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Brain, Behavior, and Immunity 120 (2024) 248–255

Contents lists available at ScienceDirect

Brain Behavior and Immunity

journal homepage: www.elsevier.com/locate/ybrbi

Full-length Article

Evidence for reduced anti-inflammatory microglial phagocytic response in late-life major depression

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ARTICLE INFO

Keywords:
Depression
Alzheimer's disease
Microglia
sTREM2
Amyloid beta
Phagocytosis

ABSTRACT

Major depressive disorder (MDD) is associated with Alzheimer's disease (AD) but the precise mechanisms underlying this relationship are not understood. While it is well established that cerebrospinal fluid (CSF) soluble levels of triggering receptor expressed on myeloid cells 2 (sTREM2) increase during early stages of AD, how sTREM2 levels behave in subjects with MDD is not known. In a longitudinal study, we measured CSF sTREM2 levels in 27 elderly cognitively intact individuals with late-life major depression (LLMD) and in 19 healthy controls. We tested the hypothesis that, similarly to what happens in early stages of AD, CSF sTREM2 would be elevated in MDD. In addition, we compared the associations of CSF sTREM2, pro- and anti- inflammatory, and AD biomarkers in LLMD and control subjects. Surprisingly, we found that mean CSF sTREM2 levels were significantly reduced in LLMD compared to controls. This reduction was no longer significant at the 3-year follow-up visit when depression severity improved. In addition, we found that CSF sTREM2 was associated with AD biomarkers and proinflammatory cytokines in controls but not in LLMD. These findings suggest that impaired microglia phagocytic response to AD pathology may be a novel link between MDD and AD.

1. Introduction

Major depressive disorder (MDD) is a risk factor of developing Alzheimer's disease (AD), but the association between MDD and AD pathology is not fully understood. Inflammatory pathways including microglial activation and reactive astrocytes have been implicated in MDD and may provide a link between these two conditions (Enache et al., 2019; Santos et al., 2016). The role of peripheral inflammation on the association between MDD and AD is also unclear. Increased levels of circulating proinflammatory cytokines, such as interleukin-6 (IL-6), interleukin-8 (IL-8), interleukin-1β (IL–1β) and tumor necrosis factor-alpha (TNF-α), have been reported in individuals with more severe depressive symptoms (Brites and Fernandes, 2015). On the other hand, older individuals with long standing MDD (Ng et al., 2018), especially

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https://doi.org/10.1016/j.bbi.2024.05.030
Received 11 December 2023; Received in revised form 19 April 2024; Accepted 20 May 2024
Available online 23 May 2024

those who are likely to be on antidepressants, have no evidence of increased pro-inflammatory cytokines and other inflammatory proteins (Colonna, 2003; Guerreiro et al., 2013; Li et al., 2023; Hansen et al., 2018; Heslegrave et al., 2016; Piccio et al., 2016).

Triggering receptor expressed on myeloid cells 2 (*TREM2*) is a type-1 protein with an ectodomain that undergoes proteolytic cleavage resulting in the release of a soluble N-terminal fragment into the extracellular space (called sTREM2) which can be measured in CSF (Colonna, 2003). Genetic studies (Guerreiro et al., 2013) have revealed that heterozygous loss-of-function mutations in the *TREM2* gene are associated with an increased risk of developing sporadic AD second only to the risk of carrying an *APOE4* allele (Guerreiro et al., 2013). TREM2 is believed to play an important role in the phenotypic changes of microglia (Li et al., 2023) the resident immune cells in the central nervous system (CNS) that are critical to normal functioning of the brain (Hansen et al., 2018).

Studies have shown that amounts of sTREM2 are elevated in the CSF of persons with AD (Heslegrave et al., 2016; Piccio et al., 2016) when compared to cognitively normal controls. Increased CSF level of sTREM2 occurs in the early pre-clinical AD stage of the disease (Suarez-Calvet et al., 2016a; 2016b); plateau in prodromal AD and then increase again in mild-to-moderate AD where they are associated with phosphorylated-tau (p-tau) (Heslegrave et al., 2016; Suarez-Calvet et al., 2016a; 2016b; Ewers et al., 2020; Morenas-Rodríguez et al., 2022; Liu et al., 2018; Fan et al., 2017). These changes in sTREM2 correspond with increased amyloid deposition and cognitive impairment (Suarez-Calvet et al., 2016b). In AD, CSF sTREM2 is also positively associated with positron emission tomography imaging of translocator protein (TSPO PET) (Venneti et al., 2009; Pascoal et al., 2021), a marker of microglial and astrocytic activation, confirming microglial response to AD pathology. Several studies have provided compelling support that higher baseline CSF levels of sTREM2 early in the AD continuum are protective and related to both the slowing of AD pathology and better longitudinal outcomes, including lower Aβ deposition and cognitive impairment (Ewers et al., 2020; Morenas-Rodríguez et al., 2022; Franzmeier et al., 2020). Pereira et al. (2022) found that in nondemented individuals, higher baseline CSF sTREM2 levels were associated with reduced PET amyloid cross sectionally, less longitudinal PET amyloid and tau accumulation as well as less cognitive decline, consistent with protective effects. Contrary to the protective effects reported in individuals with only early brain amyloid pathology, higher baseline CSF sTREM2 and the co-occurrence of Aβ, tau and microglia abnormalities were the strongest predictor of cognitive impairment (Pascoal et al., 2021). These results suggest that in the more advanced phases of AD, the microglial response may no longer be protective Thus, during the early stages of AD, CSF sTREM2 may actually reflect anti-inflammatory microglial phagocytic activation.

Exploration of sTREM2 levels may also provide insight into the link between AD risk, neuroinflammation, and MDD. Wang et al. (2022) found a significant reduction in CSF sTREM2 in individuals with "minimal depressive symptoms" (MDS), in the absence of cognitive impairment, as compared with controls. In addition, they found that in the MDS group amyloid pathology was partially mediated by CSF sTREM2 (Wang et al., 2022). These findings suggest that reduced TREM2 function may also be a factor for increased brain amyloid burden and risk for AD in depression. In the current study, we wanted to determine the levels of sTREM2 in LLMD and the role of sTREM2 in the association between CSF markers of brain Aβ deposition and pro-inflammatory and anti-inflammatory cytokines in cognitively-intact individuals with LLMD. We predicted that LLMD would be accompanied by increased CSF sTREM2 similar to the early stages of AD. Additionally, LLMD would be positively associated with both increased CSF and plasma pro-inflammatory cytokines.

