants, the same results were yielded for the associations with CSF p-Tau$_{181}$ (Supplementary Table S3). The associations with Aβ PET at baseline were retained in CU and CI participants but marginally in Aβ+ participants. In addition, there was an association between higher Aβ PET and faster rates of increase in CSF PGRN in CU participants.

Moreover, we found an interaction effect between Aβ PET and CSF p-Tau$_{181}$ on CSF microglial biomarkers across all participants (see Fig 1), in which higher Aβ PET was related to attenuated associations of higher CSF p-Tau$_{181}$ with greater baseline levels of CSF microglial biomarkers and faster rates of increase in CSF sTREM2. We also noticed a marginal interaction on the slope of CSF PGRN. Only CI participants retained the interaction on baseline CSF sTREM2 when on testing in different subgroups (Supplementary Table S4). The mediation analyses further showed that CSF p-Tau$_{181}$ mediated the associations between Aβ PET and CSF microglial biomarkers in the whole cohort (Fig 2). The direct effects of Aβ PET on CSF microglial biomarkers were opposite from the indirect effect mediated by CSF p-Tau$_{181}$, leading to the totally disappeared impact of Aβ PET. Mediation analyses generally yielded similar results in CU, CI, and Aβ+ participants (Supplementary Fig S1).

CSF sTREM2 Levels Correlate with CSF GAP-43 Independent of Aβ PET and CSF p-Tau$_{181}$

In the primary analyses, higher baseline levels of CSF microglial biomarkers were associated with the more presynaptic loss measured by greater CSF GAP-43 levels (sTREM2: $\beta_{std} = 0.38$, 95% ci = 0.31–0.45, $p < 0.001$; PGRN: $\beta_{std} = 0.22$, 95% ci = 0.15–0.30, $p < 0.001$) and faster rates of increase in CSF GAP-43 (sTREM2: $\beta_{std} = 0.37$, 95% ci = 0.26–0.49, $p < 0.001$; PGRN: $\beta_{std} = 0.19$, 95% ci = 0.07–0.32, $p = 0.002$; Fig 3).

