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sTREM2 is associated with amyloid-related p-tau increases and glucose hypermetabolism in Alzheimer's disease

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Abstract

Microglial activation occurs early in Alzheimer's disease (AD) and previous studies reported both detrimental and protective effects of microglia on AD progression. Here, we used CSF sTREM2 to investigate disease stage-dependent drivers of microglial activation and to determine downstream consequences on AD progression. We included 402 patients with measures of earliest beta-amyloid (CSF A β_{1-42}) and late-stage fibrillary A β pathology (amyloid-PET centiloid), as well as sTREM2, p-tau₁₈₁, and FDG-PET. To determine disease stage, we stratified participants into early A β -accumulators (A β CSF+/PET–; $n = 70$) or late A β -accumulators (A β CSF+/PET+; $n = 201$) plus 131 controls. In early A β -accumulators, higher centiloid was associated with cross-sectional/longitudinal sTREM2 and p-tau₁₈₁ increases. Further, higher sTREM2 mediated the association between centiloid and cross-sectional/longitudinal p-tau₁₈₁ increases and higher sTREM2 was associated with FDG-PET hypermetabolism. In late A β -accumulators, we found no association between centiloid and sTREM2 but a cross-sectional association between higher sTREM2, higher p-tau₁₈₁ and glucose hypometabolism. Our findings suggest that a TREM2-related microglial response follows earliest A β fibrillogenesis, manifests in inflammatory glucose hypermetabolism and may facilitate subsequent p-tau₁₈₁ increases in earliest AD.

Keywords Alzheimer's disease; beta-amyloid; glucose metabolism; p-tau; sTREM2

Subject Categories Biomarkers; Neuroscience

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Introduction

Alzheimer's disease (AD) is characterized by the accumulation of beta-amyloid (A β), tau, microglial activation, metabolic brain changes, neurodegeneration, and cognitive decline (Jack *et al*, 2018). According to the amyloid cascade hypothesis, A β accumulation triggers the subsequent development and spreading of hyperphosphorylated tau aggregates which is followed by neurodegeneration, metabolic decline, and eventually dementia (Karran *et al*, 2011; La Joie *et al*, 2020; Haass & Selkoe, 2022; Strom *et al*, 2022). Previously it was shown that A β -related increases in soluble hyperphosphorylated tau (i.e., p-tau), detectable in plasma (Janelidze *et al*, 2021; Moscoso *et al*, 2021) and cerebrospinal fluid (CSF) (Mattsson-Carlgen *et al*, 2020), precede tau aggregation. We have shown recently that soluble p-tau increases may in fact drive tau aggregation and spread across interconnected brain regions

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(Pichet Binette *et al*, 2022) therefore, soluble p-tau increases may be a key link between A β deposition and tau aggregation in AD. However, the underlying mechanisms that link A β and subsequent increases in soluble p-tau in CSF or plasma are not well understood.

Here, activation of microglia, the brain's innate immune system, may play a key role in modulating these initial events in the amyloid cascade (Pascoal *et al*, 2021). The Triggering Receptor Expressed on Myeloid Cell 2 (TREM2) regulates the change of microglia from a homeostatic state to a disease-associated state (Keren-Shaul *et al*, 2017; Krasemann *et al*, 2017) and is a well-established *in vivo* proxy for microglial activation in AD (Suarez-Calvet *et al*, 2016, 2019; Ewers *et al*, 2019, 2020; Franzmeier *et al*, 2020). Yet, previous studies have yielded conflicting findings on a detrimental or protective role of microglial activation or TREM2-related microglial responses in AD. For instance, recent *in vitro* studies reported that activated microglia can induce tau hyperphosphorylation and spread (Maphis *et al*, 2015) and that activated microglia can release tau seeds which can induce tau aggregation (Brelstaff *et al*, 2021). Similarly, studies in sporadic AD patients found that a TREM2-related microglial response is strongly correlated with soluble p-tau but not with A β levels (Suárez-Calvet *et al*, 2019) and that a TREM2-related microglial response may promote the development of aggregated tau pathology in AD, as measured via tau-PET (Vogels *et al*, 2019; Pascoal *et al*, 2021). Indeed, a recent post-mortem study investigating the mediating effect of microglial activation on the A β to tau association in brain tissue revealed a mediation effect of 33% of microglia for the relationship between A β and tau (Casetto *et al*, 2022). This suggests that microglial activation may be associated with tau hyperphosphorylation and therefore contribute to the development of tau pathology in AD. In addition, activated microglia have been shown to consume high levels of glucose in AD mouse models and AD patients (Xiang *et al*, 2021), which may manifest in hypermetabolic brain changes that are observed in early-stage AD, when neurodegeneration and ensuing glucose hypometabolism are not yet apparent (Oh *et al*, 2016; Gordon *et al*, 2018). Thus, glucose hypermetabolism in early AD may not reflect a compensatory mechanism, as suggested previously, but rather reflect activated microglia and neuroinflammation (Ashraf *et al*, 2015; Arenaza-Urquijo *et al*, 2017).

On the contrary, in symptomatic sporadic AD patients and patients with autosomal dominantly inherited AD, a higher TREM2-related microglial response has been associated with attenuated cognitive decline, amyloid accumulation and neurodegeneration (Ewers *et al*, 2019, 2020; Morenas-Rodriguez *et al*, 2022). This suggests a possible protective effect of chronic microglial activation on neuronal integrity and cognition that occurs once pathologic brain changes are severe enough to result in clinically manifested cognitive deficits. Similarly, an animal model has shown that TREM2 loss of function is associated with increased A β seeding, further suggesting a protective effect of microglia on A β pathology development (Parhizkar *et al*, 2019). It is therefore of utmost importance for clinical trials trying to target microglial activation as a disease-modifying approach to understand (i) what drives microglial activation in AD, (ii) whether and when microglial activation is beneficial or detrimental and (iii) whether the directionality of microglial effects on AD progression depends on disease stage.

In the present study, we used CSF soluble TREM2 (sTREM2) as an *in vivo* marker of TREM2-related microglial responses in a well-characterized sample of AD patients and controls to investigate drivers of microglial activation across early versus late-stage A β accumulation in AD and its effects on the development of downstream p-tau and metabolic brain changes. Specifically, we included data of 402 cognitively normal (CN) and mild cognitive impaired (MCI) participants from the ADNI database with available CSF A β ₁₋₄₂, p-tau₁₈₁, and sTREM2, as well as amyloid-PET, and ¹⁸F-fluorodeoxyglucose PET (FDG-PET). FDG-PET is well established for assessing cerebral glucose uptake and FDG-PET-assessed hypermetabolism has been previously linked to microglial activation (Xiang *et al*, 2021). To determine disease stage, we classified patients into A β CSF+/PET- (early A β -accumulators) and A β CSF+/PET+ (late A β -accumulators) following a previously established approach that allows stratifying individuals by showing earliest signs of A β accumulation (i.e., A β CSF+/PET-) versus showing fully developed amyloid pathology (A β CSF+/PET+) (Palmqvist *et al*, 2017). A total of 131 participants without evidence of A β pathology were included as healthy controls (A β CSF-/PET-). A subset of participants had available longitudinal p-tau₁₈₁ and sTREM2 assessments, based on which we calculated annual sTREM2 and p-tau₁₈₁ change rates. Our specific aims were to assess first, whether earliest signs of A β accumulation (i.e., in A β CSF+/PET-) are associated with a TREM2-related microglial response and second, whether this initial A β -driven microglial activation facilitates subsequent increases in soluble hyperphosphorylated tau (i.e., p-tau₁₈₁). Third, we assessed whether earliest TREM2-related microglial responses are reflected in increased FDG-PET-assessed glucose metabolism, given that activated microglia consume large amounts of glucose (Xiang *et al*, 2021). Here, we expected a higher TREM2-related microglial response to be associated with glucose hypermetabolism in patients with earliest A β accumulation, where neurodegeneration is not yet apparent, versus hypometabolism in chronic AD phases within late A β -accumulators.