2. Materials and methods

2.1. Subjects

The participants of this study were part of a larger observational study approved by the institutional review boards of the Nathan Kline Institute (NKI) for Psychiatric Research and the New York University (NYU) School of Medicine (described elsewhere, see (Pomara et al., 2022). Participants were volunteers who responded to advertisements in local newspapers and flyers or were recruited in the Memory Education and Research Initiative (MERI) Program. All participants were compensated for their participation. A total of 51 subjects completed an optional lumbar puncture (LP) at baseline. Of the 51 subjects with baseline LPs, four subjects were excluded from the O for their MRI results or MMSE score at Baseline (described elsewhere, see (Pomara et al., 2016): 28 with Late-life Major Depressive Disorder (LLMD) and 19 healthy, aged-matched controls. Subject demographics are shown in Table 1. All subjects were cognitively intact with a Mini Mental State Exam (MMSE) score of 28 and Clinical Dementia Rating Scale Score of (CDR) of 0, had no evidence of dementia, and no gross MRI abnormalities other than white matter intensities. One additional subject was excluded for having missing sTREM2 biomarker data; a total of 46 subjects were included in the analysis. Of the individuals who completed the baseline LP, a total of 39 subjects completed the optional LP at the year 3 follow-up; 36 subjects were included in the Year 3 comparison (LLMD group, N = 19; control group, N = 17).

Table 1
A) Baseline demographic information (means and standard deviations and exact p-values) for the control group (n = 19) and LLMD group (n = 28). P-values are for independent samples *t*-test comparison, except when otherwise noted. B) Means, standard deviations, and exact p-values for the CSF and Inflammatory biomarkers for the Control group and LLMD group. P-values are for Mann Whitney *U* Test comparison.

a)	Control Group (n = 19)	LLMD Group (n = 28)	p values
	Mean ± SD	Mean ± SD	t-tests
Age (years)	68.1 ± 7.3	66.5 ± 5.4	0.41
Education (years)	16.7 ± 2.7	16.5 ± 5.4	0.79
21-item HAM-D	1.2 ± 1.9	14.9 ± 8.8	<0.001**
MMSE	29.5 ± 0.5	29.8 ± 0.6	0.13
Total Recall Rating	64.4 ± 12.3	64.9 ± 13.9	0.91
Delayed Recall Rating	8.5 ± 2.8	9.5 ± 2.5	0.22
Hippocampal volume	5.2 ± 0.4	5.2 ± 0.5	0.78
			p values (χ2)
Females (n)	12(63 %)	10(36 %)	0.12
APOE ε4 positive (n)	5(26 %)	11(39 %)	0.36
b)	Mean ± SD	Mean ± SD	Mann Whitney U-tests
CSF Biomarkers			
A β1-40 (pg/mL)	6518 ± 2687	5146 ± 2369	0.072
A β1-42 (pg/mL)	335 ± 183	225 ± 125	0.032*
Ratio 42/40	0.0498 ± 0.0128	0.0430 ± 0.0115	0.0603
Total Tau (pg/mL)	329 ± 152	301 ± 184	0.3416
Phospho tau p181 (pg/mL)	51.58 ± 20.90	48.93 ± 25.87	0.501
Inflammatory biomarkers			
IL6	4.52 ± 5.08	4.433 ± 3.065	0.209
IL8	91.22 ± 34.73	87.05 ± 15.28	0.649
IL4	4.23 ± 6.81	2.43 ± 0.69	0.526
IL10	3.43 ± 2.51	3.43 ± 1.71	0.598
sTREM2 (pg/mL)	5178 ± 2787	3476 ± 2729	0.035*

*Significant $p < 0.05$.

2.2. Brief study procedures

On the first visit, the informed consent was obtained, the Hamilton Depression Scale was administered, and a blood sample was taken for APOE genotyping. At the second visit, a medical and psychiatric history and vital signs were obtained, and participants underwent a psychiatric evaluation. Global cognitive status was assessed using the MMSE and CDR. Participants were classified as either having major depressive disorder (MDD) or as controls using the Structured Clinical Interview for DSM IV (SCID) conducted by a Psychiatrist. Participants received an MRI scan of the head to quantify the magnitude of vascular brain pathology. At the Baseline Visit, subjects underwent a comprehensive clinical including the Hamilton Depression Rating Scale (Hamilton, 1960) (HAM-D), a neuropsychological assessment, and the lumbar puncture was performed. Cerebrospinal fluid (CSF) markers and plasma biomarkers were determined as described below.

2.3. CSF markers

CSF sTREM2 was determined using an in-house electrochemoluminescent immunoassay. Streptavidin-coated 96-well plates (Meso-Scale discovery (MSD)) were blocked overnight at 4 °C in block buffer (0.5 % bovine serum albumin (BSA) and 0.05 % Tween 20 in PBS (pH 7.4). The plates were next incubated with the biotinylated polyclonal goat anti-human TREM2 capture antibody (0.25 μg/ml R&D Systems BAF1828) diluted in block buffer for 1 h at room temperature. They were subsequently washed four times with wash buffer (0.05 % Tween 20 in PBS) and incubated for 2 h at room temperature with the undiluted cell culture media samples or a standard curve constructed from recombinant human TREM2 protein (4000—62.5 pg/ml Sino Biological Inc 11084-H08H) diluted in assay buffer (0.25 % BSA and 0.05 % Tween 20 in PBS (pH = 7.4). Plates were again washed three times with wash buffer before incubation for 1 h at room temperature with the detector antibody monoclonal mouse anti-human TREM2 antibody (1 μg/ml Santa Cruz Biotechnology; B-3, sc373828). After three additional washing steps, plates were incubated with the secondary antibody (SULFO-TAG–labeled anti-mouse secondary antibody, MSD) and incubated for 1 h in the dark. Lastly, plates were washed three times with wash buffer followed by two washing steps in PBS alone. The electrochemical signal was developed by adding MSD Read buffer (1 in 2) and the light emission measured using the MSD SECTOR Imager 6000. The concentration of sTREM2 was calculated using a five-parameter logistic curve fitting method with the MSD Workbench software package. Intra-assay coefficients of variation (CVs) were < 10 %, and all samples were measured on the same day using the same reagents.

CSF levels of amyloid-$\beta_{1\text{-}40}$ (Aβ40) and amyloid $\beta_{1\text{-}42}$ (Aβ42) and the proinflammatory markers IL-6 and IL-8 were determined by electrochemoluminescent detection using Meso Scale kits (Meso Scale Discovery, Gaithersburg, Md.) according to the manufacturer's instructions. We determined the total tau concentration in CSF using a sandwich enzyme-linked immunosorbent assay (ELISA) (Innotest hTAU-Ag. Innogenetics, Ghent, Belgium) specifically constructed to measure all tau isoforms, irrespective of phosphorylation status. Tau protein phosphorylated at threonine 181 was measured using a sandwich ELISA method (Innotest Phospho-Tau [181P], Innogenetics). CSF anti-inflammatory markers IL-4 and IL-10 were determined using Luminex multiplexed bead-based immunoassays according to the manufacturer's instructions. Plasma proinflammatory markers IL-6, IL-8 and TNF-α were determined using commercial ELISA assays (Innotest, Fujirebio). CSF and plasma biomarkers were analyzed in batches.