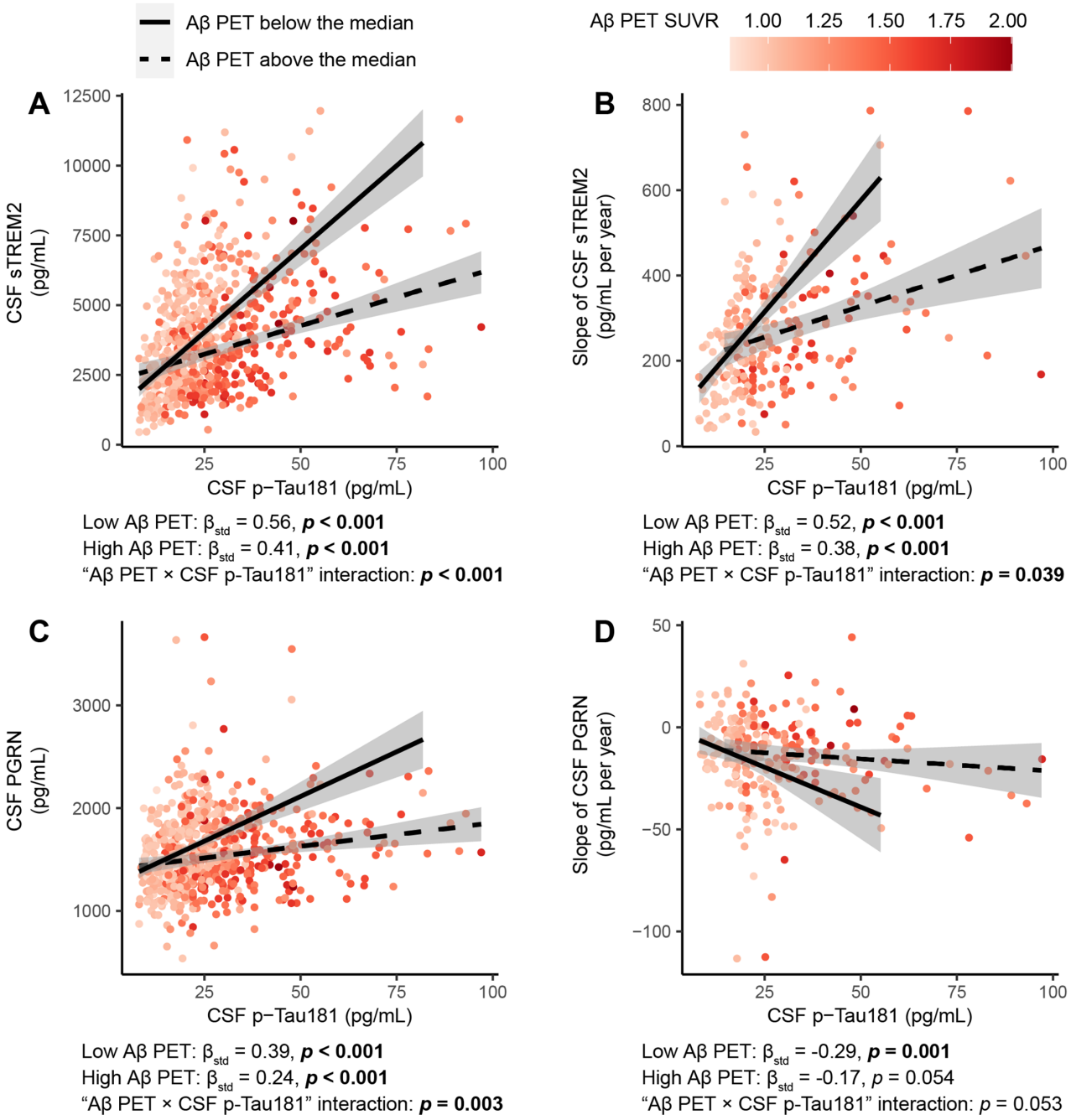

FIGURE 1: Association of cerebrospinal fluid (CSF) microglial biomarkers with CSF phosphorylated tau181 (p-Tau$_{181}$). Association of baseline CSF p-Tau$_{181}$ with baseline and longitudinal CSF soluble triggering receptor expressed on myeloid cells-2 (sTREM2; A, B) and CSF progranulin (PGRN; C, D) is shown. The dash and solid lines represent each group's regression lines. The baseline β-amyloid (Aβ) positron emission tomography (PET) × CSF p-Tau$_{181}$ interaction effect was computed. The presented *p* values were calculated using generalized linear models across all participants, controlling for age, sex, education, and *APOE-ε4* status. SUVR = standardized uptake value ratio; β$_{std}$ = standard β.

Augmented rates of increase in CSF sTREM2 and decrease in CSF PGRN were also related to faster rates of increase in CSF GAP-43 (sTREM2: $\beta_{std} = 0.37$, 95% ci = 0.26–0.48, $p < 0.001$; PGRN: $\beta_{std} = -0.16$, 95% ci = −0.28 to −0.03, $p = 0.012$). After controlling for Aβ PET and CSF p-Tau$_{181}$, the associations between CSF sTREM2 and CSF GAP-43 were preserved in the whole cohort and partially in CI and Aβ+ participants (Table 2 and Supplementary Table S5). In contrast, no association was obtained for CSF PGRN when adjusting for Aβ PET and CSF p-Tau$_{181}$. These findings suggest that CSF sTREM2 rather than CSF PGRN correlates with GAP-43-related presynaptic loss, independent of Aβ PET and CSF p-Tau$_{181}$.

