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Soluble TREM2 and Inflammatory Proteins in Alzheimer's Disease Cerebrospinal Fluid

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Abstract. The present study explores the associations of soluble TREM2, an important regulator of microglial activity linked to Alzheimer's disease (AD), with other known inflammatory proteins in cerebrospinal fluid (CSF). We studied 303 participants, including 89 controls, 135 mild cognitive impairment, and 79 AD dementia patients. Using established CSF biomarkers, subjects were classified according to the National Institute on Aging-Alzheimer's Association research framework, which groups markers into those of amyloid-β deposition (A), tau pathology (T), and neurodegeneration (N). TNFR1, TNFR2, TGF-β1, TGFβ2, IL-9, TNF-α, ICAM1, and VCAM1 showed significant concentration differences between the ATN groups, with higher concentrations in more advanced disease categories. sTREM2 was positively associated with the pro-inflammatory proteins TNF-α, TNFR1, TNFR2, ICAM1, VCAM1, and IP-10 and negatively with IL-21; also, positive associations with the anti-inflammatory proteins TGFβ1, IL-10, and IL-9 were found. Pathway enrichment analysis highlighted the involvement of sTREM2 in key functional clusters including immunoglobulin and cytokine production and cellular response to lipopolysaccharides, cytokines, and steroid hormones. Our work provides further evidence in support of TREM2 as amarker of neuroinflammatory response in AD.

Keywords: Alzheimer's disease, biomarker, functional annotation, interactions network, neurodegeneration, neuroinflammation

INTRODUCTION

Based on the assumption that neurologic disorders can be separated into different disease entities characterized by distinct mechanisms such as neurodegeneration, cerebrovascular changes, or neuroinflammation, research rarely crosses the borderlines between these categories. However, recent evidence suggests a more complex interaction between seemingly separate disease mechanisms. In Alzheimer's disease (AD), neuroinflammation, extracellular amyloid-β (Aβ) plaques, and intracellular tau neurofibrils seem to be closely linked [1].

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

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Innate immune responses are involved in most neurodegenerative disorders, as supported by their association with single nucleotide polymorphisms (SNPs) related to microglial activity. For example, SNPs in the *TREM2* gene have been associated with AD [2], Parkinson's disease, and frontotemporal dementia [3]. TREM2 was suggested to be an important regulator of microglia during neurodegeneration. Functional imaging studies in transgenic mice using positron-emission-tomography (PET) showed that TREM2 plays a role in microglial activation during normal aging and is needed to maintain physiological cerebral energy metabolism [4]. The soluble form of TREM2 (sTREM2) is accessible as a biomarker in cerebrospinal fluid (CSF) [5], and its concentrations were shown to be associated with markers of neurodegeneration and fibrillar tau pathology, but not with Aβ [6–8].

In the present study we utilized the biomarker-based classification system proposed in the recently published National Institute on Aging-Alzheimer' Association (NIA-AA) research framework [9]; this approach allowed us to contrast groups of individuals with different biomarker profiles. We tested the main hypotheses that the CSF concentrations of well-established inflammatory proteins differ between the NIA-AA groups and that these markers are associated with levels of sTREM2 within the NIA-AA groups.

MATERIALS AND METHODS

Data used in the preparation of this article were obtained from the ADNI database (http://adni.loni.usc.edu/) on January 10, 2019. The ADNI was launched in 2003 as a public-private partnership, led by Principal Investigator Michael W. Weiner, MD. The primary goal of ADNI has been to test whether serial MRI and PET, other biological markers, and clinical and neuropsychological assessments can be combined to measure the progression of mild cognitive impairment (MCI) and early clinical AD. Participants were aged between 55–90 (inclusive), considered cognitively normal (CN), MCI, or AD dementia diagnosed individuals, and underwent serial evaluations of functional, biomedical, neuropsychological, and clinical status at various intervals.

Diagnostic classification of participants

CN was defined as Mini-Mental-State Examination (MMSE) score between 25 and 30, inclusive; Clinical Dementia Rating (CDR) score of 0; no evidence of depression; and no memory complaints. MCI was defined as MMSE score between 24 and 30, inclusive; CDR score of 0.5; report of memory complaints; and no significant functional impairment; all individuals with MCI also met Petersen criteria. Finally, subjects with AD dementia met NIA-AA criteria and the diagnostic guidelines of the National Institute of Neurological and Communicative Disorders and Stroke-AD and Related Disorders Association (NINCDS–ADRDA) for AD dementia and probable AD, respectively.