2.4. Data and statistical analysis

Data were analyzed using SPSS v24.0. Since most parameters displayed non-normal distributions, non-parametric tests were used for all comparisons (Mann Whitney U) and correlations (Spearman's rank). For group comparisons, Alpha of $p < 0.05$ was used to indicate statistical significance. Due to the small sample size, no outliers were excluded for the below analyses (outliers are more than 1.5 times the interquartile range −/+ the first/third quartile when reviewing individual variable boxplots). We applied the Bonferroni-Holm correction for all correlations to control for multiple comparisons using Python Statsmodels 0.15.0 and figures were generated using Seaborn v 0.12.2. The study was originally powered ($1\text{-}\beta = 0.80$) for another outcome variable (Aβ42) to detect a medium effect at the $p < 0.05$ level. This is a secondary analysis of the collected CSF; the link between TREM2 and AD had not been described at the time the study was designed. The primary outcome of the present study was the sTREM2 concentration in CSF. With our sample size, we had a 86 % power to detect a statistically significant 50 % difference in mean CSF sTREM2 levels between LLMD and Control groups.

3. Results

There were no significant differences in sex (male/female), age, MMSE between the elderly control and LLMD groups (Table 1). *APOE* ε4 alleles were not significantly different between groups and had no effect on sTREM2 levels (ε4-positive: 3559 ± 776 pg/mL, ε4-negative: 4509 ± 494 pg/mL, p = 0.173). Levels of sTREM2 in the CSF did not depend on antidepressant use (on antidepressant medication: 3041 ± 574 pg/mL, not on medication: 4108 ± 987 pg/mL, p = 0.610). Mean (±SEM) CSF sTREM2 levels were significantly lower in LLMD than in controls (3476 ± 525 pg/mL, 5178 ± 639 pg/mL, p = 0.035) (Fig. 1a). In year 3, CSF sTREM2 no longer showed a significant reduction in LLMD (Independent Samples Mann-Whitney U Test, p = 0.616) (Fig. 1b). This observation was associated with a significant improvement in depression symptoms in the LLMD group, as previously reported (Pomara et al., 2022).

As previously reported (Pomara et al., 2021), there were no other significant differences in the commonly used AD CSF biomarkers total tau or p-tau181, nor were there differences in the levels of CSF or plasma proinflammatory cytokines IL-6 and IL-8. Additionally, there were no group differences for CSF anti-inflammatory cytokines, IL-4 and IL-10.

There was no significant correlation between sTREM2 levels and age in either controls or LLMD. In the whole group (LLMD and controls), levels of sTREM2 were negatively correlated with the baseline HAMD scores (r = -0.344, p = 0.019; Fig. 2), but this relationship was not significant when run separately (LLMD: p = 0.175, Controls: p = 0.875). In year 3, in the whole group levels of sTREM2 were not significantly correlated with HAMD scores (rho = 0.050, p = 0.772). When run separately, this relationship was not significant in LLMD (rho = -0.25, 0.302), but was significant in Controls (rho = 0.543, p = 0.024).

CSF sTREM2 levels were positively associated with CSF levels of full-length ApoE (rho = 0.323, p = 0.029). This association appears to be mainly driven by the control group (rho = 0.446, p = 0.056).

Similar to the whole group, in the control group, CSF sTREM2 was significantly positively correlated with AD biomarkers (Fig. 3a): Aβ40 (rho = 0.654, p = 0.002) and Aβ42 (rho = 0.535, p = 0.018), total tau (rho = 0.509, p = 0.026) and p-tau181 (rho = 0.568, p = 0.011). For the CSF Cytokines (Fig. 3b): sTREM2 was also positively correlated with IL-8 (rho = 0.560, p = 0.013), but not IL-6 (rho = -0.189, p = 0.437). As with the whole group, in the controls, these associations with cytokines did not survive multiple comparisons correction. In LLMD, we found no significant correlations between CSF sTREM2 and AD biomarkers (Fig. 3a) or CSF cytokines (Fig. 3b): CSF sTREM2 with Aβ40 (rho = 0.363, p = 0.063) and Aβ42 (rho = 0.260, p = 0.191), total tau (rho = 0.120, p = 0.552), p-tau181 (rho = 0.243, p = 0.221), IL-8 (rho = 0.246, p = 0.216), and IL-6 (rho = -0.232, p = 0.244). In addition, CSF sTREM2 levels were found to be positively associated with CSF IL-10 levels (rho = 0.543, p = 0.016), but not IL-4, in LLMD, but the associations did not survive multiple comparisons.