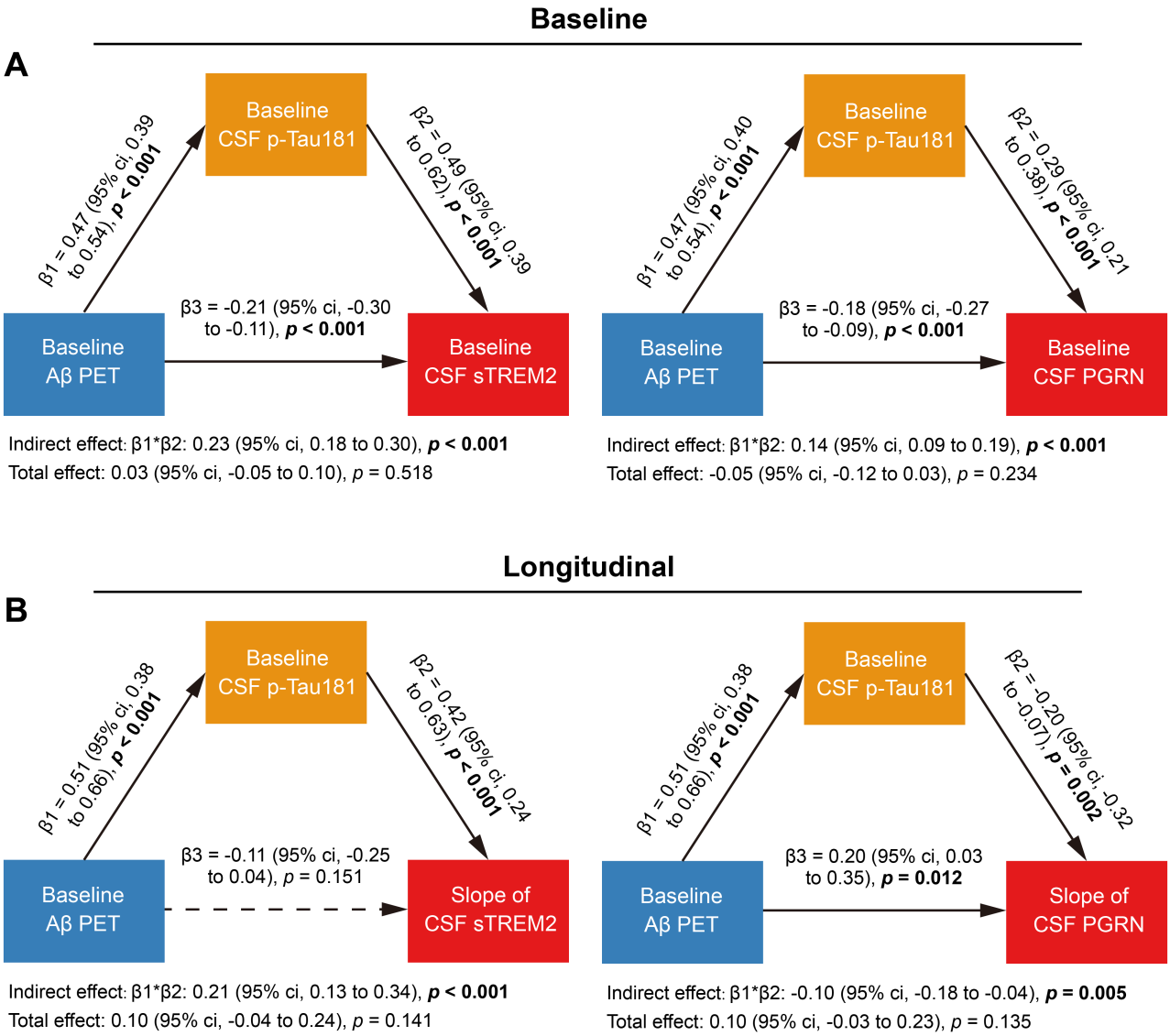

FIGURE 2: Mediation analysis of β-amyloid (Aβ) positron emission tomography (PET), CSF phosphorylated tau181 (p-Tau$_{181}$), and cerebrospinal fluid (CSF) microglial biomarkers. Baseline CSF p-Tau$_{181}$ mediated the association of baseline Aβ PET with baseline CSF soluble triggering receptor expressed on myeloid cells-2 (sTREM2) and CSF progranulin (PGRN; A) as well as slopes of CSF sTREM2 and CSF PGRN (B). The solid and the dashed lines show the significant and nonsignificant pathways, respectively. Total, direct, and indirect associations were computed by a 5,000-bootstrap procedure, controlling for age, sex, education, and *APOE-ε4* status. ci = confidence interval.

Subsequently, we further determined whether Aβ pathology modulates the associations between CSF microglial biomarkers and CSF GAP-43. Across all participants, Aβ PET had a significant interaction with CSF sTREM2 on longitudinal CSF GAP-43 (see Table 2). This showed that individuals with high levels of Aβ PET had associations of greater baseline levels and longitudinal increases in CSF sTREM2 with faster rates of increase in CSF GAP-43 (baseline: $\beta_{std} = 0.13$, 95% ci = −0.01 to 0.27; slopes: $\beta_{std} = 0.14$, 95% ci = 0.01–0.27). Conversely, individuals with low levels of Aβ PET had associations of greater baseline levels and longitudinal increases in CSF sTREM2 with slower rates of increase in CSF GAP-43 (baseline: $\beta_{std} = -0.14$, 95% ci = −0.28 to 0.004; slopes: $\beta_{std} = -0.12$, 95% ci = −0.25 to 0.01). The interactions with CSF sTREM2 were sustained in Aβ+ participants and partially in CU participants cross-sectionally and longitudinally (Supplementary Table S6). In addition, Aβ PET showed an interaction with CSF PGRN on CSF GAP-43 at baseline across all participants, in which higher Aβ PET was related to attenuated correlation between elevated levels of CSF PGRN and CSF GAP-43 (PGRN × Aβ PET interaction: $\beta_{std} = -0.06$, 95% ci = −0.11 to −0.01, $p = 0.027$) and remained significant in Aβ+ participants.

