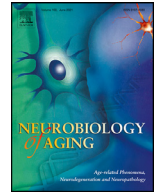




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Biological correlates of elevated soluble TREM2 in cerebrospinal fluid

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ABSTRACT

Cerebrospinal fluid (CSF) soluble triggering receptor expressed on myeloid cells-2 (sTREM2) is an emerging biomarker of neuroinflammation in Alzheimer's disease (AD). Yet, sTREM2 expression has not been systematically evaluated in relation to concomitant drivers of neuroinflammation. While associations between sTREM2 and tau in CSF are established, we sought to determine additional biological correlates of CSF sTREM2 during the prodromal stages of AD by evaluating CSF A β species (A β _{x-40}), a fluid biomarker of blood-brain barrier integrity (CSF/plasma albumin ratio), and CSF biomarkers of neurodegeneration measured in 155 participants from the Vanderbilt Memory and Aging Project. A novel association between high CSF levels of both sTREM2 and A β _{x-40} was observed and replicated in an independent dataset. A β _{x-40} levels, as well as the CSF/plasma albumin ratio, explained additional and unique variance in sTREM2 levels above and beyond that of CSF biomarkers of neurodegeneration. The component of sTREM2 levels correlated with A β _{x-40} levels best predicted future cognitive performance. We highlight potential contributions of A β homeostasis and blood-brain barrier integrity to elevated CSF sTREM2, underscoring novel biomarker associations relevant to disease progression and clinical outcome measures.

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Abbreviations: CSF, Cerebrospinal fluid; TREM2, Triggering Receptor Expressed on Myeloid Cells-2; sTREM2, Soluble Triggering Receptor Expressed on Myeloid Cells-2; AD, Alzheimer's disease; VMAP, Vanderbilt Memory and Aging Project; ADNI, Alzheimer's Disease Neuroimaging Initiative; APOE- ϵ 4, Apolipoprotein E epsilon 4; BBB, Blood-brain barrier; A β , Beta-amyloid; NVU, Neurovascular unit; MCI, Mild cognitive impairment; NC, Normal cognition; NfL, Neurofilament light chain protein.

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Data used in preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (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.

1. Introduction

Alzheimer's disease (AD) is a devastating neurodegenerative disease and the most common cause of dementia, affecting more than 1 in 9 seniors in the United States ('Alzheimer's disease facts and figures' 2022). Nosologically-defined AD pathology consists of amyloid- β (A β) plaques and neurofibrillary tangles that are thought to drive neuroinflammation, blood-brain barrier (BBB) dysfunction, and neurodegeneration resulting in cognitive decline and clinical disease (Attems and Jellinger 2014; Sweeney, Sagare, and Zlokovic 2018). The prodrome of AD can be 20+ years, whereby neuropathology begins to deposit and brain changes occur years

A complete listing of ADNI investigators can be found at: http://adni.loni.usc.edu/wp-content/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf

prior to the onset of clinical symptoms. For that reason, there has been an incredible focus on the development of biomarkers in AD, including both fluid and imaging biomarkers of AD neuropathology, neurodegeneration (Jack and Holtzman 2013; Jack et al. 2016), and more recently neuroinflammation and BBB dysfunction (Heslegrave et al. 2016; Hao et al. 2021; Bostrom et al. 2021). While cerebrospinal fluid (CSF) biomarkers of amyloid and tau are well-established, there is a pressing need to better characterize emerging biomarkers to understand their temporal dynamics and biological correlates.

One promising biomarker with a strong genetic and molecular basis is soluble triggering receptor expressed on myeloid cells-2 (sTREM2) measured in CSF. TREM2, encoding its transmembrane parent, was originally implicated as an AD risk gene through 2 large genome-wide association studies (GWAS), including the identification of the R47H missense mutation that confers an increased risk similar in magnitude to a single copy of the *APOE-ε4* allele (Guerreiro et al. 2013; Jonsson et al. 2013). TREM2 is expressed preferentially on microglia in the brain and plays a critical role in the neuroinflammatory response to AD. More specifically, functional studies of TREM2 have revealed roles in the regulation of parenchymal $A\beta$ plaque deposition (Jay et al. 2017; Kober et al. 2020; Song et al. 2018; Ulland et al. 2017; Wang et al. 2015; Wang et al. 2016; Yuan et al. 2016), progression of tau pathology (Bemiller et al. 2017; Gratuze et al. 2020; Jiang et al. 2018; Lee et al. 2021; Leyns et al. 2017), and BBB dysfunction (Wu et al. 2017; Wang, Yang, et al. 2020; Taylor et al. 2020). Beyond these roles in AD, TREM2 is also involved more generally in microglial activation (Hamerman et al. 2006; Turnbull et al. 2006), ischemia/hypoxia (Wu et al. 2017), oxidative stress responses (Linnartz-Gerlach et al. 2019; Liu, Chu, and Wang 2019), and transcriptional regulation of brain endothelial cells (Carbajosa et al. 2018), providing numerous potential avenues that could contribute to risk and progression in AD.

Cleavage of TREM2 ectodomain produces a soluble fragment (sTREM2), considered a biomarker of microglial activation, whereby increased protein levels have been reported to coincide with the transition of mild cognitive impairment (MCI) to AD dementia (Liu et al. 2018; Suarez-Calvet, Kleinberger, et al. 2016). Additionally, sTREM2 is signaling competent, thought to modulate inflammatory and phagocytic responses from microglia, and has also been shown in this manner to promote $A\beta$ clearance in 5XFAD mice (Zhong et al. 2019). Increases in sTREM2 levels during AD may mark a transition in the neuroinflammatory state that correlates with neurodegeneration and clinical progression. Thus, it is not surprising that CSF sTREM2 is strongly correlated with CSF biomarkers of tau pathology that track closely with the neurodegenerative processes in AD (Suarez-Calvet, Kleinberger, et al. 2016; Henjum et al. 2016; Heslegrave et al. 2016; Suarez-Calvet et al. 2019). In contrast, CSF sTREM2 associations with $A\beta$ and BBB dysfunction have been inconsistent (Heslegrave et al. 2016; Suarez-Calvet, Kleinberger, et al. 2016; Henjum et al. 2016; Suarez-Calvet et al. 2019; Bekris et al. 2018) and remain poorly understood. There is a need to fully characterize the biological correlates of CSF sTREM2, particularly a need to deconvolve the variance in sTREM2 levels that are explained by biomarkers of $A\beta$, tau, neurodegeneration, and BBB dysfunction, which are all thought to contribute to the neuroinflammatory milieu in AD.

It has been hypothesized that sTREM2 may be a complementary biomarker that could be useful in the context of aging and disease. Moreover, therapeutics targeting TREM2 are in active development, yet there is limited knowledge of the types of related biological processes and functions of sTREM2 itself. The goal of this

manuscript is to clarify the biological correlates and thus types of biological processes that coincide with elevated sTREM2 in CSF. A comprehensive characterization of biomarkers in the CSF compartment related to sTREM2 elevation and therefore microglial activation will build understanding of potential early neuroimmune dynamics relevant to AD. First, we fully characterize the associations between CSF sTREM2 levels and well-established biomarkers of AD pathology, neurodegeneration, and BBB dysfunction. Second, we evaluate the unique contribution of each of these biomarkers to sTREM2 levels in competitive models, evaluating whether biomarkers of BBB and $A\beta$ explain variance in sTREM2 levels above and beyond the well-established associations with CSF tau. Third, we relate residual variance in sTREM2 levels to measurements of longitudinal cognition evaluating potential clinical relevance of sTREM2 associations in CSF. Together, these analyses provide a more comprehensive picture of the biological underpinnings of elevated CSF sTREM2 in aging and disease and provide critical information for the application and interpretation of CSF sTREM2 levels in future biomarker studies.