Results

We stratified participants by evidence for early- versus late-stage A β pathology, using a previously established approach that combines CSF assessments of soluble A β ₁₋₄₂ and amyloid-PET assessments of fibrillar A β (Palmqvist *et al*, 2017). CSF A β abnormality is assumed to reflect early A β dysmetabolism and precedes amyloid-PET positivity reflecting mostly fibrillary forms of A β (Palmqvist *et al*, 2016). Therefore, we grouped participants into (i) early A β -accumulators (i.e., A β CSF+/PET-; $n = 70$; CN = 30; MCI = 40) with evidence for reduced A β ₁₋₄₂ in CSF but no suprathreshold fibrillar A β pathology on PET versus (ii) late A β -accumulators (i.e., A β CSF+/PET+; $n = 201$; CN = 41; MCI = 160), with evidence for abnormal A β in both CSF and PET. An additional pool of 131 cognitively normal subjects without evidence of abnormal A β on either CSF or PET was included as a control group. Longitudinal CSF data were available for a subset of participants for sTREM2 (early A β /late A β /controls $n = 20/75/35$) and p-tau₁₈₁ (early A β /late A β /controls $n = 20/75/35$) with an average follow-up time from baseline CSF assessment of 1.99 ± 0.09 years. Descriptive baseline statistics stratified by groups are shown in Table 1.

Table 1. Demographic and clinical data stratified by group.

	Controls (A β CSF−/ PET−)	Early A β -accumulators (A β CSF+/ PET−)	Late A β -accumulators (A β CSF+/ PET+)	P-value
Cross-sectional				
N	131	70	201	
Diagnostic (CN/MCI)	131/0	30/40	41/160	< 0.001
Sex (male/female)	64/67	47/23	112/89	0.045
Age in years	72.67 (6.39)	71.65 (7.68)	73.60 (6.53)	0.093
Years of education	16.85 (2.43) ^a	17.13 (2.24) ^a	16.00 (2.74) ^{b,c}	< 0.001
CSF A β ₁₋₄₂ (pg/ml)	1,553.122 (281.109) ^{a,c}	777.819 (147.169) ^{a,b}	656.42 (174.79) ^{b,c}	< 0.001
CSF p-tau ₁₈₁ (pg/ml)	20.241 (7.059) ^{a,c}	15.825 (7.493) ^{a,b}	32.807 (14.780) ^{b,c}	< 0.001
CSF sTREM2 (pg/ml)	4,099.933 (1,980.197) ^c	2,955.118 (1,751.746) ^{a,b}	3,984.107 (2,194.033) ^c	< 0.001
Amyloid-PET (centiloid)	−9.0315 (12.656) ^a	−2.901 (13.530) ^a	78.228 (33.871) ^{b,c}	< 0.001
FDG-PET global z-score	-	−0.132 (0.649)	−0.251 (0.532)	0.13
FDG-PET meta-ROI z-score	-	−0.214 (0.826) ^a	−0.546 (0.723) ^c	0.002
Longitudinal				
N	35	20	75	
Follow-up CSF p-tau ₁₈₁ (mean years)	1.96 (0.13) ^c	2.05 (0.08) ^{a,b}	1.99 (0.07) ^c	0.002
N	35	21	75	
Follow-up CSF sTREM2 (mean years)	1.96 (0.13) ^c	2.05 (0.08) ^b	1.99 (0.07)	0.004

Values are presented as mean (SD); P-values were derived from ANOVAs for continuous measures and from Chi-squared tests for categorical measures. Mean values significantly ($P < 0.05$, *post hoc* tests) different from—.

^aControls.

^bEarly A β -accumulators.

^cLate A β -accumulators.

Early but not late-stage A β accumulation is associated with higher CSF sTREM2

We tested first whether evidence for earliest A β abnormality in CSF but not yet in PET (i.e., early A β accumulators) is associated with a sTREM2-related microglial response and p-tau₁₈₁ increases. To this end, we used linear regression to determine the association between amyloid-PET as a marker of fibrillary A β pathology (i.e., centiloid) and sTREM2 in early A β -accumulators. Here, higher centiloid at baseline was associated with higher cross-sectional p-tau₁₈₁ ($\beta = 0.259$, $T = 2.199$, $P = 0.032$; Fig 1A, top panel) and higher sTREM2 ($\beta = 0.254$, $T = 2.268$, $P = 0.027$; Fig 1B, top panel). Further, higher sTREM2 levels were associated with higher p-tau₁₈₁ levels ($\beta = 0.587$, $T = 5.425$, $P < 0.001$). We obtained congruent results using longitudinal CSF data, showing that higher centiloid at baseline was associated with faster subsequent change rates in p-tau₁₈₁ ($\beta = 0.550$, $T = 2.975$, $P = 0.010$; Fig 1C, top panel) and faster changes in sTREM2 ($\beta = 0.535$, $T = 3.725$, $P = 0.002$; Fig 1D, top panel). In addition, higher baseline sTREM2 levels were associated with faster subsequent change rates in p-tau₁₈₁ ($\beta = 0.938$, $T = 6.286$, $P < 0.001$). Using bootstrapped mediation analyses with 1,000 iterations, we additionally found that the association between higher centiloid and higher p-tau₁₈₁ was fully mediated by sTREM2 in early A β accumulators, both for cross-sectional p-tau₁₈₁ (average causal mediation effect [ACME]: $B = 0.133$, 95% CI = 0.0039 to 0.27, $P = 0.038$; Fig 1E, top panel) as well as subsequent p-tau₁₈₁ change rates (ACME: $B = 0.450$, 95% CI = 0.1352 to 0.82, $P = 0.004$; Fig 1F,

top panel). Testing the reverse mediation models, i.e., whether p-tau₁₈₁ mediates the effect of centiloid on sTREM2 yielded a similar mediation effect for cross-sectional sTREM2 levels (ACME: $B = 0.132$, CI = 0.0084 to 0.26, $P = 0.030$) but a much lower mediation effect for subsequent sTREM2 change rates in longitudinal analyses (ACME: $B = 0.273$, CI = 0.0437 to 0.55, $P = 0.014$). These results suggest that earliest A β accumulation may induce a reactive TREM2-related microglial response which may in turn facilitate p-tau₁₈₁ increases.