There were no significant associations between sTREM2 and the

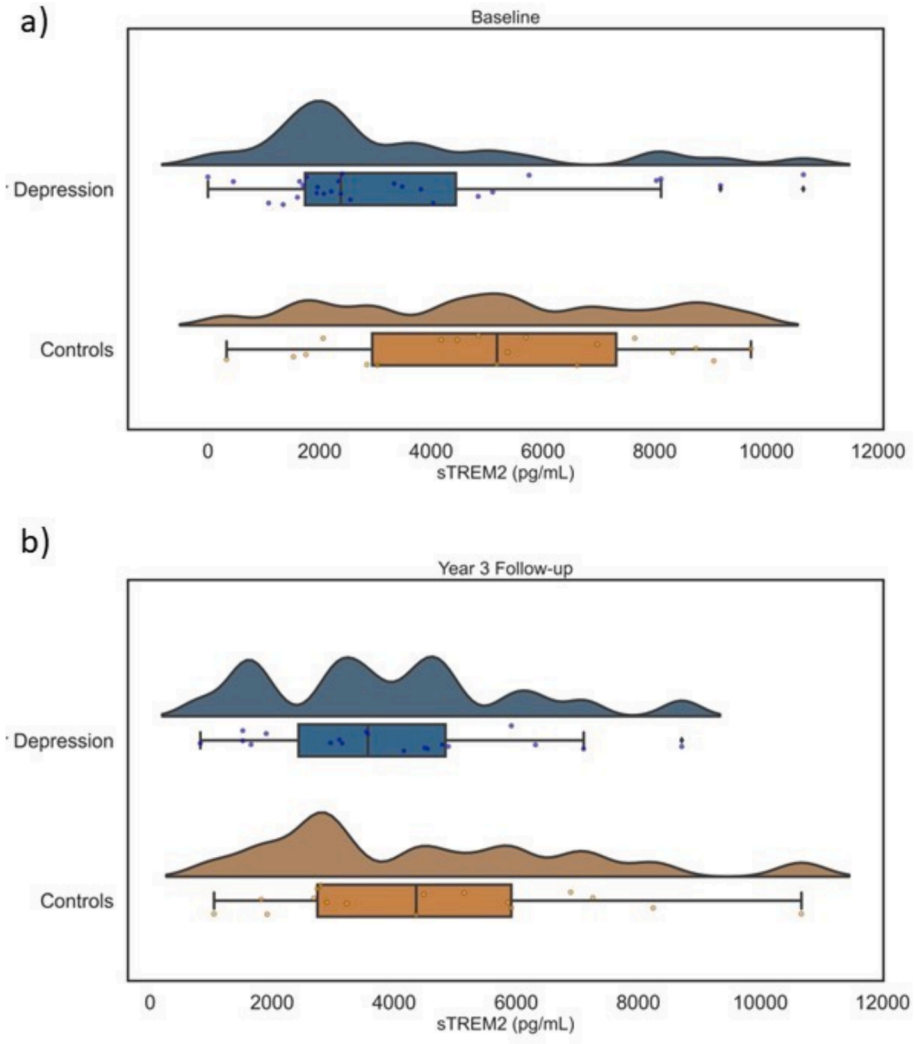

Fig. 1. A) Violinplot of csf strem2 baseline distribution by group above a box plot of CSF sTREM2 values overlayed with individual subject point plot. B) Violinplot of CSF sTREM2 Year 3 follow-up visit distribution by group above a box plot of CSF sTREM2 values overlayed with individual subject point plot. Blue represents the LLMD group. Orange represents the Control group. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

plasma biomarkers in the whole group (LLMD + Controls) nor in the control group alone. In the LLMD group, significant negative associations between sTREM2 and plasma IL-6 (rho = -0.454, p = 0.017) and marginally significant negative correlation with plasma TNF-α in LLMD (rho = -0.364, p = 0.062), but these did not survive multiple comparisons correction.

4. Discussion

The results did not support our hypothesis that CSF levels of sTREM2 would be increased in LLMD and positively associated with proinflammatory cytokines. Instead, we found lower CSF sTREM2 levels in LLMD subjects compared to controls, in the absence of associations with proinflammatory cytokines. Moreover, in LLMD, CSF sTREM2 showed no significant associations with CSF levels of ApoE, a ligand required for successful microglial activation and Aβ clearance (Keren-Shaul et al., 2017), nor with AD biomarkers. There were also no significant associations between proinflammatory cytokines, IL-6 and IL-8 and CSF sTREM2 levels in the LLMD group. Importantly, the reduction in sTREM2 at baseline was no longer significant at Year 3, when depression severity had improved (Pomara et al., 2022). There were no significant elevations in plasma cytokines in the LLMD group at baseline (Pomara et al., 2021). Additionally, although the association did not survive multiple comparisons, there was a positive trend between CSF sTREM2 and the anti-inflammatory cytokines and IL-10 in LLMD. In contrast, in healthy controls who had significantly higher CSF Aβ42 levels than the LLMD (Pomara et al., 2016; Pomara et al., 2012), there were significant associations between CSF sTREM2 and both CSF AD biomarkers and ApoE levels. This suggests microglial activation for a healthy, cognitive-normal individual is represented by less brain Aβ pathology, as reflected by higher CSF Aβ42, and a higher microglial response to Aβ deposition. The lack of associations in the LLMD group, and reduced CSF sTREM2 may represent decreased microglial reactivity against Aβ deposition (Yao et al., 2019; Xue and Du, 2021). More specifically, these findings provide support for depressive state−related impairments in microglial TREM2-mediated activation and Aβ phagocytosis, in response to brain AD pathology. This impairment in phagocytosis is consistent with a failure of anti-inflammatory (Enache et al., 2019; Wang et al., 2022; Snijders et al., 2021; Scheepstra et al., 2023; Lanz et al., 2019; Böttcher et al., 2020; Jaffe et al., 2022) microglial activation as opposed to overactive proinflammatory immune response, as has been generally suggested in the MDD literature.

Previous studies have implicated microglial activation and inflammatory pathways may play a role in MDD (Enache et al., 2019; Santos

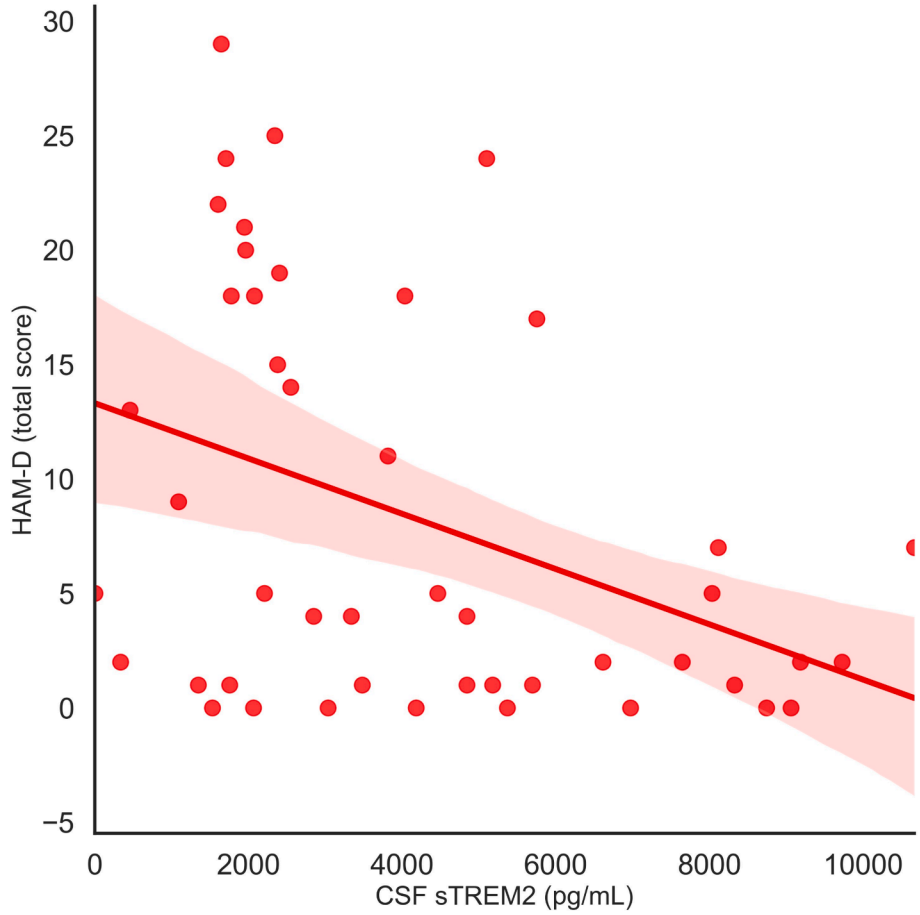

Fig. 2. Regression plot for the whole group (Control + LLMD group). Y-axis plots the total 21-item HAM-D score. X-axis plots the CSF sTREM2 baseline values.