2. Materials and methods

2.1. Study cohort

Participants were drawn from the Vanderbilt Memory and Aging Project (VMAP) launched in 2012 in Nashville, TN. VMAP is a longitudinal study of vascular health and brain aging (Jefferson et al. 2016). A total of 335 participants, 60–92 years of age, were enrolled. This included 168 with mild cognitive impairment (MCI) and 167 age-, sex-, and race-matched cognitively normal controls (NC). MCI diagnosis was determined by the National Institute on Aging/Alzheimer's Association Workgroup core clinical criteria (Albert et al. 2011). Briefly, this includes a CDR score $0 \geq 0.5$, concern of changes in cognition (reported by the participant, informant, or clinician), absence of dementia, relatively spared daily functioning, and neuropsychological functioning indicating objective impairment outside age-adjusted mean performance in 1 or more cognitive domains. Inclusion criteria required participants to speak English, have adequate auditory and visual acuity, and have a reliable study partner. Exclusion criteria included MRI contraindications, history of neurological disease or major psychiatric illness, heart failure, head injury with loss of consciousness >5 minutes, or a systemic or terminal illness. A subset of participants underwent fasting lumbar puncture for CSF collection at baseline. Written informed consent was obtained from all participants prior to data collection, and all protocols were approved by the Vanderbilt University Medical Center Institutional Review Board.

2.2. Neuropsychological composites

Participants underwent detailed neuropsychological assessment of various domains of cognitive performance at baseline and every 18 months. An episodic memory composite was calculated as a z-score from the following independent tests: California Verbal Learning Test Second Edition (CVLT-II) Total Immediate Recall, CVLT-II Delayed Recall, CVLT-II Recognition, Biber Figure Learning Test (BFLT) Total Immediate Recall, BFLT Delayed Recall, and BLFT Recognition. An executive functioning composite was calculated as a z-score from the following: Delis-Kaplan Executive Function System (D-KEFS) Number-Letter Switching Test, D-KEFS Color-Word Inhibition Test, and Letter Fluency Test (FAS). Assessments were reviewed to avoid floor and ceiling effects and composites were calculated from a latent variable model where each item was treated as a raw continuous variable loaded on a general factor, also as on a test-specific factor to reduce potential confounds (Jefferson

et al. 2016; Kresge et al. 2018). Participants with longitudinal cognition data had up to 5 measurement timepoints (mean \pm sd = 2.6 \pm 1.3 visits) and a mean follow-up period of (mean \pm sd = 4.6 \pm 1.7 years).

2.3. Blood draw and albumin measurement

Participants underwent morning fasting venous blood draw and samples were immediately stored at -80°C . Whole blood was centrifuged at 2000g and 4°C for 15 minutes and plasma was extracted and stored in ten 0.5mL aliquots. Albumin levels (plasma and CSF) were measured by immunonephelometry on a Beckman Immage Immunochemistry system (Beckman Instruments, Beckman Coulter, Brea, CA, USA). The albumin ratio was calculated as CSF albumin (mg/L) / plasma albumin (g/L).

2.4. APOE genotyping

White blood cell extraction was performed on frozen whole blood. The TaqMan single nucleotide polymorphism genotyping assay from Applied Biosystems (Foster City, CA) was applied to determine APOE genotypes by identifying the 2 single-nucleotide polymorphisms that characterize alleles $\epsilon 2$, $\epsilon 3$, and $\epsilon 4$. Polymerase chain reaction (PCR) was performed as previously described (Jefferson et al. 2016). Genotyping efficiency was $>99\%$.

2.5. Lumbar puncture and biochemical analyses

A maximum of 25mLs of CSF was drawn from a baseline, optional, fasting lumbar puncture procedure and collected with polypropylene syringes using a Sprotte 25-gauge spinal needle from an intervertebral lumbar space. CSF supernatant was immediately extracted and aliquoted in 0.5mL polypropylene tubes and stored at -80°C . Analysis of CSF total tau, p-tau₁₈₁, $A\beta_{x-40}$, $A\beta_{x-42}$, $A\beta_{1-42}$, and neurofilament light (NFL) was performed in batch using commercially available enzyme-linked immunosorbent assays (carboxy-terminal specific antibodies aided in quantification of $A\beta_{x-40}$ and $A\beta_{x-42}$ species with varying amino-terminal lengths). CSF sTREM2 concentration was measured using an in-house Meso Scale Discovery (MSD) assay (Rockville, MD), as previously described in detail (Jensen et al. 2019). Samples were processed in 1 round of experiments using 1 batch of reagents by board-certified laboratory technicians blinded to clinical information. Coefficients of variation for duplicate samples were $<10\%$ (mean 2.4%). Supplemental Table 1 contains assay kit information.

2.6. Replication of amyloid- β results using ADNI data

The AD Neuroimaging Initiative (ADNI) is a longitudinal multisite study launched in 2004 focused on the development of biomarkers for AD early detection. Participant demographics are provided in Supplemental Table 2. Baseline CSF biomarker measurement of $A\beta_{1-42}$, $A\beta_{1-40}$, and $A\beta_{1-38}$ were acquired utilizing 2D-UPLC tandem mass spectrometry. Each data point represents the average of duplicate 0.1mL aliquots from a single CSF sample. Methodology was previously validated for analysis of $A\beta_{1-42}$ (Korecka et al. 2014) and then adapted for the additional peptides by including their internal standards and re-validation of the protocol (Korecka et al. 2020). A detailed summary of the analytical method including sample preparation, parameters, and standards is publicly available for download on the ADNI database (adni.loni.usc.edu). Tau positivity was determined by the previously defined cut-off value of 23 pg/mL (Shaw et al. 2009).

An MSD platform-based assay was used for CSF sTREM2 measurement which has been previously established and validated

by Christian Haass' group and reported (Kleinberger et al. 2014; Suarez-Calvet, Araque Caballero, et al. 2016; Suarez-Calvet, Kleinberger, et al. 2016; Suarez-Calvet et al. 2019).

2.7. Statistical analyses

Statistical analyses were performed in R v.4.1.2 using R Studio IDE (<https://www.rstudio.com/>). Linear regression models were leveraged using CSF protein levels of AD biomarkers to predict CSF sTREM2 measures at baseline. Covariates included age, sex, education, and clinical diagnosis (MCI vs. NC). Following independent models for each biomarker, we performed competitive models leveraging a hierarchical linear regression approach to evaluate the unique contribution and variance explained by each significant predictor from the independent analyses. Model selection, aided by Akaike information criterion (AIC) and Bayesian information criterion (BIC) calculations, was performed using R packages *AICcmodave* and *flemix*, respectively. Residuals were then calculated from the cross-sectional models assessing variance in baseline CSF sTREM2 measurements and used to predict future cognitive performance using either a memory or executive functioning composite score as the outcome variable within a longitudinal linear mixed-effects regression.