When assessing the above-described analyses in late A β -accumulators, no association was found between centiloid and sTREM2, neither for cross-sectional sTREM2 ($\beta = 0.040$, $T = 0.565$, $P = 0.573$; Fig 1B, bottom panel) nor for longitudinal sTREM2 change rates ($\beta = 0.051$, $T = 0.450$, $P = 0.654$; Fig 1D, bottom panel). This suggests that sTREM2 increases are no longer driven by A β once fully developed fibrillar A β pathology is present. Yet, there was an association between higher baseline sTREM2, higher baseline p-tau₁₈₁ ($\beta = 0.441$, $T = 7.004$, $P < 0.001$) and longitudinal changes in p-tau₁₈₁ ($\beta = 0.439$, $T = 3.777$, $P < 0.001$), suggesting that sTREM2 is more strongly coupled to p-tau₁₈₁ in late A β -accumulators. Higher centiloid was associated with higher cross-sectional p-tau₁₈₁ ($\beta = 0.203$, $T = 2.995$, $P = 0.003$; Fig 1A, bottom panel), but no association was found between baseline centiloid and subsequent p-tau₁₈₁ change rates ($n = 75$, $\beta = 0.137$, $T = 1.165$, $P = 0.248$; Fig 1C, bottom panel). Given that there was no association between centiloid and sTREM2 in late A β -accumulators, we did not detect a mediation effect of sTREM2 for the association between

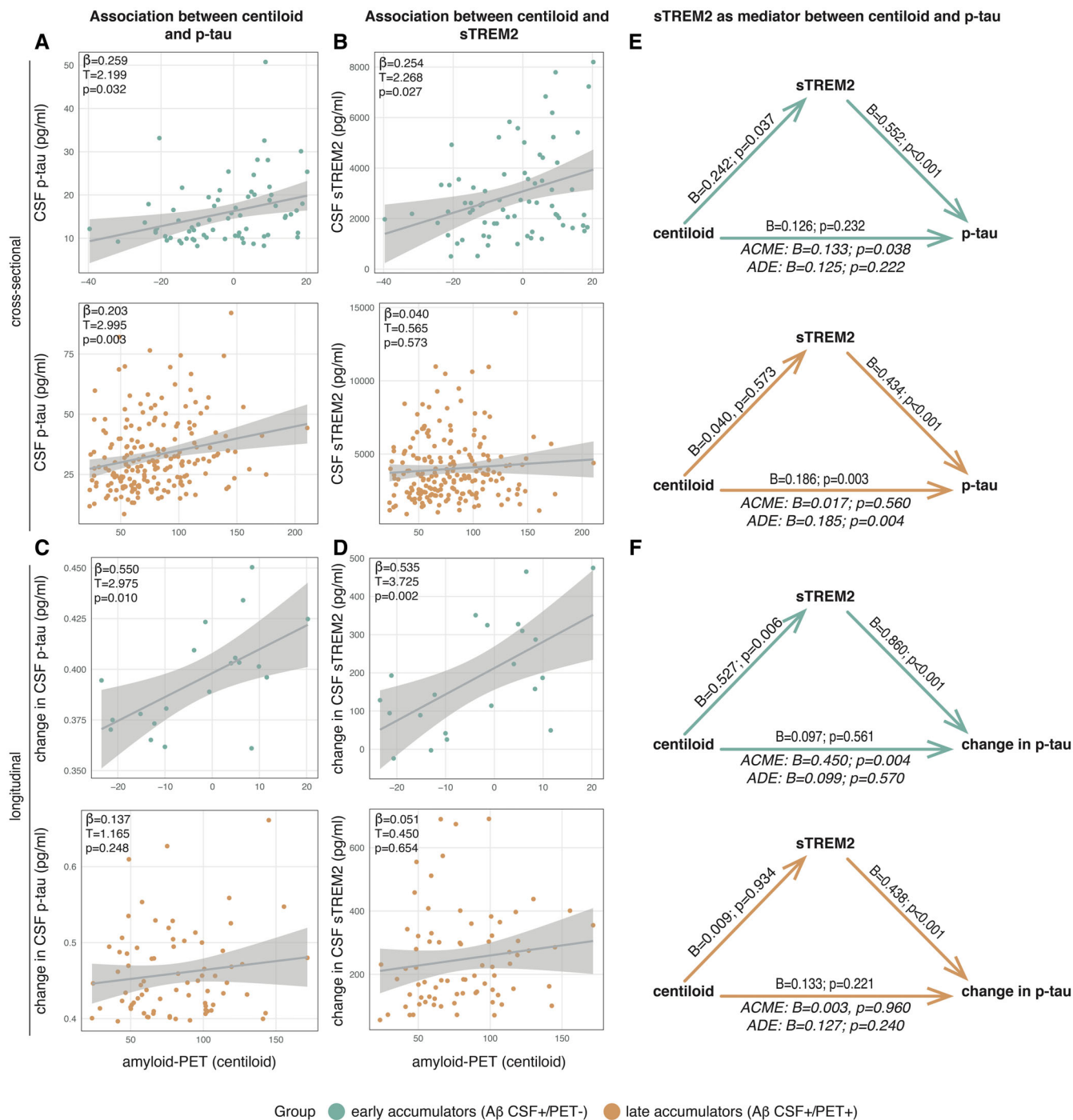


Figure 1. Cross-sectional and longitudinal analysis of the association between amyloid-PET (in centiloid), CSF p-tau₁₈₁, and CSF sTREM2 in early A β -accumulators (i.e., A β CSF+/PET-; n = 70) and late A β -accumulators (i.e., A β CSF+/PET+; n = 201).

A–D Cross-sectional linear regressions between centiloid and p-tau₁₈₁ (A) and centiloid and sTREM2 (B). Longitudinal linear regressions between centiloid and change in p-tau₁₈₁ (C) and centiloid and change in sTREM2 (D). Standardized beta-estimates (β), T-values, and P-values were derived from linear regressions.

E–F Cross-sectional mediation analyses with centiloid as predictor, sTREM2 as mediator, and p-tau₁₈₁ as dependent variable (E). Longitudinal mediation analyses with centiloid as predictor, sTREM2 as mediator, and change in p-tau₁₈₁ as dependent variable (F). Beta-estimates (B) and P-values for each path are displayed on the respective arrow. The average causal mediation effect (ACME) and the average direct effect (ADE) are displayed under each mediation triangle.

Data information: Early A β -accumulators are displayed in green, and late A β -accumulators in orange. All models are controlled for age, sex, education, and clinical status.

Source data are available online for this figure.

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centiloid and p-tau₁₈₁, neither cross-sectionally (ACME: $B = 0.017$, $CI = -0.0444$ to 0.08 , $P = 0.560$; Fig 1E, bottom panel), nor longitudinally (ACME: $B = 0.003$, $CI = -0.108$ to 0.10 , $P = 0.960$; Fig 1F, bottom panel).

Together, these findings suggest that earliest fibrillization of A β is associated with reactive sTREM2 increases, which may in turn precede increases in p-tau₁₈₁ in early A β -accumulators, while sTREM2 dynamics may uncouple from the extent of fibrillar A β pathology at later stages but rather parallel p-tau₁₈₁ increases.

All analyses remained consistent when including APOE4 genotype as a covariate (except for the cross-sectional ACME in early A β -accumulators, which only reached borderline significance ($P = 0.058$; Tables EV1 and EV2)).