Study population

The study cohort consisted of 303 participants from the ADNI1 cohort with available baseline CSF inflammatory protein profiles analyzed by Dr. William Hu and J. Christina Howell, Department of Neurology, Emory University, including 89 CN, 135 MCI, and 79 AD dementia individuals available on the ADNI database (http://adni.loni.usc.edu/). To study group differences in inflammatory CSF biomarker profiles across AD categories, participants were stratified following the ATN scheme used in the recently proposed NIA-AA research framework [9], according to which AD is defined by its pathological processes, which can be measured *in vivo* using biomarkers, and not by its clinical consequences. The relevant biomarker measures are grouped into those of Aβ deposition (A), tau pathology (T), and neurodegeneration (N), in the present study assessed by decreased $A\beta_{42}$, increased phosphorylated tau181 (ptau181) and increased total-tau (ttau) in the CSF, respectively. CSF biomarker values were binarized into normal versus abnormal for the purposes of ATN classification.

Based on the NIA-AA research framework guidelines [9], each ADNI participant was assigned to a group defined by their respective biomarker profile according to the ATN classification system, irrespective of clinical status as suggested before [10]. The aggregated tau (T) and neurodegeneration (N) groups were merged and participants were classified as TN+ if either tau or neurodegeneration were abnormal. Participants with an A-T-N- profile were considered healthy controls (N = 94). To study individuals along the AD continuum, A+TN- (N = 37) and A+TN+ (N = 151) groups were defined. Individuals with suspected non-AD pathology (SNAP) were defined as A-TN+ (N = 21). All other biomarker profiles were not considered for the

present study to exclude individuals with non-AD (co)pathologies.

Included subjects had available routine CSF proteins ($A\beta_{42}$, ttau, and ptau181) and *APOE* ε4 allele carrier status (dichotomized into carriers versus non-carriers). Additionally, included was a validated summary metric for memory (ADNI-mem; derived from: Rey Auditory Verbal Learning test, AD Assessment Scale-cognitive subscale, MMSE, and Wechsler Memory Test-logical memory I). The characteristics of the study cohort are presented in Table 1. ADNI was reviewed and approved by all host study site review boards and participants completed informed consent after receiving a comprehensive description of ADNI.

Measurement of CSF biomarkers

A detailed description of biomarker acquisition and performance measures in ADNI can be obtained by registered users at http://adni.loni.usc.edu/, with CSF collection protocols available elsewhere [11]. CSF concentrations of $A\beta_{42}$, ttau, and ptau181 were used for the present study. CSF sTREM2 [5, 12] concentrations were measured using published approaches based on the Mesoscale Discovery (MSD) electrochemiluminescence platform.

Established biomarkers

TaqMan quantitative polymerase chain reaction assays were used for genotyping *APOE* nucleotides 334 TC and 472 CT with an ABI 7900 real-time thermocycler (Applied Biosystems, Foster City, CA) using DNA freshly prepared from whole blood samples. Routine peptide CSF measures were generated from aliquot samples collected at the same time using commercially available ELISAs. Validated cut-offs were applied to a differential between normal and pathological findings for CSF Aβ, ttau, and ptau181 [11].

sTREM2 measurements

CSF sTREM2 concentrations were measured in ADNI using two different validated approaches in parallel at two different laboratories: 1) at German Center for Neurodegenerative Disorders Munich, CSF sTREM2 concentrations were measured using the MSD electrochemiluminescence platform. The assay consists of a biotinylated polyclonal goat IgG anti-human TREM2 antibody as capture antibody, raised against aminoacids 19–174 of human TREM2; a monoclonal mouse IgG anti-human TREM2 antibody as a detection antibody, raised against aminoacids 1–160 of human TREM2; and a SULFOTAG–labeled goat polyclonal IgG anti-mouse secondary antibody. Recombinant human TREM2 protein, corresponding to the extracellular domain of human TREM2 (aminoacids 1–174) is used as a standard. The electrochemical signal is measured using the SECTOR Imager 2400 (MSD). All measurements are performed in duplicate and the average is subsequently used for the statistical analyses; and 2) at Washington University, Department of Neurology, sTREM2 levels were measured using ELISA method [14, 15], which uses an anti-human TREM2 monoclonal antibody (clone 20G2) as capture antibody, a biotinylated mouse anti-human TREM-2 mAb (clone 29E3) as detection antibody and recombinant human sTREM2 (Sino Biological Inc.) to generate the standard curve. Again, all measurements are performed in duplicate and the average is subsequently used for the statistical analyses.