Given the established association between CSF tau and CSF sTREM2, we performed post hoc interaction analyses for the biomarkers that remained statistically significant in competitive hierarchical linear regression models to better understand whether the novel biomarker associations were modified according to tau status. All covariates remained the same as in our primary models above.

Sensitivity analyses included interaction models with sex, APOE- $\epsilon 4$ carrier status, and MCI diagnosis (Supplemental Table 3). Additional analyses included the date of CSF collection as a covariate to account for potential protein storage/degradation effects; however, accounting for this added variable did not have a significant impact on the main effects results (Supplemental Table 4) or competitive models (Supplemental Table 5). Further sensitivity analyses explored potential variation in results due to statistical and visual outliers as well as adjusting MCI diagnosis criteria to align with ADNI (Supplemental Tables 6–9). Scatter plots showing sTREM2 by additional biomarkers after outlier removal are provided in Supplementary Figs. 1A–D.

All models were corrected for multiple comparisons using the Benjamini & Hochberg (1995) false discovery rate.

3. Results

3.1. Participant characteristics

The VMAP discovery cohort is divided fairly equally among individuals with MCI (46%) and NC (54%), comprised mostly of males (67%), also non-Hispanic White participants (94%), and is highly educated (mean: 16 years). Baseline age and APOE- $\epsilon 4$ carrier status was similar across diagnostic groups, but years of education differed with lower levels in MCI (mean: 15 years) compared to NC (mean: 17 years) shown in Table 1.

3.2. Biomarker associations with CSF sTREM2

Main effects of AD biomarkers on sTREM2 levels (sTREM2 \sim biomarker + base covariates) were examined. First, sTREM2 associations were characterized with respect to biomarkers of $A\beta$ peptide abundance. CSF sTREM2 did not relate to $A\beta_{1-42}$ ($p = 2.59\text{e-}01$; Table 2; Fig. 1A), consistent with previous work showing weak (Suarez-Calvet, Kleinberger, et al. 2016) or no association

Table 1
VMAP cohort demographics

Characteristic	Clinical diagnosis		Total (N = 155)	p value
	Normal Cognition (N=83)	Mild cognitive impairment (N = 72)		
Male, no. (%)	58 (70)	46 (63)	104 (67)	0.535
Age (baseline)	72 ± 6.50	72 ± 6.18	72 ± 6.33	0.458
Education	17 ± 2.41	15 ± 2.94	16 ± 2.80	0.001
APOE-ε4 carriers, no. (%)	24 (29)	27 (38)	51 (33)	0.334
sTREM2 CSF pg/mL	3530 ± 1867.29	3817 ± 1759.49	3667 ± 1812.50	0.327
p-tau ₁₈₁ CSF pg/mL	56 ± 21.92	67 ± 28.59	61 ± 25.70	0.212
(% p-tau positive) ^a	(17)	(26)	(21)	
Aβ ₁₋₄₂ CSF pg/mL	760 ± 229.54	662 ± 254.02	714 ± 245.40	0.012
(% Aβ positive) ^b	(20)	(40)	(30)	

Values are presented as mean ± standard deviation, unless otherwise indicated. A student's t-test or a Pearson's χ^2 test was used to compare continuous or categorical variables, respectively, between cognitive diagnoses. Bold represents statistical significance set to a *priori* threshold $p < 0.05$. 6 participants are Black/African American; 2 American Indian/Alaska Native; 2 Asian.

^a p-tau positive ≥ 80 pg/mL and

^b Aβ positive ≤ 530 pg/mL.

Table 2
Main effects of baseline CSF biomarkers on sTREM2 measurement.

Predictor	β	SE	DF	p
Aβ _{x-40}	0.490	0.076	149	1.532e-09^a
p-tau ₁₈₁	30.513	5.126	149	1.818e-08^a
CSF/plasma albumin ratio	327.552	59.077	145	1.355e-07^a
t-tau	3.146	0.604	149	6.137e-07^a
NfL	0.969	0.263	144	3.185e-04^a
Aβ _{x-42}	1.448	0.536	149	7.728e-03^a
Aβ ₁₋₄₂	0.678	0.599	149	2.599e-01

Bold represents statistical significance set to a *priori* threshold $p < 0.05$.

^a indicates survival for multiple comparisons by FDR correction across each primary model (Benjamini & Hochberg 1995). Significance value (P), degrees of freedom (DF), standard error (SE) and estimate of coefficient (β) represented for each model.

(Heslegrave et al. 2016; Suarez-Calvet et al. 2019). In contrast, higher levels of sTREM2 robustly related to higher CSF Aβ_{x-40} ($p = 1.53e-09$; Table 2; Fig. 1B), and to a lesser degree related to higher levels of N-truncated Aβ_{x-42} species ($p = 7.72e-03$; Table 2; Fig. 1C). Second, associations with biomarkers of tau pathology and axonal injury (NfL) were assessed. As expected, higher CSF sTREM2 was associated with higher levels of both total and phosphorylated tau ($p = 6.13e-07$ and $1.81e-08$, respectively; Table 2; Figs. 1D–E). High levels of sTREM2 also associated with high NfL ($p = 3.18e-04$; Table 2; Fig. 1F). Next, associations of sTREM2 levels with a CSF biomarker of BBB integrity were investigated. Higher levels of sTREM2 protein in CSF associated with an increased CSF/plasma albumin ratio, indicating decreased BBB integrity ($p = 1.35e-07$; Fig. 2A). This association remained regardless of APOE-ε4 carrier status, an independent predictor of BBB permeability (Fig. 2B) and interaction models between the CSF/plasma albumin ratio and APOE-ε4 carrier status on sTREM2 levels (sTREM2 ~ CSF/plasma albumin ratio * APOE-ε4 + base covariates) were insignificant ($p = 0.29$; Supplemental Table 3). A correlation matrix of sTREM2 and additional biomarkers is provided in Supplemental Fig. 2.

Due to the novelty of the Aβ species results, we replicated associations of sTREM2 with both Aβ₁₋₄₀ and Aβ₁₋₃₈ in ADNI ($\beta = 0.37$, $p = 6.37e-38$ and $\beta = 1.50$, $p = 4.09e-34$, respectively; Figs. 3A–B), indicating broad elevations of Aβ peptide species concurrent with rises in sTREM2.

3.3. Competitive models highlight sTREM2 relationships beyond tau

To further deconvolve components of CSF sTREM2 signal in VMAP, competitive hierarchical linear regression models were utilized (Table 3). The base model (CSF sTREM2 ~ age + sex + education + cognitive diagnosis) explained 11.8% of variance in sTREM2 protein measurement. Independently, p-tau₁₈₁ explained 17.7%, Aβ_{x-40} explained 21.2% and the CSF/plasma albumin ratio explained 21.2% of variance in sTREM2 levels. For the purpose of model selection, p-tau was inputted first given the association is well-established in the literature. This allowed use of the hierarchical model to evaluate variance explained above and beyond p-tau and covariates. Model 1 includes p-tau₁₈₁ as a predictor explaining an additional 16.9% of variance in sTREM2 levels above and beyond the base model. The addition of Aβ_{x-40} in Model 2 explains an additional 4.6% of variance above and beyond Model 1. And the inclusion of the BBB marker in Model 3 explains 14.8% of variance above and beyond Model 2 ($R^2 = 0.4813$). When including biomarkers (p-tau, Aβ, and the CSF/plasma albumin ratio) in Model 3 all 3 remained statistically significant. Together, p-tau₁₈₁, Aβ_{x-40}, and the CSF/plasma albumin ratio explain 36% of the variance in CSF sTREM2 levels.