No sTREM2 by group interaction on CSF p-tau₁₈₁ levels were found when pooling early and late A β -accumulators (cross-sectional interaction term: $\beta = 0.022$, $T = 0.145$, $P = 0.885$; longitudinal interaction term: $\beta = -0.091$, $T = -0.345$, $P = 0.731$).

When performing the same analyses in A β CSF-/PET- healthy controls, no significant cross-sectional or longitudinal associations between centiloid and sTREM2 (cross-sectional: $\beta = 0.118$, $T = 1.273$, $P = 0.205$; longitudinal: $\beta = -0.049$, $T = -0.240$, $P = 0.812$) or p-tau₁₈₁ (cross-sectional: $\beta = 0.091$, $T = 0.967$, $P = 0.335$; longitudinal: $\beta = -0.119$, $T = -0.582$, $P = 0.565$) were observed, suggesting that the association between A β fibrillization and sTREM2 increases are specific for patients who show earliest signs of AD pathophysiology as indicated by reduced CSF A β ₁₋₄₂ levels.

Lastly, the above-described analyses were repeated using CSF A β ₁₋₄₂ instead of centiloid for testing associations between soluble A β and p-tau₁₈₁ or sTREM2. Except for an association between CSF A β ₁₋₄₂ and p-tau₁₈₁ in late accumulators ($P = 0.004$), no associations were observed (Table 2), which is in accordance with previous work (Suarez-Calvet et al, 2019). This suggests that sTREM2 increases are specifically associated with fibrillization of A β as assessed via amyloid-PET.

Microglial activation is reflected in cerebral glucose metabolism across AD stages

Next, we tested whether there is a non-linear relationship between sTREM2 levels and FDG-PET-assessed glucose metabolism across the spectrum of A β accumulation compared to controls. Microglia consume large amounts of glucose (Xiang et al, 2021), hence a

TREM2-related microglial response may result in FDG-PET hypermetabolism in the earliest stages of A β accumulation, where neurodegeneration is typically not yet apparent. In late A β accumulators, a TREM2-related microglial response parallels p-tau₁₈₁ which has been associated with subsequent neurodegeneration (Ossenkoppele et al, 2021; Pichet Binette et al, 2022). Hence higher sTREM2 may be associated with FDG-PET hypometabolism in subjects with late-stage A β accumulation. To test this, we summarized FDG-PET of early and late A β -accumulators across AD vulnerable brain regions (Landau et al, 2011) and referenced the mean FDG-PET signal to 131 controls (i.e., A β CSF-/PET-) to derive FDG-PET z-scores that allow to determine whether FDG-PET metabolism is higher or lower than in a reference group of healthy controls. Using linear regression, we observed a significant sTREM2 by group interaction on FDG-PET meta-ROI z-scores ($\beta = -0.378$, $T = -1.980$, $P = 0.049$; Fig 2A, controlled for age, sex, education, and clinical status), showing that sTREM2 was associated with higher FDG-PET in early A β -accumulators versus lower FDG-PET in late A β -accumulators. The analyses remained consistent when including APOE4 genotype as a covariate ($\beta = -0.379$, $T = -1.972$, $P = 0.050$). To exploratory map the spatial pattern of brain regions in which higher sTREM2 is associated with higher FDG-PET in early A β -accumulators, versus lower FDG-PET uptake in late A β -accumulators, we assessed the association between sTREM2 and FDG-PET z-scores for early and late A β -accumulators separately across 200 cortical brain regions included in the Schaefer brain atlas (Schaefer et al, 2018) using linear regression models controlling for age, sex, education, and clinical status. When projecting the T -values of the association between sTREM2 levels and FDG-PET to the brain surface (Fig 2B, left panel), we found consistent and brain-wide positive associations between sTREM2 and FDG-PET for early A β -accumulators, versus consistent negative associations between sTREM2 and FDG-PET for late A β -accumulators. Comparing the T -value distributions of the association between sTREM2 levels and FDG-PET between early and late A β -accumulators showed a significant group difference ($T = 35.78$, $P < 0.001$, Fig 2B, right panel). In addition, 95% CIs of the 200 T -values did not overlap (early A β -accumulators: $CI 1.086$ to 1.249 ; late A β -accumulators: $CI -1.302$ to -1.098), supporting the view that there is a non-linear relationship between sTREM2 levels and FDG-PET across the spectrum of A β deposition, where sTREM2 is associated with relative FDG-PET hypermetabolism in early A β -accumulators versus relative hypometabolism in late A β -accumulators. The brain surface projection of

Table 2. Associations between CSF A β ₁₋₄₂ and p-tau₁₈₁ or sTREM2.

	Cross-sectional			Longitudinal		
	β	T	P	β	T	P
Early A β -accumulators (A β CSF+/PET-)						
p-tau ₁₈₁ ~ A β ₁₋₄₂	-0.225	-1.911	0.061	-0.215	-0.863	0.403
sTREM2 ~ A β ₁₋₄₂	0.128	1.124	0.265	-0.006	-0.029	0.978
Late A β -accumulators (A β CSF+/PET+)						
p-tau ₁₈₁ ~ A β ₁₋₄₂	0.196	2.894	0.004	0.094	0.791	0.432
sTREM2 ~ A β ₁₋₄₂	0.069	0.979	0.329	0.134	1.197	0.236

The table displays standardized beta-estimates (β), T -values, and P -values. The regression models are controlled for age, sex, education, and clinical status.