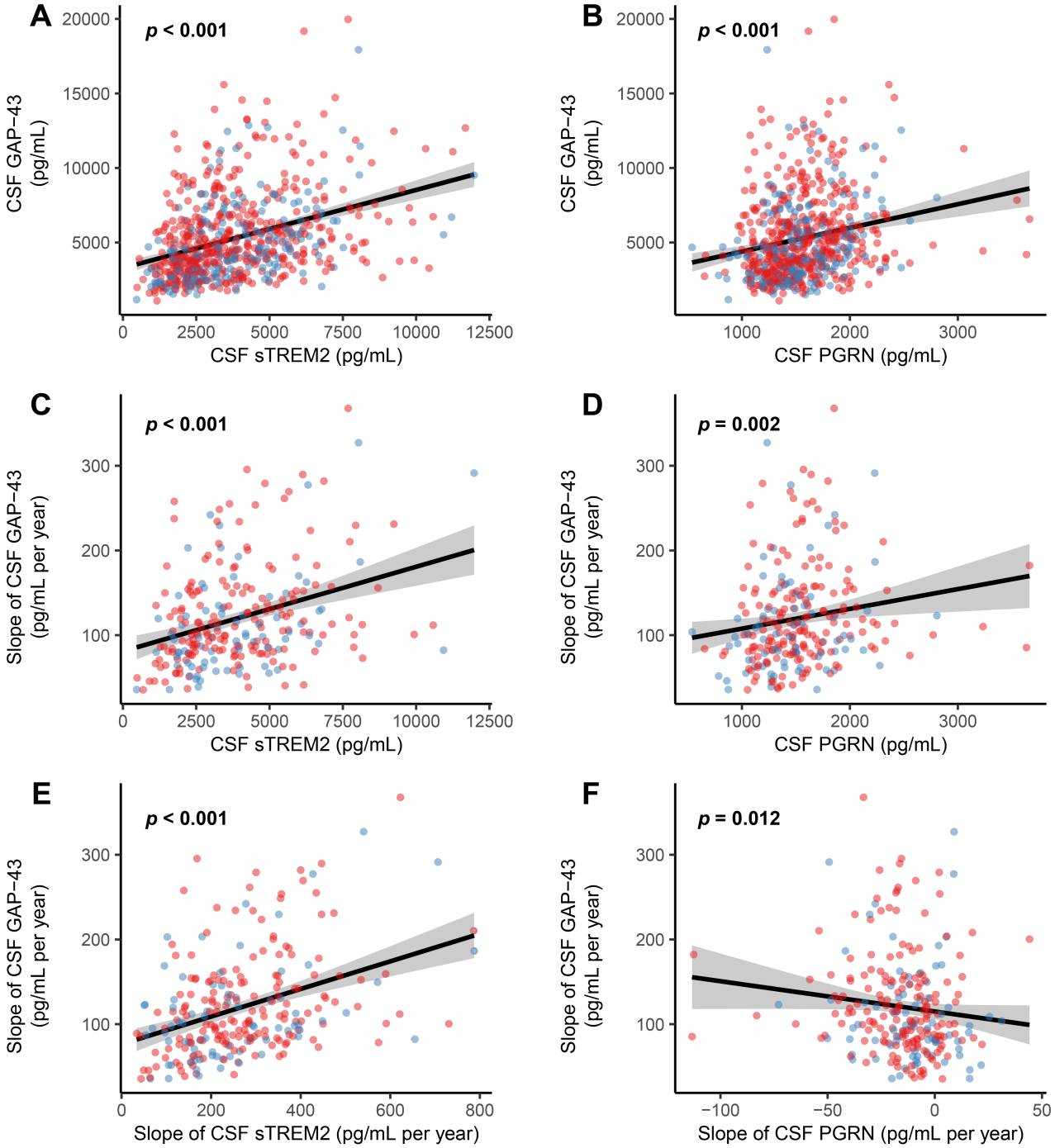

FIGURE 3: Association of cerebrospinal fluid (CSF) growth-associated protein-43 (GAP-43) with CSF microglial biomarkers. (A–D) Association of baseline and longitudinal CSF GAP-43 with baseline CSF soluble triggering receptor expressed on myeloid cells-2 (sTREM2; A, C) and CSF progranulin (PGRN; B, D). (E, F) Association of longitudinal CSF GAP-43 with longitudinal CSF sTREM2 (E) and CSF PGRN (F). The points (blue, cognitively unimpaired; red, cognitively impaired) and solid lines represent the individuals and regression lines, respectively. The presented *p* values were computed using generalized linear models across all participants, controlling for age, sex, education, and *APOE-ε4* status.