3.4. Deconvolving tau, Aβ, and BBB associations with post hoc interaction models

Given the known association between CSF tau and CSF sTREM2, we sought to better understand if the novel sTREM2 associations with Aβ and BBB differed by tau status. We did not observe statistically significant interactions between Aβ_{x-40} and p-tau₁₈₁ on sTREM2 ($p = 0.64$) or between the CSF/plasma albumin ratio and p-tau₁₈₁ ($p = 0.25$), demonstrating associations were present regardless of tau status (Figs. S3A–B). Similarly, no significant interaction between Aβ₁₋₄₀ and p-tau₁₈₁ positivity on sTREM2 in the larger ADNI dataset was observed ($\beta = 0.07$, $se = 0.06$, $p = 0.22$; Fig. S4).

3.5. Sensitivity analyses

Interactions between tau markers with cognitive diagnosis, as well as APOE-ε4 carrier status on sTREM2, survived correction for multiple comparisons suggesting a stronger association between sTREM2 and biomarkers of tau pathology amongst APOE-ε4 non-

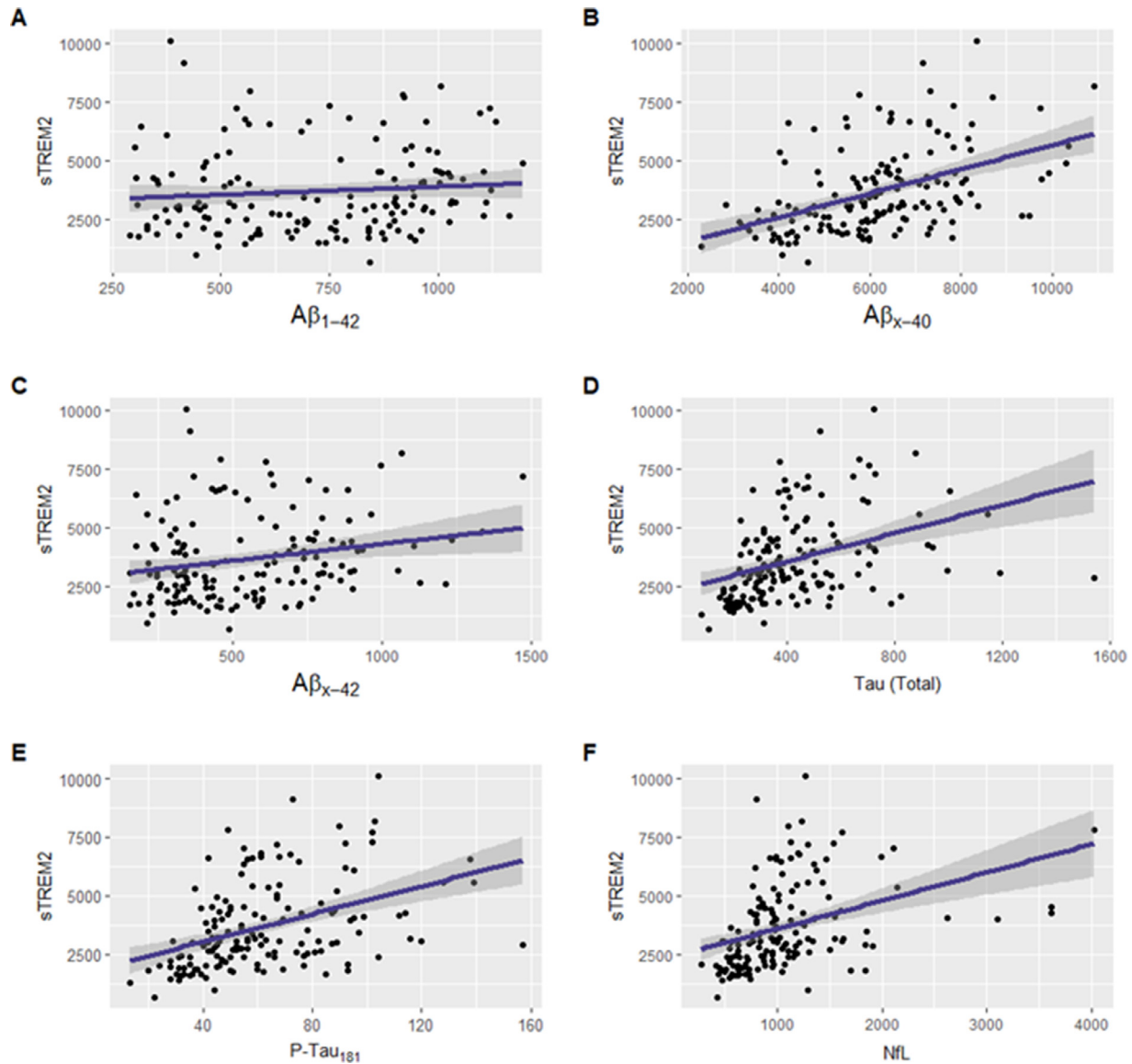


Fig. 1. Unadjusted scatter plots showing the main effects of AD CSF Biomarkers (x axis) on CSF sTREM2 (y axis). Higher sTREM2 levels relate to increases in AD biomarkers of shorter amyloid- β peptides and neurodegeneration: (A) sTREM2 levels do not significantly relate to levels of full-length $A\beta_{1-42}$, $p = 2.59e-1$. Higher sTREM2 levels significantly relate to increases in (B) $A\beta_{x-40}$, $p = 1.53e-9$; (C) $A\beta_{x-42}$, $p = 7.72e-3$; (D) total tau, $p = 6.13e-7$; (E) phosphorylated tau, $p = 1.81e-8$, and (F) NFL, $p = 3.18e-4$. Protein measurements given in pg/mL.

Table 3
Competitive hierarchical linear regression results

Model	Formula	DF	AIC	BIC	R ²	Adjusted R ²	ΔR^2
base	CSF sTREM2 ~ age + sex + education + cognitive diagnosis	150	2778	2798	0.118	0.089	N/A
1	CSF sTREM2 ~ base covariates + p-tau ₁₈₁	149	2747	2770	0.288	0.259	0.169
2	CSF sTREM2 ~ base covariates + p-tau ₁₈₁ + $A\beta_{x-40}$	148	2738	2765	0.333	0.302	0.046
3	CSF sTREM2 ~ base covariates + p-tau ₁₈₁ + $A\beta_{x-40}$ + CSF/plasma Albumin ratio	143	2636	2664	0.481	0.452	0.148

ΔR^2 = change in R² from previous nested model. Akaike information criterion (AIC) and Bayesian information criterion (BIC) calculations derived as follows: $AIC = 2K - 2\ln(L)$; where K = number of model parameters, and $\ln(L)$ = model log-likelihood. $BIC = (RSS + \log(n)d\sigma^2) / n$; where RSS = residual sum of squares, n = total observations, d = number of predictors, and σ^2 = estimate of variance of the error associated with each response measurement.

carriers compared to carriers, and among individuals with NC compared to those with MCI (Figs. 4A–D). Additionally, nominal interactions between tau and sex (Supplemental Table 3) were observed. In contrast, neither interactions between $A\beta_{x-40}$ nor the CSF/plasma albumin ratio with sex, *APOE- ϵ 4* carrier status, or diagnosis (Figs. 5A–D and Supplemental Table 3) were observed. In replication analyses using ADNI data we observed similar *APOE- ϵ 4*

($p < 0.02$) and diagnosis interactions ($p < 2.0e-05$; Supplemental Fig. 5) with tau on sTREM2 levels and did not observe such interactions with $A\beta$ species ($p > 0.16$).