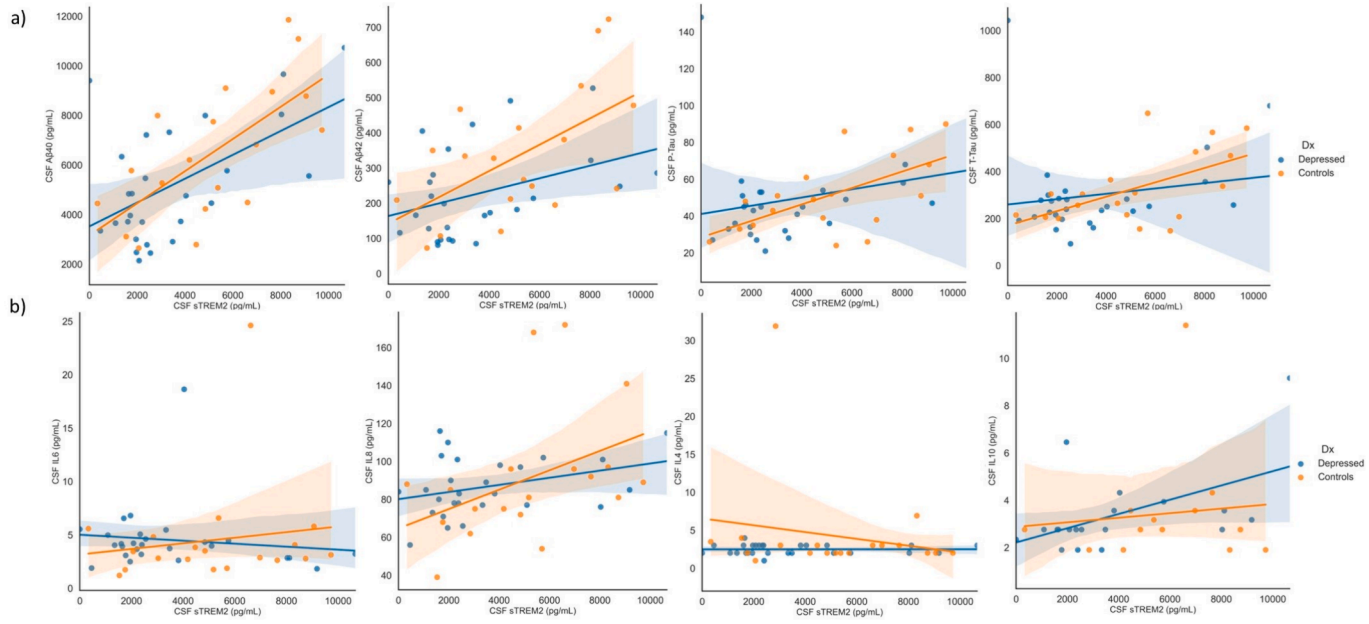

Fig. 3. Regression plot for the Control group (orange) and LLMD group (blue). a) Y-Axis plots AD Biomarkers (Aβ40, Aβ42, PTau, and Ttau). X-axis plots the CSF sTREM2 baseline values. b) Y-Axis plots Inflammatory Biomarkers (IL-6, IL-8, IL-4, and IL-10). X-axis plots the CSF sTREM2 baseline values. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

et al., 2016); however, the only study to systematically examine sTREM2 in depression included subjects with only minimal depressive symptoms (Wang et al., 2022). Our report is the first to examine this biomarker in individuals with a clinical diagnosis of MDD. When examining the whole group (LLMD and controls), we found that sTREM2 was negatively correlated with baseline score on the HAM-D, such that lower sTREM2 was associated with greater depression symptoms. These findings provide further support for the association between depressive state and reductions in sTREM2. Interestingly, this correlation was not significant when examining this association disaggregating the LLMD and control groups. In Year 3, in the whole group, the relationship between sTREM2 and HAM-D was no longer significantly related. This loss of significance may be related to the change in depression severity of the LLMD group or the loss of power, as a result of the smaller sample size at the follow-up visit.

We have previously reported that in LLMD Aβ42 CSF levels are also reduced in the same study cohort (Pomara et al., 2016; Pomara et al., 2012) and discussed how this and other evidence points to amyloid-β deposits in the brain having deleterious effects on the brains structure and function (Pomara et al., 2012). In this new study we see that CSF sTREM2 levels are also reduced in LLMD, which is contrary to what has been previously reported in AD, where a rise in levels of CSF sTREM2 has been reported (Heslegrave et al., 2016; Piccio et al., 2016). However, it has more recently been shown that higher levels of sTREM2 are associated with the early stages of AD pathology (Suarez-Calvet et al., 2016a; Suarez-Calvet et al., 2019), levels plateau in early AD, and then begins to increase in the later stages when AD is fully manifest (Fan et al., 2017). While early in the course of AD, the anti-inflammatory microglial response is functioning as expected (i.e., phagocytosing Aβ), later in the course of the disease, the proinflammatory response is set into overdrive (Fan et al., 2017; Keren-Shaul et al., 2017; Bonomi et al., 2023). Thus, we believe that LLMD is associated with a higher risk of AD because of an inability of microglia to react appropriately to early Aβ deposition, as evidenced by the lower sTREM2. Consequently, lower sTREM2 may be associated with a possible reduction in phagocytosis contributing to decreased brain Aβ clearance, increased brain amyloid burden and reduction in CSF Aβ42, which is consistent with a recent transcriptomic analysis of post-mortem brains from MDD samples (Scheepstra et al., 2023).

In line with idea that MDD as a pro-inflammatory state, several studies have reported that TSPO PET is increased in MDD (Setiawan et al., 2015), MCI due to AD (Parbo et al., 2017), and in AD (Venneti et al., 2009; Pascoal et al., 2021). Increased uptake has been thought to reflect increased microglial proinflammatory activation (Pascoal et al., 2021), particularly with the emergence of tau pathology (Fan et al., 2017; Dani et al., 2018). Evidence from the preclinical literature (Zhou et al., 2019) and human studies (Venneti et al., 2009; Pascoal et al., 2021), have reported positive associations between CSF sTREM2 and TSPO PET. In addition, TSPO PET uptake in nondemented individuals was also associated with less longitudinal brain amyloid deposition and less cognitive decline (Pereira et al., 2022). This suggests that in unimpaired populations, increased TSPO activation can also be associated with protective effects against AD and thus could also reflect protective or anti-inflammatory phagocytic microglia phenotype activation. We argue, in the case of LLMD, if lower CSF sTREM2 reflects decreased TREM2 microglial activation, we predict that TSPO PET would be negatively associated or not related to CSF sTREM2 in LLMD, especially if it reflects anti-inflammatory microglia activation (Pereira et al., 2022; Da Pozzo et al., 2019). Future studies should establish if lower CSF sTREM2 is associated with decrease TSPO PET activation.

