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Soluble TREM2, Alzheimer's Disease Pathology, and Risk for Progression of Cerebral Small Vessel Disease: A Longitudinal Study

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Abstract.
Background: Until recently, studies on associations between neuroinflammation *in vivo* and cerebral small vessel disease (CSVD) are scarce. Cerebrospinal fluid (CSF) levels of soluble triggering receptor expressed on myeloid cells 2 (sTREM2), a candidate biomarker of microglial activation and neuroinflammation, were found elevated in Alzheimer's disease (AD), but they have not been fully explored in CSVD.
Objective: To determine whether CSF sTREM2 levels are associated with the increased risk of CSVD progression.
Methods: A total of 426 individuals from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database were included in this study. All participants underwent measurements of CSF sTREM2 and AD pathology ($A\beta_{1\text{-}42}$, P-tau_{181P}). The progression of CSVD burden and imaging markers, including cerebral microbleeds (CMBs), white matter hyperintensities and lacunes, were estimated based on neuroimaging changes. Logistic regression and moderation effect models were applied to explore associations of sTREM2 with CSVD progression and AD pathology.
Results: Higher CSF sTREM2 levels at baseline were associated with increased CSVD burden (OR = 1.28 [95% CI, 1.01–1.62]) and CMBs counts (OR = 1.32 [95% CI, 1.03–1.68]). Similarly, increased change rates of CSF sTREM2 might predict elevated CMBs counts (OR = 1.44 [95% CI, 1.05–1.98]). Participants with AD pathology ($A\beta_{1\text{-}42}$ and P-tau_{181P}) showed a stronger association between CSF sTREM2 and CSVD progression.
Conclusion: This longitudinal study found a positive association between CSF sTREM2 and CSVD progression, suggesting that neuroinflammation might promote CSVD. Furthermore, neuroinflammation could be a shared pathogenesis of CSVD and AD at the early stage. Targeting neuroinflammation to intervene the progression of CSVD and AD warrants further investigation.

Keywords: Alzheimer's disease, amyloid-β, cerebral small vessel disease, microglia, neuroinflammation, sTREM2

[1]Data used in preparation for this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (https://adni.loni.usc.edu). As such, the investigators within the ADNI contributed to the design and implementation of ADNI and/or provided data but did not participate in analysis or writing of this report. A complete listing of ADNI investigators can be found at: https://adni.loni.usc.edu/wp-content/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf.

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INTRODUCTION

Cerebral small vessel disease (CSVD) is an umbrella term for dysfunction in the cerebral microvessels, predominantly diagnosed through neuroimaging techniques, such as standard magnetic resonance imaging (MRI). Typical CSVD imaging markers include cerebral microbleeds (CMBs), white matter hyperintensities (WMHs), lacunes, enlarged perivascular spaces (EPVS), and cerebral atrophy [1]. As a major cause of vascular dementia and stroke, CSVD also commonly coexists with another leading subtype of dementia in the elderly, i.e., Alzheimer's disease (AD). It also shares a series of risk factors and some common pathobiological mechanisms with AD. At present, inflammation has been increasingly considered implicated in CSVD and AD [2, 3].

During the development of CSVD, persistent or excessive inflammatory response has been demonstrated to accelerate the damage of cerebral microvessels. However, the investigation into the relationship between inflammation and the risk of CSVD is primarily limited to the use of peripheral inflammatory markers (e.g., C-reactive protein, homocysteine) [3], which may not directly reflect the inflammatory process in the brain. Therefore, accessible measurements of neuroinflammation *in vivo* are required to further our understanding of how the inflammatory process impacts CSVD.

In the brain, soluble triggering receptor expressed on myeloid cells 2 (sTREM2) is naturally released into CSF through proteolytic cleavage of a transmembrane protein, i.e., TREM2, which is mainly expressed by microglia and involved in the regulation of microglial proliferation, survival, phagocytosis, and cytokine release [4–6]. Monitoring CSF sTREM2 has been suggested to be a tool to investigate the role of microglial activation and neuroinflammation. Notably, a recent study showed that plasma sTREM2 was associated with CSVD-related white matter injury, indicating the potential role of sTREM2 in the CSVD pathogenesis [7]. Considering a strong correlation between levels of sTREM2 in the brain and blood [8, 9], the direct mechanistic links between sTREM2 in CSF and CSVD-related brain abnormalities remain to be investigated. Previous studies found that CSF sTREM2 was also involved in the neuroinflammatory process in AD [10, 11]. Therefore, CSF sTREM2 could be a potential biomarker connecting the pathophysiological mechanisms between CSVD and AD. Whether AD pathology impacts the associations between CSF sTREM2 and CSVD is still largely unexplored.