As shown before, CSF sTREM2 concentrations measured using the two different technologies are highly correlated ($\rho = 0.83$, $p < 0.001$) [6]; therefore,

Table 1
Demographic and clinical characteristics of the study population

Variable	A-T-N-(N = 94)	A+TN- (N = 37)	A+TN+(N = 151)	SNAP (N = 21)	*p*
Age, mean (SD), y	75.1 (7.1)	74.9 (6.5)	74.6 (7.7)	77.1 (6.3)	0.53[a]
Female, no (%)	36 (38.3)	18 (48.6)	63 (41.7)	9 (42.9)	0.76[b]
Education, mean (SD), y	15.8 (2.9)	15.6 (3.1)	15.5 (2.9)	15.4 (3.3)	0.87[a]
APOE genotype positive, no (%)	15 (16.0)	23 (62.2)	106 (70.2)	6 (28.6)	<0.001[b]
CSF sTREM2, mean, (SD), pg/mL	4273.88 (1954.50)	3535.54 (1550.65)	4191.52 (1803.90)	5461.93 (2098.67)	0.03[a]
CSF $A\beta_{42}$, mean (SD), ng/l	242.87 (27.80)	137.89 (22.03)	130.72 (23.77)	231.10 (33.23)	<0001[a]
CSF ttau, mean (SD), ng/l	56.77 (15.56)	59.78 (18.28)	125.52 (49.21)	102.05 (30.54)	<0.001[a]
CSF ptau181, mean (SD), ng/l	17.26 (3.96)	19.76 (4.88)	45.48 (14.21)	31.90 (7.00)	<0.001[a]
ADNI_mem, mean (SD)	0.56 (0.70)	–0.06 (0.97)	–0.42 (0.74)	0.32 (0.87)	<0.001[a]

[a]One-way ANOVA, [b]Chi square Test. CSF, cerebrospinal fluid; ttau, total-tau; ptau181, phosphorylated-tau181; $A\beta_{42}$, amyloid-β 42; sTREM2, soluble Triggering receptor expressed on myeloid cells 2; *APOE*: apolipoprotein E; ADNI_mem, summary metric for memory.

only the MSD measurements were used for all subsequent statistical analyses because of the higher sensitivity of the electrochemiluminescence platform compared to standard ELISA technology.

Inflammatory protein measurements

CSF levels of 14 inflammatory proteins were analyzed at Emory University, Department of Neurology, Atlanta, GA. Pathways related to IL-7 and IL-10 were previously reported to be associated with AD diagnosis and microglial function [16, 17]; based on these known relations, associated pathways including proinflammatory cytokines (IL-7, IL-12p40, IFN-γ), anti-inflammatory cytokines (IL-4, IL-10) as well as markers associated with T-helper cell (TH-17) activation (Transforming Growth Factor (TGF)-β), IL-6, and IL-21 & IL-22) were analyzed using commercially available multiplex immunoassays (Millipore Sigma, Burlington, MA), modified for CSF analyte levels. Furthermore, previous work also demonstrated a close relationship between neuroinflammation and TNF-α pathways [18] including Tumor Necrosis Factor Receptor 1 and 2 (TNFR1 and 2), and the analyses also included their effectors intercellular adhesion molecule 1 (ICAM1) and vascular cell adhesion molecule 1 (VCAM1), known to be associated with vascular endothelial dysfunction and blood-brain barrier (BBB) disruption as a possible pathomechanism of AD [19, 20]. Finally, Interferon Gamma-Induced Protein (IP-10), a small protein involved in inflammation and angiogenesis previously investigated as an AD biomarker, was included in the analysis [21, 22]. All samples were run in duplicate and the average was subsequently used in all further analyses. More information about the sTREM2 and inflammatory protein assays, including quality assurance measures, is available on the ADNI website (http://adni.loni.usc.edu/).