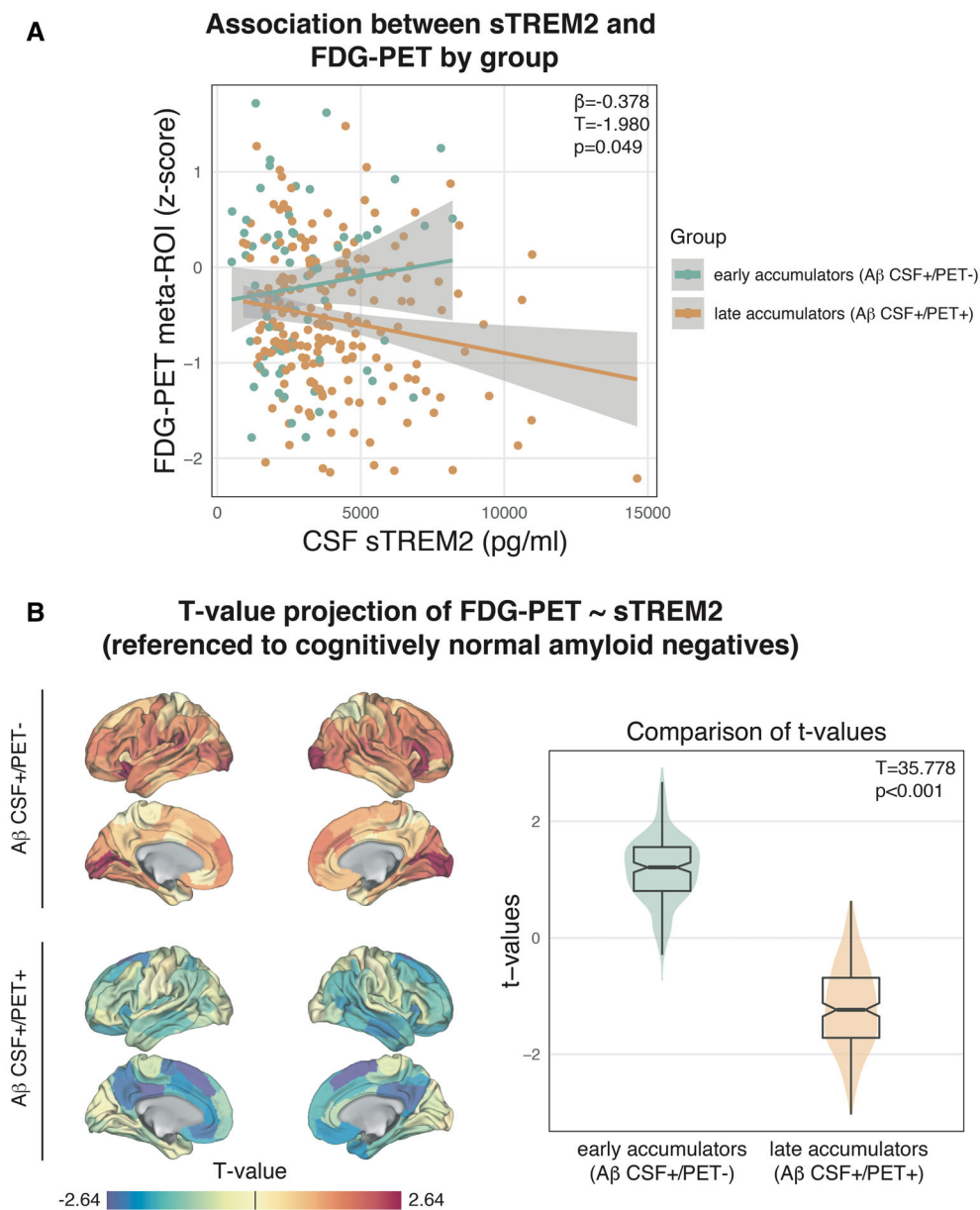
the T -values of the sTREM2 by group interaction on FDG-PET is made available in the Expanded View (Fig EV1). These findings suggest that elevated glucose metabolism in the early disease stage, before neurodegeneration is present, reflects neuroinflammation while in the later disease stage, higher sTREM2 parallels neuronal loss and tau aggregation, and therefore may manifest in metabolic decline.

Discussion

In this combined CSF biomarker and neuroimaging study, we systematically assessed the correlates of earliest sTREM2-related microglial responses in AD and its consequences on downstream changes in the amyloid cascade, including soluble p-tau₁₈₁ increases and changes in cerebral glucose metabolism. In summary, we found that in early A β -accumulators, higher fibrillar A β was associated with higher sTREM2 and that sTREM2 mediated the association between earliest PET-assessed fibrillary A β deposition and soluble p-tau₁₈₁ increases. In contrast, higher sTREM2 was no longer associated with A β but paralleled p-tau₁₈₁ increases in late A β -accumulators, suggesting that sTREM2 is more strongly coupled to the increase in soluble p-tau₁₈₁ levels once fully developed fibrillary A β pathology is present. Higher sTREM2 levels were further associated with FDG-PET-assessed glucose hypermetabolism in early A β -accumulators but with glucose hypometabolism in late A β -accumulators. This suggests that increases in glucose metabolism observed in early-stage AD may reflect A β -related neuroinflammation rather than a compensatory effect (Ashraf et al, 2015; Oh et al, 2016). Together, our results suggest that a TREM2-related microglial response is an early element of the amyloid cascade, which is closely associated with earliest p-tau₁₈₁ increases and metabolic brain changes. This is a critical point when assessing at what stage of the AD continuum we should test drugs targeting TREM2 and microglia. We previously reported protective effects of TREM2-related microglial responses on attenuated neurodegeneration and symptom progression in later disease stages (Ewers et al, 2019, 2020). Further work including longitudinal cognitive data is needed to elucidate if a protective effect also occurs on different pathological brain changes and in earlier stages before there is overt A β pathology.

Our first finding showed that the earliest sTREM2-related microglial response in AD is associated with fibrillar yet subthreshold A β increases (i.e., centiloids below 20), and that higher sTREM2 mediated the earliest A β -related increases in soluble p-tau₁₈₁ levels. In contrast, in participants showing fully developed A β pathology as indicated by combined positivity on both CSF and PET (i.e., late A β -accumulators), only associations between fibrillar A β (i.e., centiloid) and p-tau₁₈₁ or between p-tau₁₈₁ and sTREM2 were found. Notably, we and others (Suarez-Calvet et al, 2019) could not detect any associations between CSF A β levels and sTREM2, hence early insoluble but not soluble forms of A β might be associated with TREM2-related microglial responses and subsequent p-tau increases. Importantly, our results remained consistent when repeating the analysis in a subset of participants with available longitudinal CSF data, showing that sTREM2 increases at baseline predict and mediate subsequent A β -related increases in p-tau₁₈₁ in early but not in late A β -accumulators. These findings support the view that earliest A β fibrillization induces an sTREM2-related activation of microglia,

which is in turn associated with p-tau₁₈₁ increases, while sTREM2 increases may uncouple from A β severity at later stages. Our findings of microglial activation as a mediator of soluble p-tau₁₈₁ increases is in line with a recent post-mortem study in older adults showing that activated microglia partly mediated the relationship between A β and tau (Casaletto et al, 2022). In addition, several pre-clinical studies showed that activated microglia can enhance tau phosphorylation in animal models of AD and other tauopathies (Bhaskar et al, 2010; Lee et al, 2014; Maphis et al, 2015; Ising et al, 2019). Similarly, another study in a rat model of tauopathy could show that animals that are genetically prone to neuroinflammation show stronger neurofibrillary tau pathology compared to animals with less neuroinflammation (Stozicka et al, 2010). A further study using brain tissue of AD patients and AD mice could show that microglia phagocytose hyperphosphorylated tau seeds but are incapable of fully neutralizing tau seeding activity and instead release pathological tau seeds into the extracellular space, which can induce a cascade of subsequent tau hyperphosphorylation, misfolding and spread (Hopp et al, 2018). Moreover, a large-scale biomarker study in AD patients with combined amyloid-PET, tau-PET, and TSPO-PET for assessing microglial activation levels could show that elevated microglial activation may promote the aggregation and spreading of fibrillary tau deposits (Pascoal et al, 2021). However, other studies show that TREM2 loss of function is associated with a drastically elevated AD risk (Jin et al, 2014; Cheng-Hathaway et al, 2018) and with facilitated A β -associated tau seeding in AD mice expressing both A β and tau pathology (Leyns et al, 2019; Gratuze et al, 2021). Further, we reported previously that higher baseline sTREM2 levels in symptomatic AD patients are associated with lower tau-PET levels several years later (Ewers et al, 2020). Conflicting findings of microglial activation on the development of tau pathology may be explained by several factors, including the use of different animal models of AD, which recapitulate different aspects of AD pathophysiology, or using different markers of tau pathology including soluble and fibrillar forms of tau. CSF p-tau reflects hyperphosphorylated tau in its soluble form, one of the earliest tau-related changes in AD that is closely associated with A β , preceding the formation of intracellular neurofibrillary tau aggregates (Hansson, 2021; Pichet Binette et al, 2022). Therefore, microglia may have different effects on p-tau hyperphosphorylation and aggregation, which will be important to study in greater detail in future studies by combining fluid and PET biomarkers of microglial activation and tau pathology. Future investigations including more comprehensive CSF analyses should compare different CSF p-tau epitopes (e.g., p-tau₂₁₇ or p-tau₂₃₁) to test whether the effect of sTREM2 on p-tau is consistently observed across different p-tau species. Further, our study showed that the inclusion of different AD stages can have a drastic impact on the association between sTREM2 levels and downstream AD biomarkers, which may be a key confounder in previous studies. This is also supported by a previous study using an APPPS1-21 mouse model, showing that a TREM2-related microglial response can have opposing effects on A β pathology, depending on disease stage (Jay et al, 2017). Nevertheless, our findings highlight the view that microglia are crucially involved in the amyloid cascade, yet the directionality of effects may be modulated by disease stage and other pathophysiological events. It is important to stress here that microglia exist in a diverse and dynamic states, which respond to diverse physiological and