We herein assumed that active neuroinflammation indicated by elevated CSF sTREM2 could contribute to the occurrence of CSVD. Therefore, we first assessed the individual-level total burden of CSVD and investigated the longitudinal associations between CSF sTREM2 and various CSVD imaging markers. Considering that preclinical findings showed that sTREM2 dynamically changed with pathological biomarkers of AD (amyloid-β [Aβ] and phosphorylated tau [P-tau] proteins) in CSF [12, 13], we further tested the hypothesis that AD pathological proteins in CSF (i.e. $A\beta_{1-42}$ and P-tau_{181P}) may modulate the underlying association between CSF sTREM2 and progressive CSVD.

MATERIALS AND METHODS

ADNI study design

Study participants were all selected from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database, including ADNI-1, ADNI-GO, ADNI-2, and ADNI-3 (https://adni.loni.usc.edu). The ADNI was launched in 2003 as a public-private partnership and designed to test whether serial MRI, positron emission tomography, biological markers, as well as clinical and neuropsychological assessments can be combined to measure the progression of mild cognitive impairment (MCI) and early AD. For up-to-date information, see https://www.adni-info.org. ADNI was approved by the institutional review boards of all participating institutions. Written informed consent was obtained from all participants at each site.

Participants

A total of 510 participants who received measurements of CSF sTREM2 and CSVD brain imaging markers were initially included in our study (Supplementary Figure 1). According to ADNI inclusion criteria, all participants were between the ages of 55 and 90. To avoid the influence of complex AD status on the results, only individuals showing cognitive normal (CN) or MCI were included. CN participants had a Mini-Mental State Examination (MMSE) score of $\geq$24, a Clinical Dementia Rating (CDR) score of 0, and no depression. Patients with MCI had a MMSE score of $\geq$24, a CDR score of 0.5, an objective memory loss tested by the Wechsler Memory Scale Logical Memory II, and preserved activities

of daily living. Additionally, the following selection criteria were applied: 1) available baseline data of $A\beta_{1-42}$ and P-tau_{181P}; 2) at least one follow-up visit with available data of brain imaging markers, including CMBs, WMHs, and lacunes. Finally, 426 individuals (141 CN and 285 MCI) who underwent longitudinal measurements of CSVD neuroimaging biomarkers were included in this study, among whom 214 participants had available follow-up data of CSF sTREM2. The average follow-up time of the study participants was 28.0 months (range: 6–72 months). Of note, individuals with subjective memory complaints at baseline were defined as the CN group.

Measurements of CSF biomarkers

CSF sTREM2 levels were measured by an ELISA approach elaborated in previous studies [12, 14]. Detailed CSF sTREM2 assessment methods can be found online in the ADNI database (https://adni.loni.usc.edu). Significantly, we removed individuals (N = 25) that fell outside the 98% prediction tolerance level based on the comparison of the CSF sTREM2 measurements between the two centers (the Piccio group and Haass group). Finally, corrected values provided as 'MSD_sTREM2corrected' were used for the following statistical analyses.

Measurements of CSF $A\beta_{1-42}$ and P-tau_{181P} levels were performed with the Elecsys® β-amyloid (1–42) CSF and the Elecsys® phosphotau (181P) CSF immunoassays on a cobas e 601 instrument [15, 16]. The data is also available in the 'UPENNBIOMK9.csv' file in the ADNI database. Additionally, we binarized $A\beta_{1-42}$ and P-tau_{181P} in the negative (–) or positive (+) based on whether the biomarkers were normal or abnormal. In the present study, "A+" was assigned to individuals who had a CSF $A\beta_{1\text{-}42}$ <976.6 pg/ml, and "T+" was assigned to individuals who had a CSF P-tau_{181P}>21.8 pg/ml [17].

Assessments of imaging markers and CSVD burden

All participants were examined using a 3.0-Tesla MRI scanner, and the procedure was elaborated in the previous studies [18]. Details of the parameters are shown on the ADNI website (https://adni.loni.usc.edu/methods/mri-tool/mri-analysis).

CMBs

CMBs were defined as areas of 2–5 mm signal void in diameter generally (sometimes up to 10 mm), which were visible on T2*-weighted MRI or other susceptibility-weighted sequences [1]. Assessments of CMBs were based on T2* images and the status of the finding took one of three following values: possible, definite, and rescinded. Particularly, only the CMBs with definite status were selected and counted for analyses. The number of CMBs was identified by trained image analysts and further confirmed by radiologists experienced in reading T2* images. More details about the judgment of CMBs could be found online in the ADNI database (https://ida.loni.usc.edu).

WMHS

WMHs were quantified by abnormal signals of various sizes in the white matter (signals different from CSF) with hyperintensity on T2-weighted images such as fluid-attenuated inversion recovery, without cavitation [1]. In the present study, we applied the Fazekas scale to evaluate WMHs [19], including periventricular and deep WMHs separately. Total Fazekas score was calculated as the sum of periventricular and deep WMH scores, ranging from 0 to 6 points.

Lacunes

Lacunes were defined as a round or ovoid cavity (3–15 mm in diameter) fluid-filled (signals similar to CSF) and consistent with a previous acute small deep brain infarct or hemorrhage in the territory of one perforating arteriole [1]. The assessments of lacunes were based on central CSF-like hyperintensities with a surrounding rim of hyperintensities on T2 FLAIR images.