We also previously reported in this cohort that lower CSF AChE and BChE, which may reflect increased cholinergic tone and activation of the cholinergic-anti-inflammatory pathway (CAP), were both associated with lower CSF sTREM2 in both LLMD and controls (Pomara et al., 2021). In LLMD, the reduction in these enzymes was also associated with increased circulating plasma IL-6. We also found larger basal forebrain volumes at Baseline to be associated with lower CSF sTREM2 at the third follow-up visit (Teipel et al., 2021). Thus, future studies should determine how systemic inflammation and indices of cholinergic function and CAP activation may relate to different microglial activation states especially to the reduction CSF sTREM2 that we observed in depression.

In the current study, we found no relationship between current antidepressant use and CSF sTREM2. We do not know the effect of antidepressant use on different microglia phenotypes in humans (Nicolai et al., 2023), in particular how it modulates TREM2-mediated microglial activation and sTREM2. However, the majority of individuals in the LLMD group were on stable doses of antidepressants, and only a very small number of individuals in this group were not taking an antidepressant. Other medications, known to suppress microglial and astrocyte activation via inhibition of the NF-κB pathway, including over-the-counter (OTC) medications nonsteroidal anti-inflammatory drugs (NSAIDs) or aspirin (Khan et al., 2022; Lee et al., 2023; Jorda et al., 2020), were not exclusionary in this study. Thus, there is a possibility that antidepressant use or OTCs like NSAIDs may have modulated the reduction in CSF sTREM2 and its lack of associations to AD biomarkers in MDD. A larger scale study is needed to evaluate the impact of different antidepressants on sTREM2 and microglial activation states, ideally by mechanism of action, and its relationship to the depressive state. We acknowledge that a number of conditions may also influence the CSF sTREM2 and its relationship to proinflammatory cytokines. Filippello and colleagues (Filipello et al., 2022) described a number of neurological disorders such as multiple sclerosis (MS) and other factors, which influenced sTREM2 levels. In the current study, individuals with major neurological disorders, like MS, or major psychiatric disorders aside from MDD, were excluded. However, the possibility that subclinical comorbid conditions or other factors might have contributed to our findings cannot be completely excluded and these factors should be considered when designing future studies in MDD.

There are limitations to this study. Namely, the lumbar puncture was an optional procedure, which only a subset of individuals agreed to from the larger study cohort; hence, our sample size is small and even fewer individuals agreed to the follow-up LP. A second limitation is that the individuals in the LLMD group exhibited different levels of depression severity. Thus, future studies should examine how severity of MDD and even subsyndromal levels of depression influences sTREM2 levels. These analyses were exploratory in nature and several significant correlations did not survive multiple comparisons correction (i.e., the pro- and anti-inflammatory associations with sTREM2 in LLMD), limiting the interpretability of our findings. We did not adjust our correlation analyses for potentially significant covariates. In addition, CSF biomarkers, while established measures of brain AD pathology, are not direct measures of amyloid or tau burden. Future studies with PET amyloid will be required to determine if lower CSF sTREM2 is associated with increased brain amyloid burden in MDD. We did not measure sTREM2 in plasma and were not able to determine its relationship to plasma and CSF cytokines as well as CSF sTREM2 in MDD. Our study also did not account for factors that may modulate inflammation, such as stress (Madore et al., 2020) or other lifestyle factors (Casaletto et al., 2022). We collected information on smoking, alcohol use, and medications within each group, and excluded any individuals with current or history of abuse or unstable medication doses. Future studies should collect lifestyle information, including factors that modulate not only inflammation, but also AD-risk (i.e., social isolation). Lastly, a report from our team (Teipel et al., 2021) previously examined the difference between LLMD and Controls with a Bayesian analytical framework, reporting only weak evidence in favor of lower sTREM2 in LLMD. However, in the present paper, using a smaller subset of our study population, we employed a classical approach across all analyses. As it is not the goal of this report to debate the relative merits of these analytical approaches, we suggest that these somewhat mixed results regarding the different levels of sTREM2 and the lack of relationship to inflammatory markers are related to both the use of different sample and a small effect size.

Moreover, these mixed results suggest that further research is needed in analogous populations and larger sample sizes.

5. Conclusions

These preliminary results are consistent with the hypothesis that LLMD may be accompanied by a reduction in CSF sTREM2 consistent with an impairment in microglial phagocytic activation in response to early AD pathology. Future studies will need to determine if lower CSF sTREM2 levels in LLMD are associated with increased PET amyloid and tau imaging markers as well as decreased TSPO PET activation. Additionally, the relationship between CSF sTREM2, MRI and CSF cholinergic indices of CAP upregulation and pro-and anti-inflammatory cytokines levels needs to be determined.

Funding

CRP, AH, DB, JRC, SHL, RO, BPI, and NP have nothing to declare. HZ is a Wallenberg Scholar supported by grants from the Swedish Research Council (#2022–01018 and #2019–02397), the European Union's Horizon Europe research and innovation programme under grant agreement No 101053962, Swedish State Support for Clinical Research (#ALFGBG-71320), the Alzheimer Drug Discovery Foundation (ADDF), USA (#201809–2016862), the AD Strategic Fund and the Alzheimer's Association (#ADSF-21–831376-C, #ADSF-21–831381-C, and #ADSF-21–831377-C), the Bluefield Project, the Olav Thon Foundation, the Erling-Persson Family Foundation, Stiftelsen för Gamla Tjänarinnor, Hjärnfonden, Sweden (#FO2022-0270), the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 860,197 (MIRIADE), the European Union Joint Programme – Neurodegenerative Disease Research (JPND2021-00694), the National Institute for Health and Care Research University College London Hospitals Biomedical Research Centre, and the UK Dementia Research Institute at UCL (UKDRI-1003). KB is supported by the Swedish Research Council (#2017–00915 and #2022–00732), the Swedish Alzheimer Foundation (#AF-930351, #AF-939721 and #AF-968270), Hjärnfonden, Sweden (#FO2017-0243 and #ALZ2022-0006), the Swedish state under the agreement between the Swedish government and the County Councils, the ALF-agreement (#ALFGBG-715986 and #ALFGBG-965240), the European Union Joint Program for Neurodegenerative Disorders (JPND2019-466–236), the Alzheimer's Association 2021 Zenith Award (ZEN-21–848495), and the Alzheimer's Association 2022–2025 Grant (SG-23–1038904 QC).