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Figure 2. Association between CSF sTREM2 and FDG-PET in early Aβ-accumulators (i.e., Aβ CSF+/PET-; n = 70) and late Aβ-accumulators (i.e., Aβ CSF+/PET+; n = 201).

A Plot shows significant ($P < 0.05$) sTREM2 by group interaction on FDG-PET z-scores within an FDG-PET meta-ROI (Landau et al, 2011) using a linear regression.

B T-value projection of the association between sTREM2 on FDG-PET, stratified by group.

Data information: FDG-PET z-scores were derived by referencing FDG-PET SUVRs to cognitively normal controls (i.e., $n = 131$; Aβ CSF-/PET-). The models are controlled for age, sex, education, and clinical status. Boxplots are displayed as median (center line) ± inter-quartile range (box boundaries) with whiskers including observations falling within the 1.5 interquartile range. 200 Regions of interest are displayed per group.

Source data are available online for this figure.

pathological conditions (i.e., Aβ accumulation). The different biomarkers of microglia (e.g., TSPO, CSF sTREM2) may reflect different aspects of the microglial response to the pathology. A current limitation is the lack of other microglial biomarkers that reflect the wide repertoire of microglia states. Therefore, we believe that it is crucial to better understand how microglia and sTREM2 are involved in the molecular progression of tau pathology, from tau

hyperphosphorylation and increases in soluble p-tau to the development and spread of fibrillary tau pathology, in order to evaluate microglia and specifically TREM2-related pathways as a treatment target.

As a second finding, we report that a TREM2-related microglial response was associated with increased FDG-PET-assessed glucose metabolism in early Aβ-accumulators, and with decreased glucose

metabolism in late A β -accumulators compared to healthy controls. This observation aligns well with previous work, showing that activated microglia consume high levels of glucose (Xiang *et al*, 2021) and should thus be reflected in increased FDG-PET. The metabolic drop that we observed in later disease might in contrast reflect neurodegenerative processes and neuronal death (Strom *et al*, 2022). Increased glucose metabolism has been previously reported in early AD stages (Ashraf *et al*, 2015; Oh *et al*, 2016) and interpreted as possible compensatory neuronal activity (Ashraf *et al*, 2015), however, proof of an actual advantage of these compensatory mechanisms has been lacking. A match between regional hypermetabolism in early disease stages followed by hypometabolism in later stages has been observed in a previous cross-sectional study (Oh *et al*, 2016), where cognitively normal older adults with higher levels of A β showed glucose hypermetabolism in those regions, which were most susceptible to AD-related hypometabolism in advanced disease. In addition, in a study including the DIAN cohort, the biggest database of autosomal dominant AD patients, increased FDG-PET signal was detected \sim 25 years before estimated symptom onset in mutation carriers compared to non-carriers. With advanced disease, hypometabolism was mostly observed in regions that were previously hypermetabolic (Benzinger *et al*, 2013). However, the authors caution the low number of participants ($n = 11$) which were included in the analysis. Importantly, we detected statistically significant associations between a TREM2-related microglial response and glucose metabolism depending on disease stage. Considering previous work, our findings suggest that different stages of microglial responses and neurodegeneration may induce a non-linear trajectory of metabolic brain changes in AD. This is also supported by a recent study showing associations between astrogliosis, i.e., another soluble marker of neuroinflammation, and higher FDG-PET metabolism in earliest AD (Salvado *et al*, 2022). Future studies using more advanced TSPO-PET for the regional assessment of neuroinflammation should further investigate whether regions affected by high A β load show increased signs of neuroinflammation and whether this is coupled by glucose hypermetabolism in earliest AD.

Our findings have important implications for microglial-related treatment strategies. Previous trials focusing on the reduction of A β plaques have mostly failed to significantly reduce cognitive dysfunction, despite some recent positive phase III trials (Knopman *et al*, 2021). Thus, treatments that are directly targeting the pathogenesis of tau, which is much closer linked to neurodegeneration and cognitive decline than A β (Bennett *et al*, 2004; Jack *et al*, 2013; Fleisher *et al*, 2015; Ossenkoppele *et al*, 2016; Wang *et al*, 2016; Jack *et al*, 2018), might be more likely to result in a clinical benefit. In the present study, we showed that in earliest A β accumulation a TREM2-related microglial response moderates the association between A β and tau, thus, a treatment that targets microglial activation might reduce increases in p-tau₁₈₁ and therefore, prevent tau aggregates and following neurodegeneration. Here, the time window for an optimal treatment effect will be critical, since our models showed that a TREM2-related microglial response only mediates p-tau₁₈₁ increases in early A β -accumulators, while in later disease stages, beneficial effects of activated microglia on cognition could be observed (Ewers *et al*, 2020; Franzmeier *et al*, 2020). Therefore, future studies are highly needed to reveal the ideal time windows for microglial-related treatment that might target reduction or enhancement of microglia, depending on disease stage.