Lastly, sensitivity analyses assessed the impact of MCI diagnosis criteria between VMAP and ADNI as well as statistical and visual outliers. Specifically, VMAP MCI diagnosis criteria was updated to resemble more closely that of ADNI. This included the

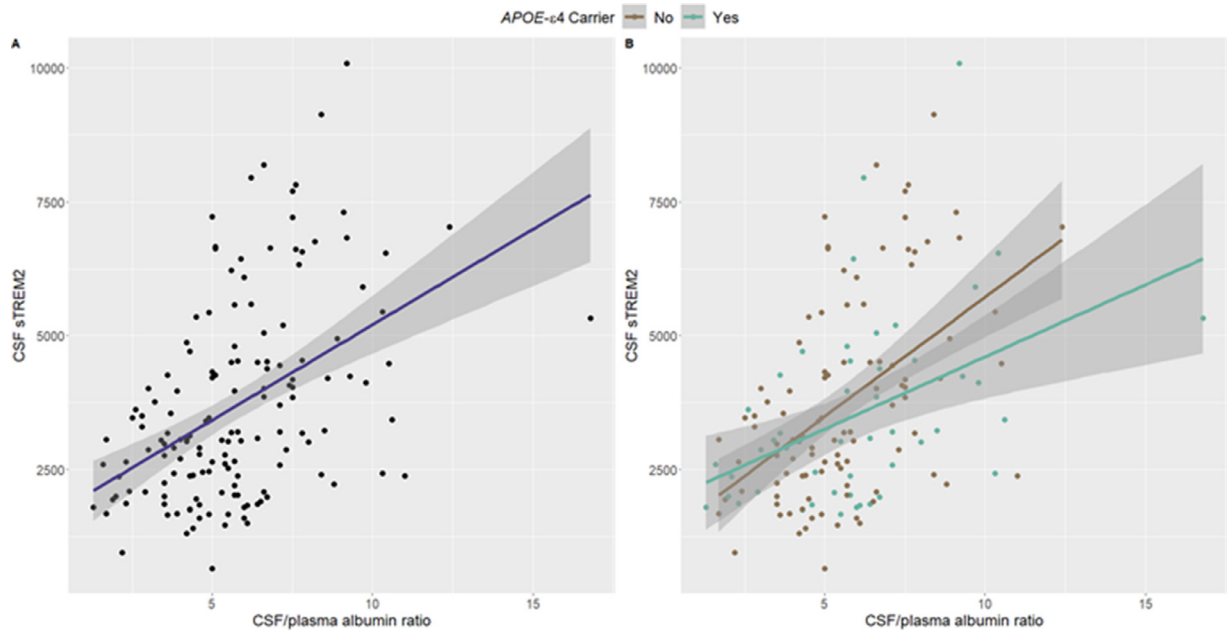


Fig. 2. Unadjusted plots showing higher CSF sTREM2 levels relate to an increased CSF/plasma albumin ratio independent of *APOE-ε4* carrier status: (A) Main effect of the CSF/plasma albumin ratio on sTREM2 ($p = 1.35e-07$). (B) Interaction of CSF/plasma albumin* *APOE-ε4* carrier status on sTREM2 ($p.int. = 0.28$). sTREM2 protein measurements given in pg/mL.

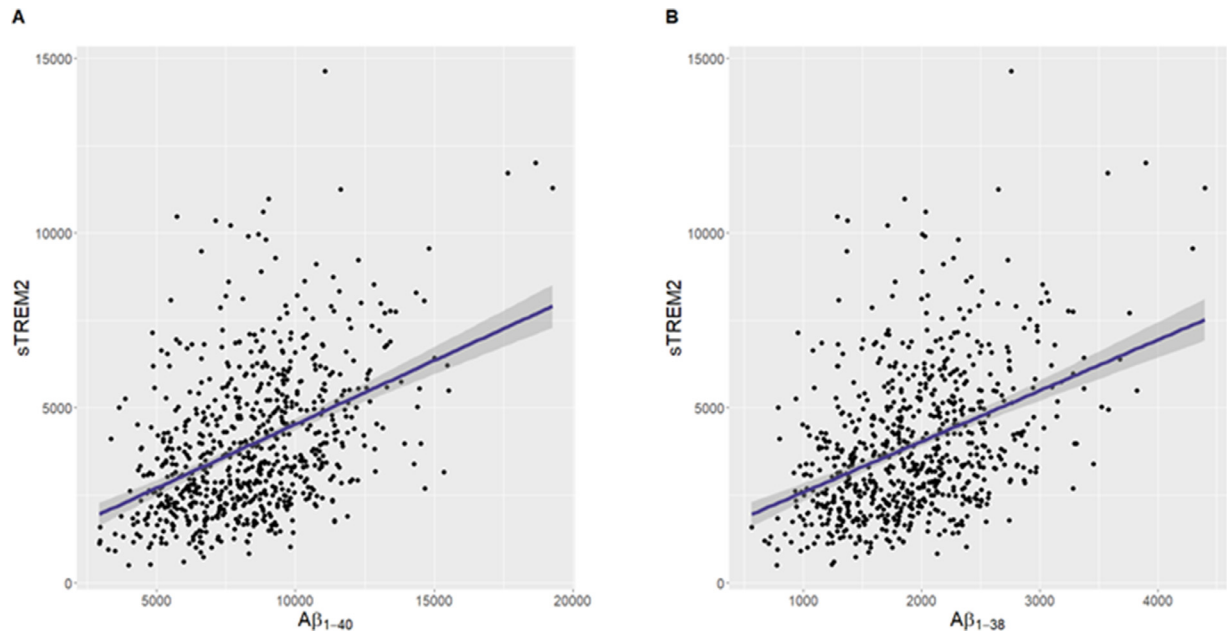


Fig. 3. Unadjusted plots demonstrating main effects of shorter $A\beta$ species on sTREM2 using ADNI data. Higher CSF sTREM2 levels relate to increases in CSF (A) $A\beta_{1-40}$ and (B) $A\beta_{1-38}$ ($\beta = 0.37$, $p = 6.37e-38$ and $\beta = 1.50$, $p = 4.09e-34$, respectively). Protein measurements given in pg/mL.

removal of 13 individuals with a Clinical Dementia Rating (CDR) of 0 or a Montreal Cognitive Assessment (MoCA) score of 17 or less (a MoCA score of 18 corresponds to a MMSE score of 24) (Trzepacz et al. 2015). These analyses yielded similar results and are provided in Supplemental Tables 6–9.