Association of CSF sTREM2 with Aβ, p-Tau, and Presynaptic Loss in the Validation Cohort

In the independent CSF samples (n = 65) obtained from the GHABS cohort, higher levels of both CSF $A\beta_{42}$ and CSF p-Tau_{181} were marginally associated with higher CSF sTREM2 levels (Aβ: $\beta_{std} = 0.22$, 95% ci = −0.01 to 0.46, $p = 0.065$; p-Tau: $\beta_{std} = 0.22$, 95% ci = −0.02 to 0.45, $p = 0.072$) when we conducted multivariate analyses with CSF $A\beta_{42}$ and CSF p-Tau_{181} as predictors in one model adjusted for age and sex. Similar to the ADNI

cohort, there was an interaction between CSF $A\beta_{42}$ and CSF p-Tau_{181} on CSF sTREM2, in which lower CSF $A\beta_{42}$ was related to attenuated association of CSF p-Tau_{181} with CSF sTREM2 (Fig 4A). Mediation analysis also showed a marginally direct effect of CSF $A\beta_{42}$ on CSF sTREM2 opposite from the indirect effect mediated by CSF p-Tau_{181} (Supplementary Fig S2).

Similar to the ADNI cohort, higher CSF sTREM2 was related to elevated CSF GAP-43 ($\beta_{std} = 0.37$, 95% ci = 0.15–0.59, $p = 0.001$; see Fig 4B) adjusted for age and sex. The association remained significant after controlling for CSF $A\beta_{42}$ and CSF p-Tau_{181} ($\beta_{std} = 0.45$, 95% ci = 0.23–0.67, $p < 0.001$). No interaction effect on GAP-43 was obtained, which may be because most of the

TABLE 2. Interaction between CSF Microglial Biomarkers and Aβ PET on CSF GAP-43

	Main Effect		Aβ PET Interaction	
	β_{std} (95% ci)	*p*	β_{std} (95% ci)	*p*
Baseline CSF GAP-43				
Baseline CSF sTREM2	0.08 (0.02 to 0.14)	**0.005**	0.05 (−0.01 to 0.10)	0.079
Baseline CSF PGRN	0.05 (−0.01 to 0.10)	0.094	−0.06 (−0.11 to −0.01)	**0.027**
Slope of CSF GAP-43				
Baseline CSF sTREM2	0.10 (0.01 to 0.20)	**0.036**	0.11 (0.03 to 0.19)	**0.007**
Baseline CSF PGRN	0.05 (−0.03 to 0.14)	0.229	−0.03 (−0.11 to 0.05)	0.483
Slope of CSF sTREM2	0.11 (0.02 to 0.20)	**0.016**	0.10 (0.02 to 0.19)	**0.011**
Slope of CSF PGRN	−0.04 (−0.12 to 0.05)	0.390	0.04 (−0.04 to 0.12)	0.311

The bold *p* values refer to significant effect.
Aβ = β-amyloid; ci = confidence interval; CSF = cerebrospinal fluid; GAP-43 = growth-associated protein-43; PET = positron emission tomography; PGRN = progranulin; sTREM2: soluble triggering receptor expressed on myeloid cells-2.