A clear strength of the present study is its multi-modal design including CSF biomarkers and PET imaging. When interpreting the data, however, several caveats should be addressed. First, A β , p-tau₁₈₁, and sTREM2 are colinear in the early phase and the mediation analysis was significant both ways, with sTREM2 as mediator for subsequent p-tau₁₈₁ increases and vice versa. However, the mediation effect was much higher for sTREM2 as mediator than for p-tau₁₈₁ ($B = 0.450$ compared to $B = 0.273$), suggesting that albeit both, A β and p-tau₁₈₁ may have activating effects on sTREM2, sTREM2 might play a driving role in subsequent p-tau₁₈₁ elevations. Second, we used CSF A β ₁₋₄₂ levels for patients' classification, however, we recognize that a normalization of A β using the A β ₄₂/A β ₄₀ ratio would be the preferable measure which should be applied once more data become available in ADNI or other datasets. Further, we cannot ensure that all participants of the early A β -accumulators group will progress to fully developed fibrillar A β pathology due to a lack of longitudinal amyloid-PET data. To minimize the risk of wrong allocation, participants showing an unexpected A β biomarker pattern (i.e., CSF-/PET+) or those who already have dementia were excluded from this study (Palmqvist *et al*, 2017). Third, sample sizes were considerably small for early A β -accumulators when including longitudinal CSF sTREM2 ($n = 21$) and CSF p-tau₁₈₁ ($n = 20$) data, however, results were consistent for both cross-sectional and longitudinal associations. Further, we could only assess cross-sectional relationships between sTREM2 and FDG-PET. Here, it will be critical to assess longitudinal associations between sTREM2 and changes in FDG-PET once larger longitudinal datasets become available. Fourth, we could not assess p-tau₁₈₁ by sTREM2 interactions on tau aggregates since tau-PET data were limited and thus not sufficient to reliably assess relationships with sTREM2 in the current dataset. Here, it would be of high interest to assess the association between tau aggregates, sTREM2, and FDG-PET. Although we observed group differences (early vs. late A β -accumulators) for the association between sTREM2 and FDG-PET, the current study cannot disentangle whether sTREM2 plays a moderating role for the association between tau-PET and FDG-PET. Nevertheless, we encourage future studies to assess, whether sTREM2 in later disease stages shows attenuating effects on tau accumulation, thereby preventing neuronal death and atrophy which might explain previous findings on protective effects of sTREM2 on neurodegeneration and cognitive decline (Ewers *et al*, 2019). In addition, the current study is to our knowledge the first to assess associations between early versus late-stage A β accumulation, sTREM2, p-tau, and changes in glucose metabolism. Therefore, replication of our findings in an independent cohort will be an important future endeavor, however, at the time of this study, no comparable dataset was available to us for replication. Finally, for generalization of the results, our analysis should be replicated in ethnically diverse cohorts, since ethnicity has been shown to influence markers of microglial activation (Schindler *et al*, 2021).

Conclusions

Our findings support disease stage-dependent effects of microglial responses on AD disease progression. Besides previously reported beneficial effects of a TREM2-related microglial response in

advanced AD, we show that in patients with earliest A β abnormalities, microglial activation is associated with increases in p-tau₁₈₁ and neuroinflammation which is reflected in glucose hypermetabolism. Our findings have important clinical implications as they suggest that targeting enhancement of TREM2-related microglial responses may have opposing effects on the progression of AD pathophysiology in early versus later stages of AD.

Materials and Methods

Participants

We included 402 participants from the Alzheimer's Disease Neuroimaging Initiative (ADNI) with available CSF A β ₁₋₄₂, p-tau₁₈₁, and sTREM2, ¹⁸F-florbetapir/¹⁸F-florbetaben amyloid-PET, FDG-PET as well as demographics (sex, age, education) and clinical status. Baseline CSF and PET data had to be obtained within a time window of 6 months. Clinical status was classified by ADNI investigators as cognitively normal (CN; Mini Mental State Examination [MMSE] \geq 24, Clinical Dementia Rating [CDR] = 0, non-depressed), mild cognitive impairment (MCI; MMSE \geq 24, CDR = 0.5, objective memory-impairment on education-adjusted Wechsler Memory Scale II, preserved activities of daily living) or dementia (MMSE = 20–26, CDR \geq 0.5, NINCDS/ADRDA criteria for probable AD). ADNI inclusion/exclusion criteria can be found at https://adni.loni.usc.edu/wp-content/uploads/2010/09/ADNI_GeneralProceduresManual.pdf. Participants with dementia were excluded from this study, since we followed a previous approach of disease stage stratification (Palmqvist *et al*, 2017), excluding later stages of AD. To determine disease stage, participants were stratified into early or late A β -accumulators based on their A β CSF and amyloid-PET status (Palmqvist *et al*, 2017). A β CSF positivity was determined as CSF A β ₁₋₄₂ < 976.6 pg/ml (Suarez-Calvet *et al*, 2019) and amyloid-PET positivity was determined as ¹⁸F-florbetapir > 1.11 SUVR (Landau *et al*, 2012) or ¹⁸F-florbetaben PET > 1.08 SUVR (Royse *et al*, 2021). Participants were then grouped as early A β -accumulators (A β CSF+/PET–; CN/MCI n = 30/40) and as late A β -accumulators (CSF+/PET+; CN/MCI n = 41/160). Participants which were classified as A β CSF–/PET+ were excluded (n = 39). In addition, A β CSF–/PET– participants were included as a healthy reference group. One participant had abnormal A β CSF levels (~4,000 pg/ml) and was thus excluded, resulting in 131 controls comprising of CN participants only. A subset of participants had available longitudinal p-tau₁₈₁ (early/late/controls n = 20/75/35) and sTREM2 (early/late/controls n = 21/75/35) assessments, based on which we calculated annual p-tau₁₈₁ and sTREM2 change rates. Ethical approval was obtained by ADNI investigators, and all study participants provided written informed consent.

Ethics approval and consent to participate

Ethics approval was obtained by the ADNI investigators from the local ethical committees of all involved sites. The study was conducted in accordance with the Declaration of Helsinki and all study participants provided written informed consent. All work complied with ethical regulations for work with human participants.

CSF biomarkers

A β ₁₋₄₂ and p-tau₁₈₁ levels were assessed by the ADNI biomarker core team at the University of Pennsylvania. An electrochemiluminescence immunoassays Elecsys on a fully automated Elecsys cobas e 601 instrument and a single lot of reagents for each biomarker were used. For the assessment of sTREM2, a previously described ELISA approach was applied (Kleinberger *et al*, 2014; Suarez-Calvet *et al*, 2016; Ewers *et al*, 2019). sTREM2 data are provided in the ADNI_HAASS_WASHU_LAB.csv file available in the ADNI database (variable “MSD_STREM2CORRECTED”). A detailed description of the methods is found online (<https://ida.loni.usc.edu>).

MRI and PET acquisition and preprocessing

3T structural MRI was obtained by ADNI employing T1-weighted MPRAGE sequences using unified scanning protocols (<http://adni.loni.usc.edu/methods/mri-tool/mri-analysis/>). Amyloid-PET was recorded 50–70 min after ¹⁸F-florbetapir injection in 4 \times 5 min frames or 90–110 min after ¹⁸F-florbetaben injection in 4 \times 5 min frames. FDG-PET was recorded 30–60 min after ¹⁸F-fluorodeoxyglucose injection in 6 \times 5 min frames. To obtain mean images, recorded time frames were motion corrected and averaged (see also <http://adni.loni.usc.edu/methods/pet-analysis-method/pet-analysis/>). Using the Advanced Normalization Tools (ANTs; Avants *et al*, 2011) high-dimensional warping algorithm, nonlinear spatial normalization parameters to Montreal Neurological Institute (MNI) space were estimated based on structural skull-stripped T1-weighted images. Amyloid-PET and FDG-PET images were then co-registered to native-space T1-weighted images and subsequently normalized to MNI space by applying the ANTs-derived normalization parameters. Amyloid-PET SUVRs were intensity normalized to the whole cerebellum and FDG-PET SUVRs to the pons. To harmonize between both amyloid-PET tracers, global amyloid-PET SUVRs across ¹⁸F-florbetapir and ¹⁸F-florbetaben were transformed to centiloid using equations provided by ADNI (Klunk *et al*, 2015).

Statistical analyses

All statistical analyses were computed using R statistical software version 4.0.2 (<http://www.R-project.org/>; R Core Team, 2021).

Baseline characteristics between groups were compared using ANOVAs for continuous and chi-squared tests for categorical data.