3.6. Associations with cognition and clinical progression

Given that CSF p-tau₁₈₁, CSF $A\beta_{x-40}$, and the CSF/plasma albumin ratio all explain independent variance in sTREM2, next, it was explored which component of the sTREM2 variance is also associated

with cognitive performance. This was evaluated by regressing out variance in sTREM2 associated with each biomarker sequentially and assessing the association between sTREM2 residual variance (when accounting for a given biomarker) with cognition. If regressing out a biomarker alters the association with cognition, it was concluded that the variance in sTREM2 associated with that particular biomarker is also relevant to cognitive performance. Baseline CSF sTREM2 levels predicted longitudinal memory in VMAP ($\beta = 1.44e-05$, $se = 6.80e-06$, $p = 0.03$), similar to previous reports (Ewers et al. 2019; Edwin et al. 2020) but not longitudinal executive functioning ($\beta = -3.22e-06$, $se = 7.32e-06$, $p = 0.66$). Due

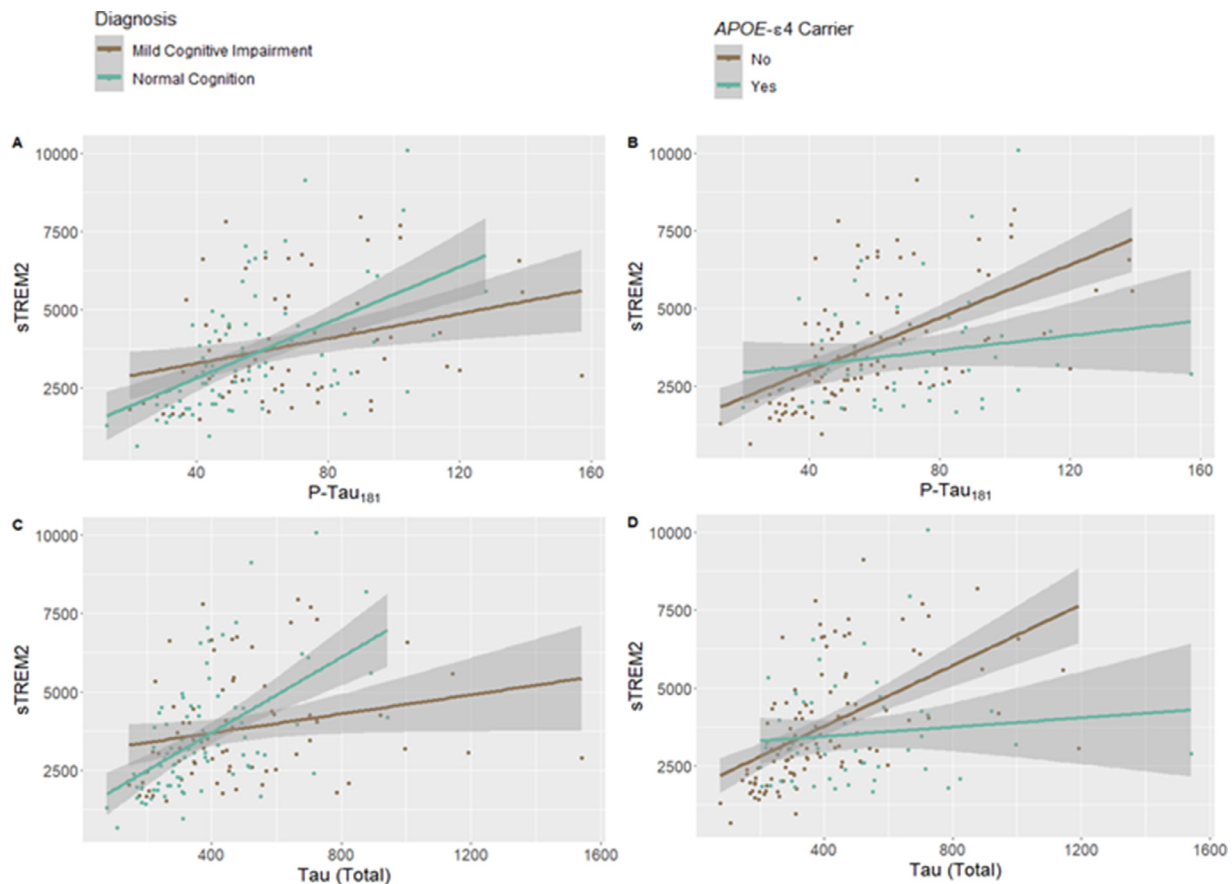


Fig. 4. Unadjusted plots showing tau biomarkers interact with cognitive diagnosis as well as *APOE-ε4* carrier status on sTREM2 levels in CSF: (A) phosphorylated tau*diagnosis, $p.int. = 0.033$, (B) phosphorylated tau**APOE-ε4*, $p.int. = 0.006$, (C) total tau*diagnosis, $p.int. = 0.003$, and (D) total tau* *APOE-ε4*, $p.int. = 0.002$. Protein measurements given in pg/mL.

to the well-established association between tau levels and cognitive decline, we wanted to determine whether this association was due simply to covariance with tau. When regressing out the variance in sTREM2 associated with p-tau₁₈₁, the residual variance in sTREM2 remained associated with longitudinal memory decline ($\beta = 1.68e-05$, $se = 7.28e-06$, $p = 0.02$). Similarly, when regressing out the variance in sTREM2 associated with both the CSF/plasma albumin ratio and p-tau₁₈₁ the residual variance remained significantly associated with longitudinal memory decline ($\beta = 2.21e-05$, $se = 8.15e-06$, $p = 6.83e-03$). In contrast, when regressing out the variance in sTREM2 associated with CSF $A\beta_{x-40}$, the residual variance was not associated with longitudinal cognitive performance ($\beta = 1.37e-05$, $se = 7.41e-06$, $p = 0.06$). These results suggest that variance in sTREM2 that is related to $A\beta$ species may indeed be relevant to cognitive trajectory.

4. Discussion

The present results provide an in-depth characterization of CSF sTREM2 expression in nondemented older adults. We provide strong evidence that fluid biomarkers of tau pathology, $A\beta$ abundance, and BBB dysfunction independently relate to sTREM2 levels, and that together these biomarkers explain substantial variance in CSF sTREM2. Specifically, we recapitulate previously reported associations between CSF sTREM2 and both biomarkers of BBB integrity and tau pathology demonstrating for the first time that all 3 biomarkers ($A\beta_{x-40}$, the CSF/plasma albumin ratio, and p-tau) explain unique variance in sTREM2 using competitive mod-

els. Importantly, our results provide novel evidence that sTREM2 relates to elevated CSF $A\beta$ species and that the variance in baseline CSF sTREM2 levels associated with $A\beta_{x-40}$ predicts future cognitive performance. Together, our results highlight the need to better understand sTREM2 in relation to the complex intersection of AD neuropathology, microglial activation, and BBB dysfunction to characterize its utility as both a dynamic biomarker of disease and therapeutic target.