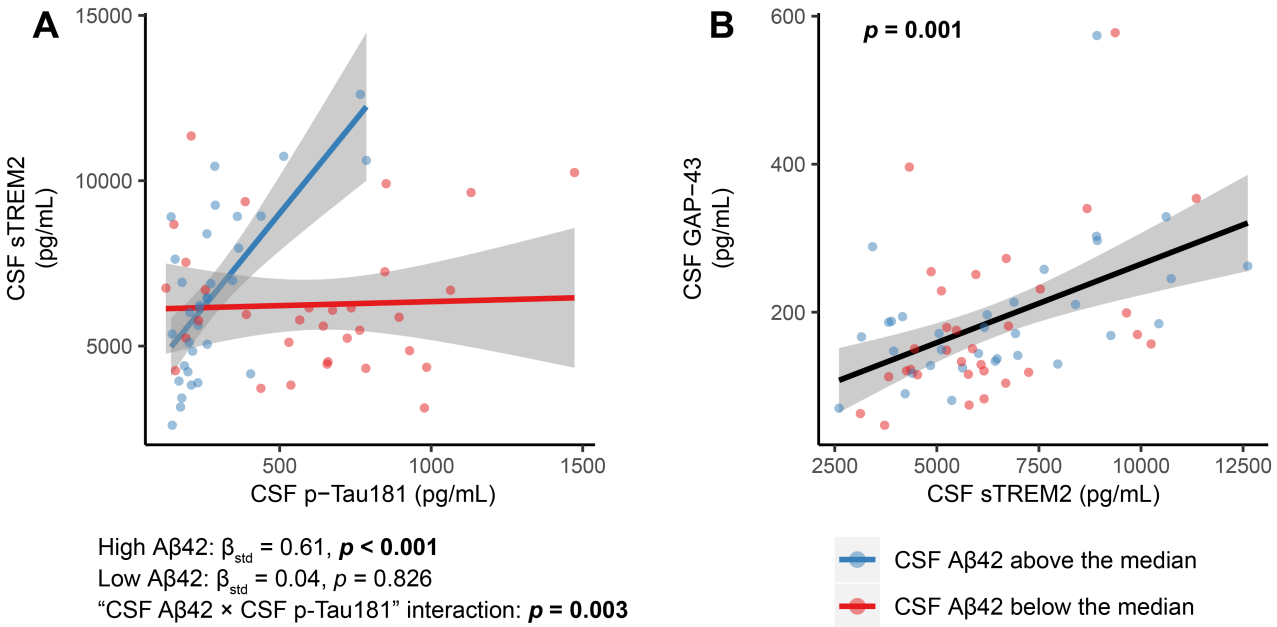

FIGURE 4: Association of cerebrospinal fluid (CSF) soluble triggering receptor expressed on myeloid cells-2 (sTREM2) with CSF phosphorylated tau181 (p-Tau_{181}) and CSF growth-associated protein-43 (GAP-43). Association of CSF sTREM2 with CSF p-Tau_{181} (A) and CSF GAP-43 (B) at baseline in the validation cohort is shown. The points and solid lines represent each group's individuals and regression lines. The baseline CSF $A\beta_{42}$ × CSF p-Tau_{181} interaction effect was computed. The presented *p* values were calculated using generalized linear models across all participants, controlling for age and sex.

participants were CI (88%) in the validation cohort. The limitation of sample sizes did not allow meaningful analyses among different subgroups.

Discussion

In this study, we showed that elevated CSF p-Tau$_{181}$ was related to higher CSF microglial biomarkers. The effect size of p-Tau$_{181}$-associated CSF microglial biomarker increases was attenuated by higher Aβ burden. Furthermore, mediation analyses showed that the direct effects of Aβ PET on CSF microglial biomarkers were opposite from the indirect effect mediated by CSF *p*-Tau$_{181}$. Independent of Aβ PET and CSF *p*-Tau$_{181}$, higher CSF sTREM2 but not CSF PGRN was associated with elevated CSF GAP-43 and faster rates of CSF GAP-43 increase. Given that CSF sTREM2 and CSF PGRN may reflect expression levels of TREM2-dependent and PGRN-dependent signaling in microglia,[11,13] these findings provide novel insights into TREM2-related and PGRN-related microglial responses to primary AD pathologies and modulation on subsequent presynaptic loss (Fig 5).

Several preclinical studies in genetic knockout mice reported that *Trem2* deficiency locked microglia in a homoeostatic signature, whereas *Pgrn* deficiency led to microglial hyperactivation,[7,15,32] indicating that TREM2 and PGRN may participate in triggering and restricting microglial activation, respectively. This was favored by the finding that TPSO PET signals assessing microglial activation decreased in *Trem2*-deficient mice but increased in *Pgrn*-deficient mice,[15] and loss of TREM2 function could suppress microglial hyperactivation and phagocytosis in *Pgrn*-deficient mice.[7] We and colleagues[21] found significant longitudinal increases in CSF sTREM2 over time. Intriguingly, we first observed more rapid decreases in CSF PGRN over time parallel with higher baseline levels in this study. The opposite longitudinal changes correspond with the different effects of TREM2 and PGRN signaling in regulating microglial reactivity.[7,15,32] Supporting this, we further noticed an association between faster increases in CSF sTREM2 and decreases in CSF PGRN. Given that both sTREM2 and PGRN expression increased upon microglial activation,[33–38] it is likely that the earliest TREM2-related microglial responses to toxic proteins are protective by simultaneously enhancing PGRN-dependent signaling to suppress microglial hyperactivation. In the later stage, excessive pathological burdens disrupt the microglial homeostasis, eventually reducing PGRN-dependent signaling and increasing detrimental cellular subtypes of microglia.