To test whether a TREM2-related microglial response is associated with soluble p-tau₁₈₁ increases in early A β -accumulators, we first assessed cross-sectional disease stage-dependent (i.e., early vs. late A β -accumulators) associations between centiloid and CSF sTREM2, and between centiloid and CSF p-tau₁₈₁, using linear regressions. Here, we used sTREM2 or p-tau₁₈₁ as the dependent variable, and centiloid as the independent variable. To assess longitudinal disease stage-dependent associations, we first calculated slope estimates for annual change rates of CSF sTREM2 and CSF p-tau₁₈₁ for those participants with available longitudinal sTREM2 and p-tau₁₈₁ data. Here, linear mixed models were fitted using sTREM2 or p-tau₁₈₁ as dependent variable and time (i.e., years from baseline) as independent variable, adjusting for subject-specific random slope and intercept. Subsequently, we performed linear regressions

using annual change rates of sTREM2 or p-tau₁₈₁ as the dependent variable and baseline centiloid as the independent variable. In addition, analyses were repeated using CSF A β instead of centiloid for testing associations between soluble A β and sTREM2 or p-tau₁₈₁. The regression models were controlled for age, sex, education, and clinical status (i.e., CN or MCI). The models testing the control group were not controlled for clinical status since the group only comprised of CN participants. Finally, bootstrapped mediation analyses using 1,000 iterations were applied using the mediation package in R (<https://cran.r-project.org/web/packages/mediation/mediation.pdf>) for assessing cross-sectional and longitudinal associations, using centiloid as the predictor variable, p-tau₁₈₁ (i.e., cross-sectional) or p-tau₁₈₁ change rate (i.e., longitudinal) as the dependent variable, and sTREM2 as the mediator. Subsequently, we tested the reverse associations (i.e., p-tau₁₈₁ as the mediator of centiloid's effect on sTREM2). All mediation analyses were controlled for age, sex, education, and clinical status. Lastly, we combined early and late A β -accumulators and tested for an sTREM2 by group interaction on cross-sectional and longitudinal p-tau₁₈₁ levels, controlling for age, sex, education, and clinical status.

In the next step, we assessed whether a sTREM2-related microglial response manifests in glucose hypermetabolism in early AD stages when neurodegeneration is typically not yet apparent (i.e., A β CSF+/PET-) versus glucose hypometabolism in later disease stage (i.e., A β CSF+/PET+). To this end, we first calculated FDG-PET z-scores by referencing FDG-PET signals to 131 CN A β - controls. We then tested for an sTREM2 by group (i.e., A β CSF+/PET- vs. A β CSF+/PET+) interaction on FDG-PET signal in a meta-ROI that typically captures AD-related glucose metabolism changes (Laudau *et al*, 2011). For *post hoc* exploration, we repeated the analysis in a brain wide manner across 200 ROIs that cover the entire neocortex (Schaefer *et al*, 2018), in order to map the pattern of microglial activation effects on glucose hyper- versus hypometabolism depending on disease stage. To that end, we computed linear regressions for the associations between sTREM2 and each of the 200 FDG-PET ROIs. Note that the latter analysis was only exploratory to assess the general pattern between sTREM2 and FDG-PET in early versus late A β -accumulators and thus not corrected for multiple comparisons. To test whether early/late A β -accumulators differ regarding sTREM2-related glucose metabolism, *T*-values of the association between sTREM2 and FDG-PET were compared between groups using *t*-tests. For non-parametric comparison, we determined 95% confidence intervals (CI) for the 200 *T*-values for early and late A β -accumulators. The models were controlled for age, sex, education, and clinical status. Finally, the main analyses were repeated including APOE4 status as a covariate to assess whether APOE4 influences the effect of sTREM2 on p-tau₁₈₁ or glucose metabolism. Subjects were classified as APOE4 risk allele carriers when at least one ϵ 4 allele was detected.

Data availability

All data used in this manuscript are available from the ADNI database (adni.loni.usc.edu) upon registration and compliance with the data use agreement. The data that support the findings of this study are available on reasonable request from the corresponding author. Source data of the figures of this manuscript are available online on

The paper explained

Problem

Besides beta-amyloid (A β) and tau accumulation, microglial activation plays a role in the pathogenesis of Alzheimer's disease (AD). Previous studies reported both detrimental and protective effects of microglia on AD progression, thus, it is critical to investigate at which AD stages microglial activation could be protective or detrimental to evaluate microglia as a treatment target. To address this, we used CSF sTREM2 (i.e., soluble Triggering receptor expressed on myeloid cells 2) to investigate disease stage-dependent drivers of microglial activation and to determine downstream consequences on AD biomarker progression.

Results

We first stratified groups into early and late A β -accumulators to investigate disease stage-dependent effects of CSF sTREM2 on AD progression. To that end, A β CSF positive but still amyloid-PET negative participants were classified as early A β -accumulators and participants that were both A β CSF and PET positive were classified as late A β -accumulators. We found that in early A β -accumulators, higher amyloid-PET was associated with cross-sectional/longitudinal CSF sTREM2 and CSF p-tau₁₈₁ increases, suggesting reactive microglial and p-tau increases in response to earliest A β fibrillization. Further, higher CSF sTREM2 mediated the association between amyloid-PET and cross-sectional/longitudinal CSF p-tau₁₈₁ increases and higher CSF sTREM2 was associated with FDG-PET hypermetabolism in line with previous findings of increased glucose consumption of activated microglia. In late A β -accumulators, we found no association between amyloid-PET and CSF sTREM2 but a cross-sectional association between higher CSF sTREM2, higher CSF p-tau₁₈₁ and glucose hypometabolism, suggesting that sTREM2 parallels tau and neurodegeneration rather than A β once fully developed A β pathology is present.

Impact

Our findings suggest that sTREM2-related microglial activation occurs in response to earliest A β fibrillization, manifests in inflammatory glucose hypermetabolism and may facilitate subsequent p-tau increases in earliest AD, while previous reports of protective sTREM2 effects may occur in later AD stages.

the BioStudies database (<https://www.ebi.ac.uk/biostudies/studies/S-BSST954>).

Expanded View for this article is available [online](#).

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Author contributions

Davina Biel: Conceptualization; data curation; formal analysis; investigation; writing – original draft; writing – review and editing. **Marc Suárez-Calvet:**

Writing – review and editing. **Paul Hager**: Data curation; writing – review and editing. **Anna Rubinski**: Writing – review and editing. **Anna Dewenter**: Writing – review and editing. **Anna Steward**: Writing – review and editing. **Sebastian Roemer**: Writing – review and editing. **Michael Ewers**: Writing – review and editing. **Christian Haass**: Data curation; writing – review and editing. **Matthias Brendel**: Conceptualization; writing – review and editing. **Nicolai Franzmeier**: Conceptualization; data curation; formal analysis; investigation; writing – original draft; writing – review and editing. Open Access funding enabled and organized by Projekt DEAL.

Disclosure and competing interests statement

MSC has served as a consultant and at advisory boards for Roche Diagnostics International Ltd and has given lectures in symposia sponsored by Roche Diagnostics, S.L.U and Roche Farma, S.A. NF has served as a consultant for Merck Sharp & Dohme. The authors declare no further competing interests.

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