4.1. Unique contribution of tau, BBB dysfunction, and $A\beta$ abundance to sTREM2 levels

Together, findings suggest that a heterogeneous set of biological correlates in the aging brain likely contributes to sTREM2 changes in CSF, including independent associations with tau, BBB dysfunction, and the most abundant $A\beta$ species (Table 2; Figs. 1–2). Given the previously described associations between CSF biomarkers of tau pathology and CSF sTREM2, it is not surprising that CSF p-tau₁₈₁ explained significant variance in sTREM2 levels. Previous work has demonstrated not only that sTREM2 relates to CSF p-tau, but also that the ratio of sTREM2 to p-tau is predictive of future cognitive decline (Ewers et al. 2019). Although this was not recapitulated in our smaller cohort, we do see a significant interaction between total tau and cognitive diagnosis on sTREM2 levels (sTREM2 \sim t-tau*diagnosis + base covariates) while p-tau performs similarly but does not reach statistical significance (Fig. 4), demonstrating a stronger association between tau biomarkers and sTREM2 in cognitively normal individuals compared to those with

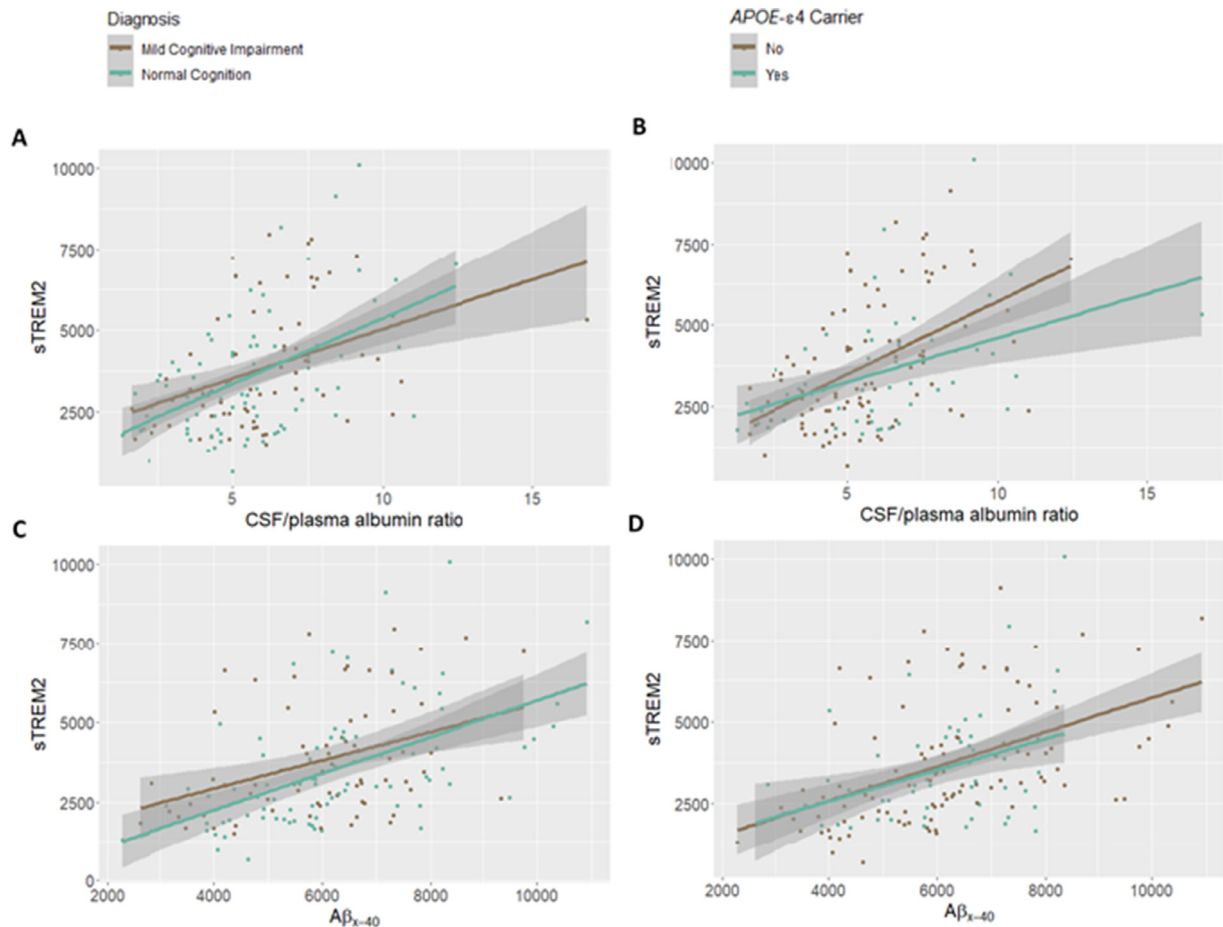


Fig. 5. Unadjusted plot demonstrating $A\beta$, and BBB biomarkers do not significantly interact with cognitive diagnosis nor $APOE-\epsilon 4$ carrier status on sTREM2 levels in CSF: (A) CSF/plasma albumin ratio * diagnosis, $p.int. = 0.62$, (B) CSF/plasma albumin ratio * $APOE-\epsilon 4$, $p.int. = 0.28$, (C) $A\beta_{x-40}$ * diagnosis, $p.int. = 0.36$, and (D) $A\beta_{x-40}$ * $APOE-\epsilon 4$, $p.int. = 0.78$. Protein measurements given in pg/mL.

MCI. Likewise, using ADNI data, the association between $p\text{-tau}_{181}$ and sTREM2 differs by diagnosis where tight coupling is attenuated in both MCI and AD compared to cognitively normal individuals (Fig. S5). Therefore, the sTREM2/ $p\text{-tau}$ ratio likely predicts longitudinal cognitive outcomes because of this decoupling of sTREM2 and tau as the disease progresses. In contrast to $p\text{-tau}$, $A\beta_{x-40}$ explains a slightly larger percentage of variance in sTREM2, but this association does not differ by diagnosis. Interestingly, even when removing the variance in sTREM2 that is due to $p\text{-tau}$, the variance in sTREM2 that is associated with $A\beta_{x-40}$ remains associated with future cognitive decline, suggesting that the complex interplay between $A\beta$ abundance, tau pathology, and sTREM2 is needed to properly interpret the clinical relevance of this emerging biomarker. Finally, the CSF/plasma albumin ratio explained an additional 14.8% of variance in CSF sTREM2 beyond covariates, CSF $p\text{-tau}_{181}$, and $A\beta_{x-40}$, highlighting the potential importance of the neurovascular unit (NVU) to changing CSF sTREM2 levels (Table 3). While previous work has discussed the potential drivers of tau associations with sTREM2 (Suarez-Calvet et al. 2019; Ulrich et al. 2017; Gratuze et al. 2020), less is known about the independent associations with BBB and $A\beta$ abundance, so we expand our discussion of those 2 relationships below.