Pathway enrichment analysis

For inflammatory proteins showing a statistically significant correlation, we conducted a pathway enrichment analysis using gene-sets derived from the Gene Ontology biological process (GO:BP) annotation database (http://geneontology.org/). We considered only gene-sets with a minimum of three and a maximum of 500 genes. The Benjamini-Hochberg-False-Discovery-Rate method was applied to control for multiple testing. Significance threshold was set at a $\alpha \leq 0.005$. We then visualized the statistically significant enriched gene-sets (GO terms depth level = 1) and their overlap in Cytoscape using the plugin Enrichment Map (baderlab.org/Software/EnrichmentMap). The Jaccard and overlap combined coefficient threshold was set to 0.5.

Statistical analysis

The statistical analyses were performed in R (Version 3.5.1, The R Foundation for Statistical Computing) and IBM SPSS Statistics (Version 20, IBM, New York, USA). Statistical significance level for all tests was $\alpha = 0.05$ (two-sided). Boxplots were used to identify extreme values and measurements were excluded if they fell more than three times the interquartile range above the third quartile or below the first quartile (TNRF2: N = 2; TGF-β1: N = 3; TGF-β2: N = 3; TGF-β3: N = 28; IL-21: N = 1; IL-6: N = 14; IL-7: N = 5; IL-9: N = 1; IL-10: N = 2; TNF-α: N = 2; IL-12P40: N = 2; ICAM1: N = 9; VCAM1: N = 4; ttau: N = 2; ptau: N = 2). Normal distribution was tested using visual histogram inspection and Kolmogorov-Smirnov test for each biomarker per group. Variables with significant Kolmogorov-Smirnov test ($p < 0.05$) were log10-transformed before applying subsequent statistical tests. Group comparisons were performed using paired one-way ANOVA or chi square test with Bonferroni-corrected *post-hoc* tests, as appropriate. Partial correlations (as described in [23]) were calculated to test for associations between different proteins, adjusting for age, sex, APOE, and ADNI_mem. All correlation results were Bonferroni corrected for multiple comparisons.

RESULTS

Differences between ATN groups

Most of the inflammatory proteins (TNFR1, TNFR2, TGF-β1, VACM1) followed a similar pattern of changes, with a slight concentration decrease compared to healthy controls in Aβ positive but aggregated tau and neurodegeneration negative individuals (A+TN-), intermediate levels in Aβ and tau/neurodegeneration positive participants (A+TN+), and highest levels in SNAP individuals (Table 2 and Fig. 1). A similar pattern, without the initial decrease in A+TN- was observed for ICAM1. For the remaining markers (TGF-β2, IL9, TNF-α, IP-10), the highest concentrations were also found in SNAP, but without any other significant differences between the groups. As shown previously [6], sTREM con-

Table 2
CSF inflammatory protein concentrations per ATN group

Variable	A-T-N-	A+TN-	A+TN+	SNAP	*p*
TNFR1	828.64 (178.96)	708.16 (154.02)	900.37 (245.46)	1144.10 (294.53)	<0.001*
TNFR2	956.45 (209.54)	856.08 (182.35)	1090.68 (286.02)	1314.92 (310.89)	<0.001*
TGF-β1	100.78 (29.44)	83.26 (23.26)	110.64 (36.94)	118.75 (42.39)	<0.001*
TGF-β2	159.41 (42.17)	158.57 (40.07)	159.62 (40.36)	137.91 (56.34)	<0.01*
TGF-β3	2.82 (0.54)	2.75 (0.42)	2.83 (0.51)	2.78 (0.55)	0.88
IL-21	10.43 (10.28)	13.87 (14.66)	10.73 (10.69)	16.06 (17.83)	0.97
IL-6	4.35 (2.11)	4.36 (2.05)	4.20 (2.07)	4.27 (1.40)	0.88
IL-7	1.05 (0.79)	0.97 (0.70)	1.20 (0.83)	1.22 (0.68)	0.25
IL-9	3.30 (1.47)	2.88 (1.49)	3.45 (1.71)	4.99 (1.83)	<0.01*
IL-10	5.92 (2.81)	5.46 (2.64)	5.43 (2.08)	6.37 (2.25)	0.23
TNF-α	1.66 (0.44)	1.57 (0.49)	1.74 (0.50)	2.11 (0.60)	<0.01*
IP-10	5410.36 (1771.52)	5790.53 (2217.51)	4948.05 (2217.51)	6476.88 (1887.34)	<0.01*
IL-12P40	1.39 (1.08)	1.44 (1.03)	1.20 (0.83)	1.52 (1.08)	0.99
ICAM1	314.18 (130.09)	327.32 (159.82)	368.48 (155.40)	461.96 (153.93)	<0.001*
VCAM1	40507.68 (17728.62)	31379.33 (11271.59)	42536.53 (20641.60)	58584.71 (20353.40)	<0.001*

Mean concentrations of CSF inflammatory proteins (SD) and results of one-way ANOVA group comparisons; *significant ($p < 0.05$) after Bonferroni correction for multiple comparisons.