CSVD burden

In the present study, the CSVD burden was assessed by the CSVD score generated as described previously [20, 21]. One point was given for each presence of three imaging markers (CMBs, WMHs, and lacunes) of CSVD: the presence of CMBs and lacunes were defined as the presence of one or more CMBs (1 point if present) or any lacune (1 point if present); the presence of WMHs was defined as either (early) confluent deep WMHs (Fazekas score

2 or 3) or irregular periventricular WMHs extending into the deep white matter (Fazekas score 3) (1 point if present). Therefore, the CSVD score had a range from 0 to 3.

Definition of the progression of CSVD

Individuals who manifested a worsening CSVD burden were ascertained based on an increased score of total CSVD burden during the follow-up period. Similarly, the progressive events of three CSVD markers were determined by an increase in CMBs counts, total Fazekas score, and lacunes counts [22–25].

Statistical analyses

We standardized continuous variables (baseline CSF sTREM2, CSF $A\beta_{1\text{-}42}$, and CSF P-tau_{181P}) by z-score transformation and excluded outlier values outside four standard deviations (SDs) before conducting the following analyses. The characteristics of participants were summarized using descriptive statistical methods (mean ± SD). Differences in characteristics between CSVD progression and non-progression groups were assessed using the Mann-Whitney U test (continuous variables) and the χ^2 test (categorical variables). Spearman rank correlation was applied to test associations between baseline CSF sTREM2 levels and demographic characteristics, including age, sex, MMSE, educational levels, *apolipoprotein E* ε4 (*APOE* ε4) carrier status, and vascular risk factors.

Considering the follow-up period was relatively short, we first applied binomial logistic regression (LR) to explore longitudinal associations between levels of baseline CSF sTREM2 with progressive events (yes versus no) of total burden and each imaging marker of CSVD. Furthermore, to estimate the change rates of CSF sTREM2, subject-specific intercepts and slopes for the time were included in the linear mixed-effects model (LMR) as random effects that allowed for heterogeneity among subjects accounting for repeated assessments on the same subject, and time from baseline was included as fixed effects. Sensitivity analyses were run to assess the robustness by constructing various LR models: 1) adjustment for age and sex (Model 1); 2) additional adjustment for educational levels, MMSE, *APOE* ε4 carrier status, and intracranial volume (ICV) (Model 2, as the priority model in the present study); 3) correcting covariates in Model 2 and vascular risk factors including history of hypertension (yes or no), hyperlipidemia (yes or no), coronary heart disease (yes or no), diabetes (yes or no), smoking status (yes or no), and alcohol habit (yes or no).

In addition, to explore the moderation effects of critical confounders, including age (<70 years versus ≥70 years), sex (females versus males), APOE ε4 carrier status (carriers versus non-carriers), and cognition diagnosis (CN versus MCI), we introduced multiplicative interaction terms into multivariable-adjusted logistic models. Subgroup analyses were conducted if there existed possible strata effects (p for interaction <0.05).

Given the dynamic changes of CSF sTREM2 accompanying accumulating brain amyloidosis and tau pathology during CSVD progressive processes [13, 17], we specially investigated the role of AD pathology in the associations between CSF sTREM2 (baseline levels or longitudinal changes) and CSVD progressive events. Confounding and modification effects of AD pathological biomarkers in CSF (baseline levels of $A\beta_{1\text{-}42}$ and P-tau_{181P}) were thus scrutinized in both. Potentially enhanced associations were finally ascertained through *post-hoc* stratification analyses by baseline A/T status (A+ versus A–; T+ versus T–; A–T– versus A+T–/A–T+ versus A+T+).

Statistical analyses were conducted using R version 4.0.2 and SPSS version 25. The statistical significance threshold was set at a 2-tailed $p < 0.05$.

RESULTS

Participants characteristics

We totally studied 426 participants (141 CN and 285 MCI) in the ADNI database. The overall sample was in their late-life stage (aged 71.9 ± 7.0 years, 187 females) and showed a relatively good cognitive performance (mean MMSE score = 28.4 ± 1.6). Baseline participants' characteristics were summarized in Table 1. CSVD non-progression and progression groups were comparable in age, MMSE score, educational levels, proportions of females, APOE ε4 carriers, smokers, and drinkers, and prevalence of cardiovascular diseases at baseline. As expected, individuals with progressive events showed higher baseline levels of CSF sTREM2 (4279.2 ± 1960.8 pg/mL) compared to non-progression controls (3777.5 ± 1951.0 pg/mL) ($p = 0.017$) but presented comparable levels of CSF biomarkers of AD pathology (including $A\beta_{1\text{-}42}$ and