For Aβ pathology in the brain, early studies revealed that different Aβ aggregates could induce glial activation, but the smaller soluble Aβ oligomers showed a far the smaller soluble Aβ oligomers induced microglia reactivity

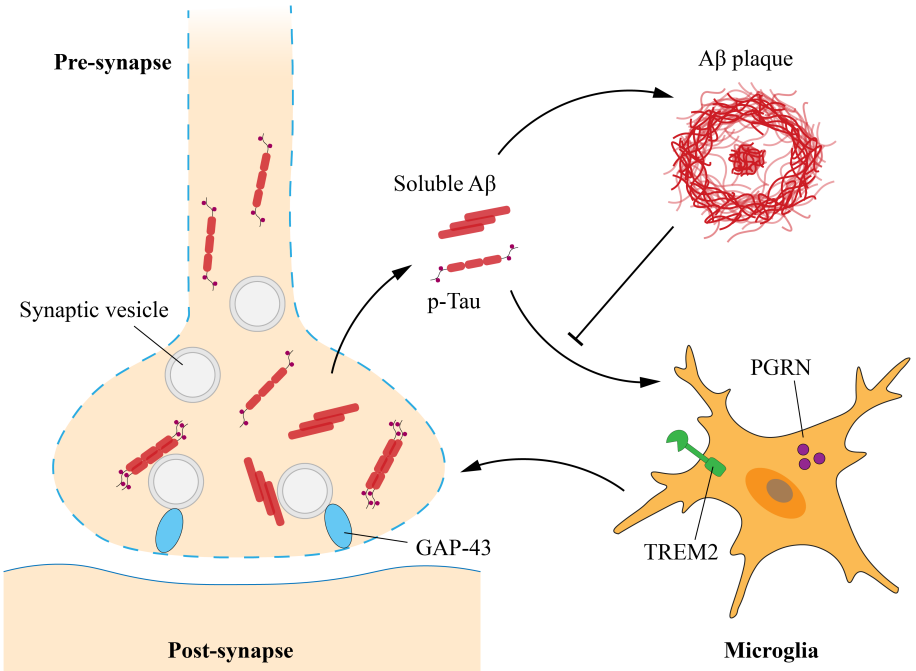

FIGURE 5: Interlinking schematic among β-amyloid (Aβ), phosphorylated tau (p-Tau), microglial reactivity, and presynaptic loss. The findings in the current study suggest that early p-Tau pathology may trigger triggering receptor expressed on myeloid cells-2 (TREM2)-dependent and progranulin (PGRN)-dependent microglial reactivity, which is attenuated by higher Aβ pathology. In addition, TREM2-dependent microglial reactivity may independently correlate to growth-associated protein-43 (GAP-43)-related presynaptic loss.

and neurotoxicity far more than other Aβ species.[1,39] In return, reactive microglia was found to protect against Aβ pathology in amyloidosis mice, especially in the early Aβ seeding stage in a TREM2-dependent manner.[40–43] Supporting this, two longitudinal studies in sporadic and familial AD reported the association of increased CSF sTREM2 with slower Aβ accumulation.[20,21] In line with our findings, two observational studies reported that individuals with evidence of Aβ pathology only had decreased CSF sTREM2 levels in preclinical and symptomatic AD.[18,44] Similarly, trend-level decreases in CSF PGRN were observed in preclinical AD with Aβ pathology only.[13] It may be explained by the formation of Aβ plaques sequestering soluble Aβ oligomers,[39] thereby limiting their potential to induce microglial reactivity. An alternative explanation may be that reactive microglia are recruited and form a barrier around plaques; thereby, the sTREM2 and PGRN generated from microglia are restricted within plaques.[18,45] Regarding tau-related pathology, we and others[13,18,44] observed positive associations between CSF p-Tau_{181} and CSF microglial biomarkers at baseline. Our findings further showed that elevated CSF p-Tau_{181} predicted longitudinal increases in CSF sTREM2 and decreases in CSF PGRN over time. However, another study in familial AD found no relationship between CSF p-Tau_{181} and longitudinal CSF sTREM2 increases.[21] The conflicting results could be explained by several factors, including the differentiative TREM2-related microglial responses to the pathophysiology between sporadic and familial AD or the larger sample sizes and greater age in the current study. Age-related increases in CSF sTREM2 and activated microglia were characterized in both human brains and mouse models,[46–48] implying more susceptible microglia responding to brain pathology in older adults. Intriguingly, the p-Tau effect on CSF microglial biomarker increases was suppressed by higher Aβ deposition in this study. Mediation analyses further demonstrated the opposite associations of Aβ PET and CSF p-Tau_{181} with changes in CSF microglial biomarkers. Similar results for CSF sTREM2 were yielded using CSF $Aβ_{42}$ as a predictor instead of Aβ PET in the validation cohort. These results suggest that Aβ PET and CSF p-Tau pathologies have opposite relationships with the levels of microglial TREM2 and PGRN signaling measured by CSF microglial biomarkers.