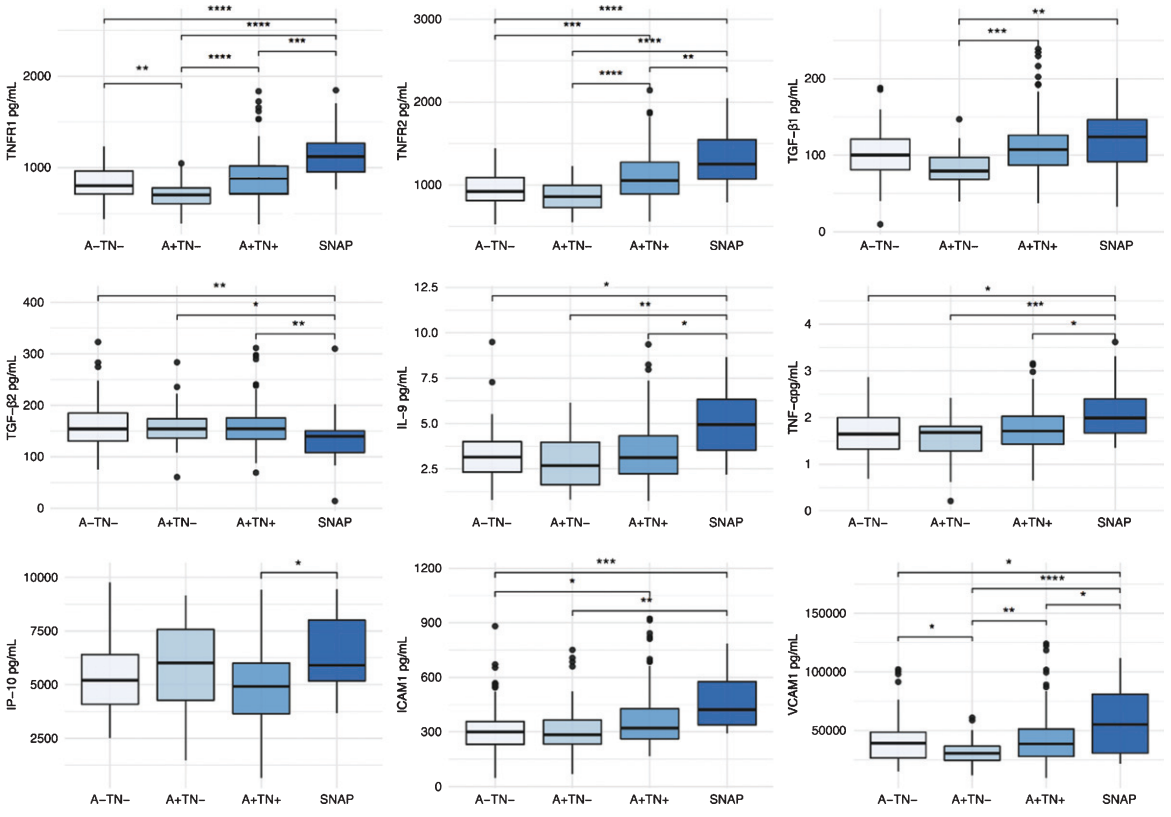

Fig. 1. Boxplots of CSF inflammatory protein concentrations per ATN group. Only biomarker showing significant ANOVA results are presented. An asterisk represents significant Bonferroni corrected group differences in *post-hoc* tests with *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.