Table 1
Participants characteristics at baseline

Characteristic		All participants	CSVD non-progression	CSVD progression	*p*
Number of participants		426	344	82	
Demographic characteristic					
Age (y)	Mean (SD)	71.9 (7.0)	71.8 (7.3)	72.2 (5.9)	0.702
Sex	% Female	187 (43.9)	151 (43.9%)	36 (43.9%)	0.999
Education (y)	Mean (SD)	16.4 (2.6)	16.4 (2.6)	16.1 (2.6)	0.412
APOE ε4 carrier status	% Present	179 (42.0)	145 (42.2%)	34 (41.5%)	0.910
MMSE	Mean (SD)	28.4 (1.6)	28.4 (1.7)	28.5 (1.5)	0.959
Vascular risk factors					
Hypertension	% Present	190 (44.6%)	157 (45.6%)	33 (40.2%)	0.377
Hyperlipidemia	% Present	215 (50.5%)	178 (51.7%)	37 (45.1%)	0.281
Coronary heart disease	% Present	26 (6.1%)	20 (5.8%)	6 (7.3%)	0.609
Diabetes	% Present	52 (12.2%)	41 (11.9%)	11 (13.4%)	0.710
Smoking status	% Present	120 (28.2%)	100 (29.1%)	20 (24.4%)	0.397
Alcohol consumption	% Present	7 (1.6%)	6 (1.7%)	1 (1.2%)	0.737
Image features					
CMBs	% Present	109 (25.6)	93 (27.0%)	16 (19.5%)	0.161
WMHs	% Present	153 (35.9)	129 (37.5%)	24 (29.3%)	0.163
WMHs score (0–6)	Mean (SD)	1.35 (1.61)	1.34 (1.63)	1.40 (1.55)	0.364
Lacunes	% Present	130 (30.5)	109 (31.7%)	21 (25.6%)	0.283
CSVD score (0–3)	Mean (SD)	0.92 (0.96)	0.96 (1.00)	0.74 (0.75)	0.170
ICV (cm^3)	Mean (SD)	1513.2 (153.5)	1513.3 (151.8)	1513.2 (161.2)	0.936
CSF biomarkers					
sTREM2 levels (pg/mL)	Mean (SD)	3874.1 (1960.6)	3777.5 (1951.0)	4279.2 (1960.8)	0.017
$A\beta_{1-42}$ levels (pg/mL)	Mean (SD)	1205.1 (615.8)	1191.7 (612.4)	1261.0 (630.6)	0.400
P-tau_{181P} levels (pg/mL)	Mean (SD)	25.1 (13.2)	24.7 (13.1)	26.7 (13.5)	0.095

p values were calculated using *t*-test for continuous variables or Chi-square test for categorical variables. WMHs score was calculated using total Fazekas score. *APOE* ε4, apolipoprotein E ε4; Aβ, amyloid-β; CMBs, cerebral microbleeds; CSF, cerebrospinal fluid; CSVD, cerebral small vessel disease; ICV, intracranial volume; MMSE, Mini-Mental State Examination; P-tau, phosphorylated tau; sTREM2, soluble TREM2; SD, standard deviation; WMHs, white matter hyperintensities.

P-tau_{181P}) and similar burdens of CSVD imaging features.

Spearman rank correlation analyses showed that CSF sTREM2 levels were positively associated with age ($\rho = 0.182$, $p < 0.001$) but presented a negative relationship with MMSE ($\rho = -0.169$, $p < 0.001$) at baseline. No significant correlation was found with sex, educational levels, APOE ε4 carrier status, or vascular risk factors.

Associations of CSF sTREM2 with CSVD burden and imaging markers

LR models with adjustment for age and sex demonstrated that increased baseline CSF sTREM2 levels might predict accelerated worsening of CSVD burden (OR = 1.28 [95% CI, 1.01–1.62]) and the progression of CMBs (OR = 1.32 [95% CI, 1.03–1.68], Model 1, Supplementary Table 1). However, high progressive risks for WMHs (OR = 1.23 [95% CI, 0.97–1.56]) and lacunes (OR = 1.07 [95% CI, 0.84–1.36]) were indicated by increased baseline sTREM2 levels but yielded non-significant estimates. Similarly, rapid change rates of CSF sTREM2 levels substantially elevated the CMBs counts in subsequent follow-up (OR = 1.44 [95% CI, 1.05–1.98], Model 1, Supplementary Table 2).

Besides, after correcting educational levels, MMSE, APOE ε4 carrier status, ICV, and vascular risk factors, sensitivity analyses provided robust findings that predictive efficacies of baseline and change rates of CSF sTREM2 levels for progressive outcomes of CSVD were not explained by cognitive performance, genetic risk, and vascular risk factors (Model 3, Fig. 1, Supplementary Table 2).