CRediT authorship contribution statement

Chelsea Reichert Plaska: Writing – review & editing, Formal analysis, Data curation. **Amanda Heslegrave:** Writing – original draft, Methodology, Formal analysis, Writing – review & editing. **Davide Bruno:** Writing – review & editing, Writing – original draft, Formal analysis. **Jaime Ramos-Cejudo:** Writing – review & editing, Conceptualization. **Sang Han Lee:** Formal analysis, Writing – review & editing. **Ricardo Osorio:** Writing – review & editing. **Bruno P. Imbimbo:** Writing – review & editing. **Henrik Zetterberg:** Writing – review & editing, Methodology, Conceptualization. **Kaj Blennow:** Writing – review & editing, Methodology, Conceptualization. **Nunzio Pomara:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

CRP, AH, DB, JRC, SHL, RO, BPI, and NP have nothing to declare. HZ has served at scientific advisory boards and/or as a consultant for Abbvie, Acumen, Alector, Alzinova, ALZPath, Annexon, Apellis, Artery Therapeutics, AZTherapies, Cognito Therapeutics, CogRx, Denali, Eisai, Nervgen, Novo Nordisk, Optoceutics, Passage Bio, Pinteon Therapeutics, Prothena, Red Abbey Labs, reMYND, Roche, Samumed, Siemens Healthineers, Triplet Therapeutics, and Wave, has given lectures in symposia sponsored by Alzecure, Biogen, Cellectricon, Fujirebio, Lilly, and Roche, 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 and at advisory boards for Acumen, ALZPath, BioArctic, Biogen, Eisai, Lilly, Moleac Pte. Ltd, Novartis, Ono Pharma, Prothena, Roche Diagnostics, and Siemens Healthineers; has served at data monitoring committees for Julius Clinical and Novartis; has given lectures, produced educational materials and participated in educational programs for AC Immune, Biogen, Celdara Medical, Eisai and Roche Diagnostics; and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program, outside the work presented in this paper.

Data availability

Data will be made available on request.

Acknowledgements

This work was supported in part by National Institute of Health (NIH) grant [R01 MH-080405] awarded to NP. We also acknowledge the financial support for personnel salaries and other operational expenses, including administrative support, data analyses and manuscript preparation provided by NIH grants R01 AG070821, R01AG056031, R01AG056531, and P30AG008051. We would like to thank Stefan Teipel, MD, for his constructive feedback on this manuscript. We would also like to thank Jefferson Ortega for his help with the Python Code for generating the figures. Additionally, we would like to thank Yadong Huang, MD, PhD and his lab, Gladstone Institute, for determining CSF ApoE levels. We would also like to thank Frank Martiniuk, PhD, formerly CTSI NYU School of Medicine, for determination of CSF cytokines. Lastly, we would like to thank Drs. Raymundo Hernando, Antero Sarreal, and Mrs. Vita Pomara for their help with data collection and data entry and the staff at NYU Bellevue for assisting with carrying out the study.

References

Bonomi, C.G., et al., 2023. Cerebrospinal Fluid sTREM-2, GFAP, and β-S100 in Symptomatic Sporadic Alzheimer's Disease: Microglial, Astrocytic, and APOE Contributions Along the Alzheimer's Disease Continuum. J. Alzheimers Dis. 1–13.

Böttcher, C., et al., 2020. Single-cell mass cytometry of microglia in major depressive disorder reveals a non-inflammatory phenotype with increased homeostatic marker expression. Transl. Psychiatry 10, 310.

Brites, D. & Fernandes, A. Neuroinflammation and Depression: Microglia Activation, Extracellular Microvesicles and microRNA Dysregulation. *Front Cell Neurosci* **9**, 476 (2015). https://doi.org:10.3389/fncel.2015.00476.

Casaletto, K.B., et al., 2022. Microglial correlates of late life physical activity: Relationship with synaptic and cognitive aging in older adults. J. Neurosci. 42, 288–298.

Colonna, M., 2003. TREMs in the immune system and beyond. Nat Rev Immunol 3, 445–453. https://doi.org/10.1038/nri1106.

Da Pozzo, E., et al., 2019. Microglial pro-inflammatory and anti-inflammatory phenotypes are modulated by translocator protein activation. Int. J. Mol. Sci. 20, 4467.

Dani, M., et al., 2018. Microglial activation correlates in vivo with both tau and amyloid in Alzheimer's disease. Brain 141, 2740–2754.

Enache, D., Pariante, C.M., Mondelli, V., 2019. Markers of central inflammation in major depressive disorder: a systematic review and meta-analysis of studies examining cerebrospinal fluid, positron emission tomography and post-mortem brain tissue. Brain Behav. Immun. 81, 24–40.

Ewers, M., et al., 2020. Higher CSF sTREM2 and microglia activation are associated with slower rates of beta-amyloid accumulation. EMBO Mol. Med. 12, e12308.

Fan, Z., Brooks, D.J., Okello, A., Edison, P., 2017. An early and late peak in microglial activation in Alzheimer's disease trajectory. Brain 140, 792–803.

Filipello, F., et al., 2022. Soluble TREM2: Innocent bystander or active player in neurological diseases? Neurobiol. Dis. 105630.

Franzmeier, N., et al., 2020. Higher CSF sTREM2 attenuates ApoE4-related risk for cognitive decline and neurodegeneration. Mol. Neurodegener. 15, 1–10.