Growing evidence supports the role of microglia in synaptic elimination during aging and AD. One postmortem study[49] demonstrated that microglial processes contained synaptic elements in the hippocampus of AD patients, and the amyloidosis mice experienced greater synaptic engulfment by microglia compared to wild-type mice. Animal studies showed that *Trem2* deficiency was linked to reduced microglial phagocytosis and increased dendritic spine densities and synaptic proteins in the brains of aged and amyloidosis mice.[9,50] Supporting this, another study found that loss of function in TREM2 markedly reduced synaptic engulfment by microglia in in vivo and in vitro experiments,[8] and TREM2 overexpression in the hippocampus could also induce synaptic loss.[9] These findings in animal models suggest that TREM2 signaling plays an active role in microglia-mediated synaptic removal. Importantly, our results in two independent cohorts provided further evidence from living humans by showing positive associations between CSF sTREM2 and CSF GAP-43 cross-sectionally and longitudinally independent of Aβ PET and CSF p-Tau pathologies, supporting the opinion on microglia-associated presynaptic elimination in a TREM2-dependent manner.[8,9] Moreover, it is crucial to note that cortical Aβ deposition significantly modulated the association between CSF sTREM2 and CSF GAP-43. Specifically, higher levels of Aβ PET were related to augmented relationships between CSF sTREM2 and longitudinal increases in CSF GAP-43, suggesting a destructive role of Aβ pathology on microglial TREM2 signaling-related presynaptic dysfunction. This was in accordance with a previous study in nonhuman primates showing that administration of oligomeric Aβ could trigger an increased uptake of the cortical synapses by microglia paralleled by neuroinflammation.[51]

In this study, we systematically investigated the association of CSF microglial biomarkers with Aβ PET, CSF p-Tau_{181}, and presynaptic loss measured by CSF GAP-43 in a large dataset. The crucial findings were validated in an independent cohort. However, some caveats should be addressed when interpreting the current results. First, CSF sTREM2 and CSF PGRN are only indirect microglial TREM2 and PGRN signaling measurements. Thus, the levels of microglial activation cannot be regarded as conclusive unless confirmed by autopsy or PET imaging. Nevertheless, our findings may reflect, at least partly, the TREM2-related and PGRN-related microglial reactivity, given that both sTREM2 and PGRN in CSF are primarily shedding from microglia in the brain.[10,15,25] To the best of our knowledge, this study is the first to explore the association between biomarker-based evidence of microglial reactivity and presynaptic integrity in living humans. Therefore, more validations in independent cohorts with large sample sizes are needed in the future, especially by using different synaptic biomarkers (ie, postsynaptic protein neurogranin[52] and presynaptic protein SNAP-25,[53] whose sample sizes are limited in the ADNI cohort). Finally, the current study is observational in nature, and causal associations should not be drawn from our findings.

In conclusion, this study demonstrated that Aβ pathology may attenuate p-Tau-related increases in microglial TREM2 and PGRN signaling, which are

independently linked to GAP-43-related presynaptic loss. These findings extend the understanding of the association among primary AD pathology, microglial reactivity, and presynaptic dysfunction in AD and other neurodegenerative diseases, which may have important clinical implications for developing therapeutic strategies targeting microglial TREM2 to prevent the progression of AD.

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Author Contributions

T.G. and G.L. contributed to the conception and design of the study. T.G., G.L., X.C., J.Y., Y.C., P.S., A.L., and Y.Z. contributed to the acquisition and analysis of data. T.G., G.L., Z.L., and S.M. contributed to drafting the manuscript and preparing the figures.

Potential Conflicts of Interest

Nothing to report.

Data Availability

The data used in the current study were obtained from the ADNI database (available at https://adni.loni.usc.edu) and the GHABS cohort. Derived data are available from the corresponding author on request by any qualified investigator subject to a data use agreement.

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