Modification effects by demographic factors, genetic risk, and cognitive status

Of note, substantial modification effects of age stages and APOE ε4 carrier status were shown through performing interaction analyses (p for

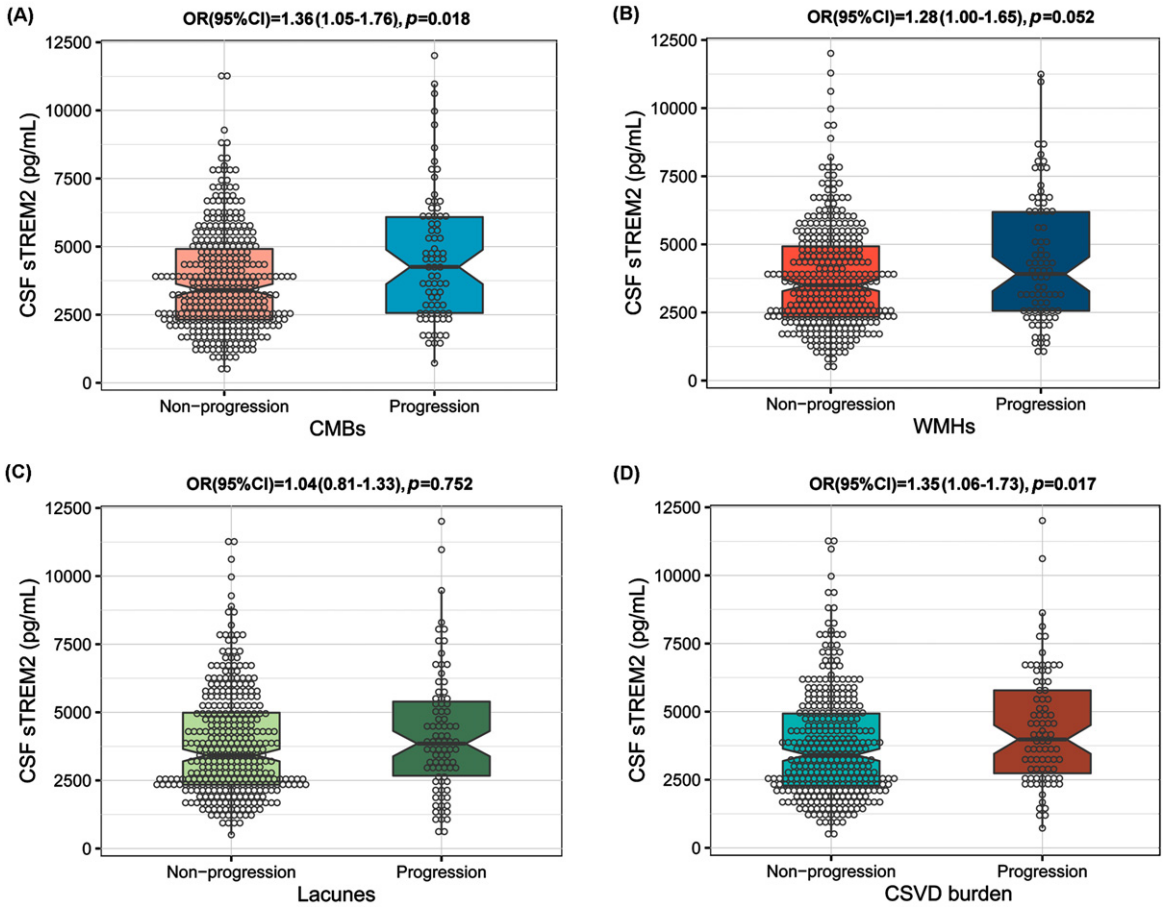

Fig. 1. Longitudinal associations of progressive risk for CSVD imaging markers with baseline CSF sTREM2. Baseline CSF sTREM2 levels predicted the risk for progressive CMBs (A) and total burden of CSVD (D) but were not associated with the risk of progressive WMHs (B) and lacunes (C). p values were assessed by LR models with full adjustment for age, sex, educational level, *APOE* ε4 carrier status, MMSE score, ICV, and vascular risk factors (including a history of hypertension, hyperlipidemia, coronary heart disease, diabetes, smoking status, and alcohol habit). *APOE* ε4, apolipoprotein E ε4; CI, confidence interval; CMBs, cerebral microbleeds; CSF, cerebrospinal fluid; CSVD, cerebral small vessel disease; ICV, intracranial volume; MMSE, Mini-Mental State Examination; OR, odds ratio; sTREM2, soluble TREM2; WMHs, white matter hyperintensities

interaction <0.05, Supplementary Table 3). More pronounced associations of baseline CSF sTREM2 with the progression outcomes of CSVD burden and CMBs were observed in the younger participants (age <70 years, Supplementary Figure 2) and APOE ε4 carriers (Supplementary Figure 3). But sex and cognitive status seemed unlikely to modify these potential associations. Regarding the longitudinal changes of CSF sTREM2, baseline cognitively unimpaired individuals presented a strengthened association of an accelerated change rate with an increased risk of progressive CMBs as compared to MCI persons, indicating a potential of sTREM2 as the treatment target at an early stage (Supplementary Table 4). No obvious interaction effects by demographic factors and genetic risk were found for associations between change rates of CSF sTREM2 and CSVD progression (Supplementary Table 3).