Guerreiro, R., et al., 2013. TREM2 variants in Alzheimer's disease. N. Engl. J. Med. 368, 117–127. https://doi.org/10.1056/NEJMoa1211851.
Hamilton, M., 1960. A rating scale for depression. J. Neurol. Neurosurg. Psychiatry 23, 56.
Hansen, D.V., Hanson, J.E., Sheng, M., 2018. Microglia in Alzheimer's disease. J. Cell Biol. 217, 459–472.
Heslegrave, A., et al., 2016. Increased cerebrospinal fluid soluble TREM2 concentration in Alzheimer's disease. Mol Neurodegener 11, 3. https://doi.org/10.1186/s13024-016-0071-x.
Jaffe, A.E., et al., 2022. Decoding shared versus divergent transcriptomic signatures across cortico-amygdala circuitry in PTSD and depressive disorders. Am. J. Psychiatry 179, 673–686.
Jorda, A., et al., 2020. Action of low doses of Aspirin in Inflammation and Oxidative Stress induced by aβ1-42 on Astrocytes in primary culture. Int. J. Med. Sci. 17, 834.
Keren-Shaul, H. *et al.* A unique microglia type associated with restricting development of Alzheimer's disease. *Cell* **169**, 1276-1290. e1217 (2017).
Khan, H., Sharma, K., Kumar, A., Kaur, A., Singh, T.G., 2022. Therapeutic implications of cyclooxygenase (COX) inhibitors in ischemic injury. Inflamm. Res. 71, 277–292.
Lanz, T.A., et al., 2019. Postmortem transcriptional profiling reveals widespread increase in inflammation in schizophrenia: a comparison of prefrontal cortex, striatum, and hippocampus among matched tetrads of controls with subjects diagnosed with schizophrenia, bipolar or major depressive disorder. Transl. Psychiatry 9, 151.
Lee, D., Ryoo, J.E., Hong, S., Kim, H.Y., Kim, Y., 2023. Carprofen alleviates Alzheimer-like phenotypes of 5XFAD transgenic mice by targeting the pathological hallmarks induced by amyloid-β aggregation. Sci. Rep. 13, 10889.
Li, Y., et al., 2023. TREM2: Potential therapeutic targeting of microglia for Alzheimer's disease. Biomed. Pharmacother. 165, 115218.
Liu, D., et al., 2018. Soluble TREM2 changes during the clinical course of Alzheimer's disease: A meta-analysis. Neurosci. Lett. 686, 10–16.
Madore, C., Yin, Z., Leibowitz, J., Butovsky, O., 2020. Microglia, lifestyle stress, and neurodegeneration. Immunity 52, 222–240.
Morenas-Rodríguez, E., et al., 2022. Soluble TREM2 in CSF and its association with other biomarkers and cognition in autosomal-dominant Alzheimer's disease: a longitudinal observational study. The Lancet Neurology 21, 329–341.
Ng, A., et al., 2018. IL-1β, IL-6, TNF- α and CRP in Elderly Patients with Depression or Alzheimer's disease: Systematic Review and Meta-Analysis. Sci Rep 8, 12050. https://doi.org/10.1038/s41598-018-30487-6.
Nicolai, L., Nettesheim, P., de Witte, L.D., Snijders, G., 2023. Antidepressants as a potential candidate to reduce microglia activation in neurodegenerative diseases. A systematic review and meta-analysis of preclinical studies. Journal of Affective Disorders Reports 11, 100465.
Parbo, P., et al., 2017. Brain inflammation accompanies amyloid in the majority of mild cognitive impairment cases due to Alzheimer's disease. Brain 140, 2002–2011.
Pascoal, T.A., et al., 2021. Microglial activation and tau propagate jointly across Braak stages. Nat. Med. 27, 1592–1599.
Pereira, J.B., et al., 2022. Microglial activation protects against accumulation of tau aggregates in nondemented individuals with underlying Alzheimer's disease pathology. Nature Aging 1–7.
Piccio, L., et al., 2016. Cerebrospinal fluid soluble TREM2 is higher in Alzheimer disease and associated with mutation status. Acta Neuropathol. 131, 925–933. https://doi.org/10.1007/s00401-016-1533-5.
Pomara, N., et al., 2012. Lower CSF amyloid beta peptides and higher F2-isoprostanes in cognitively intact elderly individuals with major depressive disorder. Am J Psychiatry 169, 523–530. https://doi.org/10.1176/appi.ajp.2011.11081153.
Pomara, N., et al., 2016. State-dependent alterations in cerebrospinal fluid Aβ42 levels in cognitively intact elderly with late-life major depression. Neuroreport 27, 1068–1071.
Pomara, N., et al., 2021. Evidence of upregulation of the cholinergic anti-inflammatory pathway in late-life depression. J. Affect. Disord. 286, 275–281.
Pomara, N., et al., 2022. Plasma Amyloid-β dynamics in late-life major depression: a longitudinal study. Transl. Psychiatry 12, 301.
Santos, L.E., Beckman, D., Ferreira, S.T., 2016. Microglial dysfunction connects depression and Alzheimer's disease. Brain Behav. Immun. 55, 151–165.
Scheepstra, K.W., et al., 2023. Microglia transcriptional profiling in major depressive disorder shows inhibition of cortical grey matter microglia. Biol. Psychiatry 94, 619–629.
Setiawan, E., et al., 2015. Role of translocator protein density, a marker of neuroinflammation, in the brain during major depressive episodes. JAMA Psychiat. 72, 268–275.
Snijders, G.J., et al., 2021. Distinct non-inflammatory signature of microglia in post-mortem brain tissue of patients with major depressive disorder. Mol. Psychiatry 26, 3336–3349.
Suarez-Calvet, M., et al., 2016a. Early changes in CSF sTREM2 in dominantly inherited Alzheimer's disease occur after amyloid deposition and neuronal injury. Sci Transl Med 8, 369ra178. https://doi.org/10.1126/scitranslmed.aag1767.
Suarez-Calvet, M., et al., 2016b. sTREM2 cerebrospinal fluid levels are a potential biomarker for microglia activity in early-stage Alzheimer's disease and associate with neuronal injury markers. EMBO Mol. Med. 8, 466–476. https://doi.org/10.15252/emmm.201506123.
Suarez-Calvet, M., et al., 2019. Early increase of CSF sTREM2 in Alzheimer's disease is associated with tau related-neurodegeneration but not with amyloid-beta pathology. Mol Neurodegener 14, 1. https://doi.org/10.1186/s13024-018-0301-5.
Teipel, S., et al., 2021. Association of CSF sTREM2, a marker of microglia activation, with cholinergic basal forebrain volume in major depressive disorder. J. Affect. Disord. 293, 429–434.
Venneti, S., Wiley, C.A., Kofler, J., 2009. Imaging microglial activation during neuroinflammation and Alzheimer's disease. J. Neuroimmune Pharmacol. 4, 227–243.
Wang, Z.-B., et al., 2022. sTREM2 mediates the associations of minimal depressive symptoms with amyloid pathology in prodromal Alzheimer's disease: The CABLE study. Transl. Psychiatry 12, 140.
Xue, F., Du, H., 2021. TREM2 mediates microglial anti-inflammatory activations in Alzheimer's disease: lessons learned from transcriptomics. Cells 10, 321.
Yao, H., et al., 2019. Distinct signaling pathways regulate TREM2 phagocytic and NFκB antagonistic activities. Front. Cell. Neurosci. 13, 457.
Zhou, J., et al., 2019. Imbalance of microglial TLR4/TREM2 in LPS-treated APP/PS1 transgenic mice: a potential link between Alzheimer's disease and systemic inflammation. Neurochem. Res. 44, 1138–1151.