4.2. CSF sTREM2 relates to broad peptide species of CSF amyloid- β

CSF sTREM2 associations with $A\beta$ have been inconsistent and focused on $A\beta_{1-42}$ (Heslegrave et al. 2016; Suarez-Calvet, Klein-

berger, et al. 2016; Henjum et al. 2016; Suarez-Calvet et al. 2019). However, we observed a robust, novel association between CSF sTREM2 and shorter species including truncated CSF $A\beta$ (Fig. 1B–C; Table 2) as well as $A\beta_{1-40}$ and $A\beta_{1-38}$ using ADNI data (Fig. 3) that may explain some of the discrepant reports in the literature. One possibility is that the positive correlation between $A\beta_{x-40}$ and sTREM2 concentration reflects a direct beneficial role of sTREM2 in facilitating amyloid clearance described in the animal model literature. Specifically, injection of recombinant sTREM2 in Trem2-knockout and wild-type mice, as well as in culture, induced inflammatory responses in microglia, significantly increasing IL-1 β , IL-10, IL-6, and TNF cytokine production and enhancing microglial survival (Zhong et al. 2017). Additionally, sTREM2 stereotaxic injection in the hippocampus has been shown to ameliorate $A\beta$ plaque load in 5XFAD mice, suggesting a beneficial role for sTREM2 in AD (Zhong et al. 2019). However, we did not observe associations with $A\beta_{1-42}$ or the $A\beta_{42/40}$ ratio (Fig. S2), both of which are thought to be the most sensitive markers of AD neuropathology, suggesting the associations with other $A\beta$ species may not reflect brain amyloidosis. This is particularly interesting given previous evidence that sTREM2 levels decline in the early preclinical stages of AD in amyloid positive individuals before elevating later in the disease cascade (Suarez-Calvet et al. 2019), a pattern recapitulated in the present cohort. It may be that the tau association masks an independent $A\beta_{1-42}$ association as pathology begins to emerge, but the data presented cannot speak to such a possibility.

Beyond a direct role in $A\beta$ processing or clearance, it is also possible that the association between sTREM2 and $A\beta$ reflects a compensatory alteration in $A\beta$ abundance in response to glial activation and/or altered neuronal activity. Synaptic activity has indeed been linked to the regulation of soluble $A\beta$ abundance in interstitial fluid (ISF) in vivo and in vitro (Bero et al. 2011; Cirrito et al. 2005). And this activity-dependent modulation of $A\beta$ production has been proposed as a compensation to neuronal hyperactivity (Kamenetz et al. 2003; Wei et al. 2010). This compensation hypothesis aligns with our data suggesting the relationship of $A\beta_{x-40}$ to sTREM2 may indeed be important to cognitive functioning. It is possible that prior to the onset of neurodegeneration, sTREM2 is elevated concurrently with an inflammatory response that promotes glymphatic trafficking of free and abundant $A\beta$ peptide. As $A\beta_{40}$ is the most abundant species, this provides for a more sensitive window in which it is possible to detect alterations of abundance in the CSF compartment by regulators of ISF/CSF flow. Functional studies will be needed to evaluate these mechanistic hypotheses and understand the interplay more thoroughly between $A\beta$ species and TREM2 proteins. Finally, it is also possible that sTREM2 levels rise concurrently with a parallel mechanism of $A\beta$ abundance that is causally unrelated or driven by a third unmeasured variable. Regardless of the mechanism, our results provide strong evidence that sTREM2 levels are correlated with the abundance of $A\beta$ species in a manner that does not appear to be specific to plaque deposition and that does not change with clinical disease.

4.3. CSF sTREM2 relates to BBB integrity

We also identified an association of the TREM2 axis with BBB permeability, recapitulating one other report in the literature (Bekris et al. 2018) whereby higher levels of CSF sTREM2 were associated with greater BBB permeability as indicated by the CSF/plasma albumin ratio (Fig. 2A; Table 2). This association was independent of APOE- ϵ 4 carrier status (Fig. 2B), suggesting an alternative pathway of cerebrovascular injury coincides with elevations in CSF sTREM2. Notably, our analyses revealed that BBB integrity, as measured by the CSF/plasma albumin ratio, explained a substantial proportion of variance in CSF sTREM2 levels (Table 3). BBB breakdown allows blood-derived accumulation of toxic proteins (i.e., fibrin and thrombin), microbial agents, as well as peripheral immune cells within the brain parenchyma (Sweeney, Sagare, and Zlokovic 2018). In turn, this remodeling drives microglial alterations associated with elevated sTREM2. Moreover, it is possible that the high levels of CSF/plasma albumin and sTREM2 reflect a compensatory change in barrier permeability in response to deposition of amyloid as early neuroinflammation may also serve to stave off plaque formation. Similarly, the pro-inflammatory role of sTREM2 in activating microglia may drive cerebrovascular dysregulation as microglia are known to regulate critical NVU mechanisms such as the recruitment of peripheral immune cells and the integrity of tight junction proteins (da Fonseca et al. 2014). This balance of neuronal-glial-vascular communication is a critical component of AD pathophysiology and subsequent heterogeneity. A better understanding of the role of sTREM2 and BBB function is critical, particularly as modulation of TREM2 is being actively pursued as a treatment target for AD pathogenesis (Wang, Mustafa, et al. 2020). Future studies including longitudinal measurement of sTREM2, and markers of cerebrovascular injury are needed to determine whether early elevations of sTREM2 predict changes in other NVU processes over the course of disease.

4.4. Strengths and limitations

Our deeply characterized cohort provides rich timepoints early in the disease process providing a unique opportunity to understand changes in sTREM2 within the preclinical period. Moreover, we were able to replicate previous associations and provide independent replication for the novel associations reported herein. Despite these strengths, our focus on baseline biomarker measurements precludes the temporal resolution and experimental control needed to determine cause and effect. Finally, it should be noted that our sample is enriched for highly educated, non-Hispanic White individuals, limiting our ability to generalize to other populations.

4.5. Conclusions

Taken together, we demonstrate that CSF sTREM2 relates to biomarkers of concomitant pathological processes in AD including $A\beta$ peptide abundance, tau pathology, and BBB dysfunction. We highlight multiple novel and independent pathways that are relevant to sTREM2 levels in aging and AD, enhancing its characterization as a biomarker and therapeutic target. Results suggest sTREM2 is relevant to cognitive progression with a heterogeneous etiology that must be further explored if it is going to have future clinical utility.

Authors' contributions

TH, RW and AJ designed the research framework. RW performed the analyses, analyzed the data, created tables and figures. RW, TH, LD, KB, HZ, AJ, KG and KP contributed to the writing and editing of the manuscript. TH, AJ, and KG obtained the funding. All authors read and approved the final manuscript.

Ethics declarations

Ethics approval and consent to participate.

All protocols were conducted with approval by the Vanderbilt University Medical Center Institutional Review Board after written informed consent from all subjects was obtained.

Consent for publication

All authors have given consent to the publication of this manuscript.

Availability of data and material

VMAP data can be requested within our data sharing portal and will be made freely available to qualified investigators (<https://www.vmacdata.org/vmap/data-requests>).

Disclosure statement

HZ has served at scientific advisory boards and/or as a consultant for Alector, Eisai, Denali, Roche Diagnostics, Wave, Samumed, Siemens Healthineers, Pinteon Therapeutics, Nervgen, AZTherapies, CogRx and Red Abbey Labs, has given lectures in symposia sponsored by Cellectricon, Fujirebio, Alzecure and Biogen, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program (outside submitted work). KB has served as a consultant, at advisory boards, or at data monitoring committees for Abcam, Axon, Biogen, JOMDD/Shimadzu, Julius Clinical, Lilly, MagQu, Novartis, Prothena,

Roche Diagnostics, and Siemens Healthineers, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program (all outside the work presented in this paper). TH is a member of the scientific advisory board for Vivid Genomics (also outside the work presented herein).

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.neurobiolaging.2022.06.013.

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