Enhanced associations by cerebral amyloid and tau pathology

As expected, substantial correlations at baseline were confirmed between sTREM2 and AD pathological biomarkers (i.e., $A\beta_{1\text{-}42}$ and P-tau_{181P}), independent of age, sex, educational level, APOE ε4 gene, and general cognition. Supplementary Figures 4 and 5 depict the linear relations as evidenced by significant standardized coefficients ($A\beta_{1\text{-}42}$: $\beta = 0.19$, $p < 0.0001$; P-tau_{181P}: $\beta = 0.44$,

$p < 0.0001$). Therefore, sensitivity analyses after correcting confounding effects of $A\beta_{1\text{-}42}$ and P-tau_{181P} demonstrated a robust risk for aggravating CSVD total burden among participants with an elevated CSF sTREM2 (OR = 1.61 [95% CI, 1.15–2.26], $p = 0.005$, Supplementary Table 5).

Intriguingly, effect modification analyses illustrated significant risk differences in the association between CSF sTREM2 and the risk of progressive CSVD events by A/T status (p for interaction <0.1, Fig. 2, Supplementary Table 6). In detail, individuals who had increased baseline CSF sTREM2 levels and evidence of cerebral amyloid pathology (A [+]) bear a higher progressive risk for CSVD total burden (OR = 2.02 [95% CI, 1.38–2.96], $p < 0.001$) when compared to those without amyloidosis evidence (A [–]). Regarding the various CSVD imaging markers, we also noted stronger associations with progressive CMBs (OR = 2.17 [95% CI, 1.46–3.21], $p < 0.001$) and WMHs (OR = 1.60 [95% CI, 1.12–2.28], $p = 0.010$) in sub-populations of A (+). Besides, similar stratified effects were found by tau-related pathological status: higher baseline CSF sTREM2 might predict higher likelihoods for progressive CSVD total burden (OR = 1.42 [95% CI, 1.01–2.00], $p = 0.042$) as well as worsening outcomes of CMBs (OR = 1.50 [95% CI, 1.06–2.12], $p = 0.022$) and WMHs (OR = 1.54 [95% CI, 1.09–2.20], $p = 0.016$) between subjects identified with definite accumulating tau pathology (T [+]). After performing subgroup analyses in A–T–, A+T–/A–T+, and A+T+ categorized subgroups, similar results were also observed in the A+T+ subgroup (Supplementary Table 6).

Simultaneously, longitudinal associations between CSF sTREM2 change rates and the progressive events of CSVD showed similar stratified effects to the above analyses (Table 2). A (+) evidence strengthened the associations of higher change rates with higher possibilities of progressive CSVD total burden (OR = 1.82 [95% CI, 1.12–2.95], $p = 0.016$) as well as CMBs (OR = 2.29 [95% CI, 1.31–3.99], $p = 0.004$) and lacunes progression (OR = 1.90 [95% CI, 1.19–3.02], $p = 0.007$). Rapid change rates of CSF sTREM2 may predict higher progressive risks for CSVD total burden and various image makers in T (+) group when compared to T (–) group, but with some non-significant estimates (CSVD burden, OR = 1.46 [95% CI, 0.94–2.28]; CMBs, OR = 1.51 [95% CI, 0.96–2.40]; WMHs, OR = 1.41 [95% CI, 0.90–2.20], lacunes: OR = 1.81 [95% CI, 1.13–2.89]).

DISCUSSION

We found that elevated CSF sTREM2 levels were positively associated with CSVD progression, particularly with CMBs, suggesting that the severity of neuroinflammation might predict a high risk of CSVD progression. AD pathology was demonstrated to modulate the associations between CSF sTREM2 and CSVD progression. CSF sTREM2 as a neuroinflammatory marker could be an intersection between the pathogenesis of CSVD and AD.

The remarkable longitudinal associations in our study indicated that elevated neuroinflammation at an early stage could contribute to subsequent progressive CSVD. Two relevant animal studies showing a reversal of white matter damage after using drugs targeting inflammation were also consistent with our findings [26, 27]. However, characteristics observed in MRI are more likely to reflect late-stage manifestations of CSVD. Therefore, further investigations are needed to explore the associations between neuroinflammation and the earlier pathological changes of CSVD. Our study showed that the association between sTREM2 and CSVD was more evident in younger individuals, which might be explained by the age-related decrease in microglial function and heavier baseline CSVD burden in the elders [28]. As a major genetic risk factor for both CSVD and AD [2], APOE $\varepsilon 4$ was demonstrated to moderate the association between neuroinflammation and CSVD in our study, which was consistent with previous studies showing that APOE $\varepsilon 4$ could enhance inflammatory response by impairing macrophage efferocytosis and potentiating apoptosis [29].

Among the imaging markers of CSVD, sTRME2 showed an association with progressive CMBs rather than WMHs and lacunes. This difference in the associations of neuroinflammation with CSVD imaging markers provided new insights into the mechanisms of neuroinflammation-mediated CSVD, given that different CSVD imaging markers are involved in various pathophysiological etiologies of CSVD. Loss of blood-brain barrier (BBB) integrity is a prominent feature of CSVD and is suggested as the initiating event in CSVD [30, 31]. Notably, BBB disruption has been found associated with CMBs and EPVS among the CSVD imaging markers [3]. The presence of CMBs indicates the previous extravasation of red blood cells into the brain parenchyma resulting from increased BBB permeability in the context of chronic hypertension or cerebral amyloid accumulation

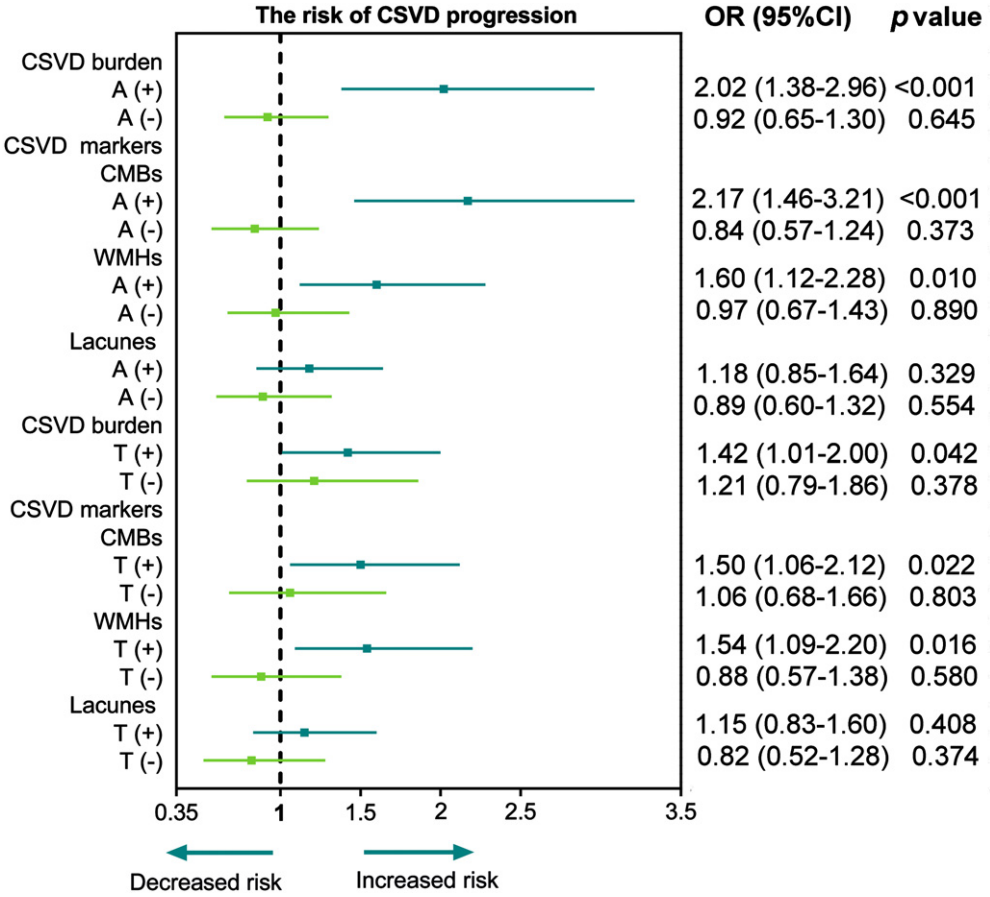

Fig. 2. Enhanced prediction by cerebral amyloid and tau pathology for progressive events of CSVD based on baseline CSF sTREM2 levels. LR models were approached to calculate ORs and 95%CI with adjustment for covariates in the main model (including age, sex, educational level, *APOE* ε4 carrier status, MMSE score, and ICV). *APOE* ε4, apolipoprotein E ε4; CI, confidence interval; CMBs, cerebral microbleeds; CSF, cerebrospinal fluid; CSVD, cerebral small vessel disease; ICV, intracranial volume; MMSE, Mini-Mental State Examination; OR, odds ratio; sTREM2, soluble TREM2; WMHs, white matter hyperintensities.

Table 2
Enhanced associations of CSF sTREM2 change rates and progressive CSVD by abnormal amyloid- and tau-related deposition

Variables (P versus NP)	CSF sTREM2 change rates (1-SD increase)				*p* for interaction
	A (–)		A (+)		
	OR (95% CI)	*p*	OR (95% CI)	*p*	
CSVD burden	0.81 (0.50–1.30)	0.378	1.82 (1.12–2.95)	0.016	0.031
CSVD markers					
CMBs	1.02 (0.63–1.63)	0.948	2.29 (1.31–3.99)	0.004	0.037
WMHs	0.99 (0.56–1.76)	0.972	1.50 (0.95–2.36)	0.079	0.222
Lacunes	0.91 (0.51–1.62)	0.741	1.90 (1.19–3.02)	0.007	0.053
Variables	T (–)		T (+)		*p* for interaction
(P versus NP)	OR (95% CI)	*p*	OR (95% CI)	*p*	
CSVD burden	0.85 (0.67–1.56)	0.604	1.46 (0.94–2.28)	0.095	0.088
CSVD markers					
CMBs	1.46 (0.80–2.65)	0.218	1.51 (0.96–2.40)	0.077	0.872
WMHs	0.69 (0.35–1.36)	0.282	1.41(0.90–2.20)	0.130	0.112
Lacunes	0.90 (0.50–1.63)	0.727	1.81 (1.13–2.89)	0.014	0.072

Models were adjusted for age, sex, educational level, *APOE* ε4 carrier status, MMSE score, and ICV. A, amyloid-β; *APOE* ε4, apolipoprotein E ε4; CMBs, cerebral microbleeds; CI, confidence interval; CSF, cerebrospinal fluid; CSVD, cerebral small vessel disease; ICV, intracranial volume; MMSE, Mini-Mental State Examination; NP, non-progressive event; OR, odds ratio; P, progressive event; sTREM2, soluble TREM2; T, phosphorylated tau; WMHs, white matter hyperintensities.

[28, 32]. Neuroinflammation is thought to promote vessel occlusion and endothelial dysfunction, leading to an increase of BBB permeability. And increased BBB permeability causes erythrocyte exudation from small cerebral vessels, which is the primary characteristic of CMBs [9, 33]. Additionally, the dysfunction of BBB itself also causes chronic neuroinflammatory responses and tissue damage, resulting in a vicious cycle of pathological processes [34]. Collectively, the damage of neuroinflammation to BBB could be an early pathological event of CSVD, especially CMBs.

Remarkably, although CSF sTREM2 is generally accepted as a downstream product of neuroinflammatory response, it also could induce inflammatory responses and lead to microglial activation, independently of the full-length TREM2 [35]. Thus, targeting CSF sTREM2 could be a novel pathway for treating CSVD. However, neuroinflammation, which was initially found as a protective mechanism against microvascular dysfunction, was also demonstrated to reduce amyloid pathology and toxicity in AD patients [36]. Therefore, microglial function served as a double-edged sword in CSVD and AD. More future studies on the role of neuroinflammatory responses in the development of CSVD are warranted to determine the suitable time to intervene.

Amyloid and tau deposition modulated the association between sTREM2 and CSVD progression. Neuroinflammation could be a shared pathogenesis of AD and CSVD at the early stage. There are two potential mechanisms underlying the modulation effects. Firstly, the aggregation of Aβ (especially $A\beta_{1\text{-}42}$) can impair the clearance system of Aβ in the brain, and then destroy the dynamic equilibrium of different forms of Aβ, including $A\beta_{1\text{-}40}$ which is the primary form of vascular Aβ deposition in a CSVD subtype of cerebral amyloid angiopathy (CAA) [37, 38]. Secondly, owing to the accumulation of toxic tau and Aβ, microglial activation and the subsequent release of pro-inflammatory cytokines contribute to a chronic inflammatory condition within the brain [36] and increase BBB permeability [39], which in turn impairs the clearance system of Aβ in the brain. Therefore, AD core pathology could induce or promote microglial activation, leading to a chronic neuroinflammatory state *in vivo* and greater susceptibility of those with AD core pathology to CSVD.

Our study was the first to investigate the association between CSVD and neuroinflammation *in vivo* using CSF sTREM2. Several limitations in our study should also be acknowledged. Firstly, we did not screen the included individuals for possible TREM2 mutations as TREM2 mutation carriers showed impaired microglia activation and clustering [40]. However, given the low prevalence of TREM2 mutations in the population [41], TREM2 mutations were unlikely to bias our results. Secondly, our study lacked data on some CSVD imaging markers such as EPVS and cerebral atrophy, and thereby the associations of sTREM2 with such markers should be investigated in further studies. Thirdly, although some previous studies reported that CSF sTREM2 levels significantly correlated with CSF albumin/serum albumin ratio, a BBB integrity biomarker, our study lacked the measurements of BBB dysfunction and the subsequent correlation analyses to support the association between neuroinflammation and BBB [9]. Moreover, the follow-up period was relatively insufficient. Consequently, these findings should be verified in larger populations and cohorts with longer follow-up periods.

Conclusion

Our study presents the first investigation on CSVD and *in vivo* neuroinflammation using CSF sTREM2. The findings suggested that neuroinflammation may promote the development of CSVD and is likely to be modulated by AD pathology (brain amyloidosis and tau pathology). Neuroinflammation could be a shared pathogenesis of CSVD and AD at the early stage, suggesting a complex role of neuroinflammation as a targeting therapeutic pathway in AD and CSVD.

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CONFLICT OF INTEREST

The authors have no conflict of interest to report.

DATA AVAILABILITY

The dataset generated and analyzed in the current study is available from the corresponding author on reasonable request.

SUPPLEMENTARY MATERIAL

The supplementary material is available in the electronic version of this article: https://dx.doi.org/10.3233/JAD-220731.

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