Table 3
Associations between CSF inflammatory proteins and sTREM2

Variable	A-T-N-	A+TN-	A+TN+	SNAP	All participants
TNFR1	0.638**	0.533*	0.650**	0.277	0.629**
TNFR2	0.635**	0.740**	0.637**	0.464	0.634**
TGF-β1	–0.078	–0.040	0.471**	0.276	0.244*
TGF-β2	–0.260	–0.112	0.201	0.132	–0.015
TGF-β3	0.001	–0.060	0.119	–0.222	0.053
IL-21	–0.131	–0.396	–0.039	–0.940**	–0.136
IL-6	0.096	0.149	0.052	–0.645	0.050
IL-7	–0.069	0.174	0.040	–0.893*	0.005
IL-9	0.397*	0.490	0.403*	–0.257	0.401**
IL-10	0.343*	0.328	0.242	0.406	0.284**
TNF-α	0.226	0.293	0.288*	–0.345	0.256**
IP-10	0.371	–0.060	0.314*	–0.087	0.257*
IL-12P40	–0.042	–0.016	0.171	–1.000**	0.024
ICAM1	0.304	0.637*	0.152	0.146	0.291**
VCAM1	0.332*	0.302	0.514*	0.590	0.459**

Results of the partial correlation of CSF inflammatory proteins with sTREM2 controlled for *APOE*, age, sex and ADNI_mem score, all results are Bonferroni corrected for multiple testing. Significance: $^{*}p < 0.05$; $^{**}p < 0.001$.

centrations were higher in tau and neurodegeneration positive Aβ groups, and highest in SNAP.

Associations between CSF inflammatory proteins and sTREM2

To assess associations of CSF inflammatory proteins with sTREM2, partial correlation analyses were performed. In the entire study cohort, sTREM2 was significantly correlated with TNFR1, TFNR2, TGF-β, IL-9, IL-10, IP-10, and VCAM1; in A+TN- with TNFR1, TNFR2, IL-9, and VCAM1; in A+TN+ with TNFR1, TNFR2, TGF-β1, IL-9, TNF-α, IP-10, and VCAM1; in SNAP with IL-21, IL-7, and IL-12IP40; and in healthy controls with TNFR1, TNFR2, IL-9, IL-10, and VCAM1. The full correlation analysis results are shown in Table 3 and depicted in Figs. 2 and 3.

Pathway enrichment analysis

For proteins significantly correlated with TREM2, we identified a total of 80 enriched gene-sets in GO:BP. For the anti-inflammatory proteins TGF-β1, IL-9, and IL-10, we found 50 enriched gene-sets among which, those related to immunoglobulin production were the most strongly enriched ($p < 0.001$). We found 30 significantly enriched gene-sets for the pro-inflammatory proteins ICAM1, VCAM1, IP-10, and Il-21. Here, *membrane to membrane docking* ($p < 0.001$), *leukocyte cell-cell adhesion* ($p < 0.01$), *leucocyte migration* ($p < 0.01$), and *T-cell activation* ($p < 0.01$) were among the most enriched pathways. The complete list of enriched gene-sets can be found in Supplementary Tables 1 and 2.

Enriched pathways for TREM2 were visualized as interaction networks in Cytoscape using Enrichment Map (Fig. 4, Supplementary Figure 1 for node labels). We grouped each of these pathways into manually defined functional clusters (list of pathways per functional clusters can be found in Supplementary Table 3). The clusters related to immunoglobulin and cytokine production and cellular response to stimuli such as lipopolysaccharide, cytokine, or steroid hormone were the most represented. There was a high overlap among gene sets of the *B cell regulation and activation, immuglobulin production, and cellular response to stimuli* clusters. Some clusters contained gene sets only enriched for anti-inflammatory proteins such as *B cell regulation and activation and immuglobulin production.* In contrast, clusters such as *membrane docking* and *cell killing* contained gene sets only enriched for pro-inflammatory proteins.

DISCUSSION

A growing body of evidence suggests that neuroinflammation plays a crucial role in AD. Various markers seem to differ between patients and controls, but the results are often inconsistent (or even contradictory) due to heterogeneity introduced by the use of clinical (rather than biomarker-based) diagnoses and an insufficient separation of different disease categories. Given the crucial role of inflammatory processes in AD pathogenesis, a better understanding of the interrelations between the different inflammatory makers is important.

We explored the associations between a panel of established CSF inflammatory proteins and the more recently highlighted microglial activation marker sTREM2. This new biomarker is believed to play a crucial role in neuroinflammation in the context of neurodegeneration, but its neuroprotective versus neurodegenerative role is not yet well delineated. To determine how the CSF concentrations of the studied inflammatory proteins differed between individuals across different AD categories, we applied a biomarker-based stratification, recently proposed in the NIA-AA research framework. This approach allowed us to explore inflammatory responses in relation to the underlying presence of AD pathophysiology.

The three main findings of our study are: 1) several inflammatory proteins show increased CSF

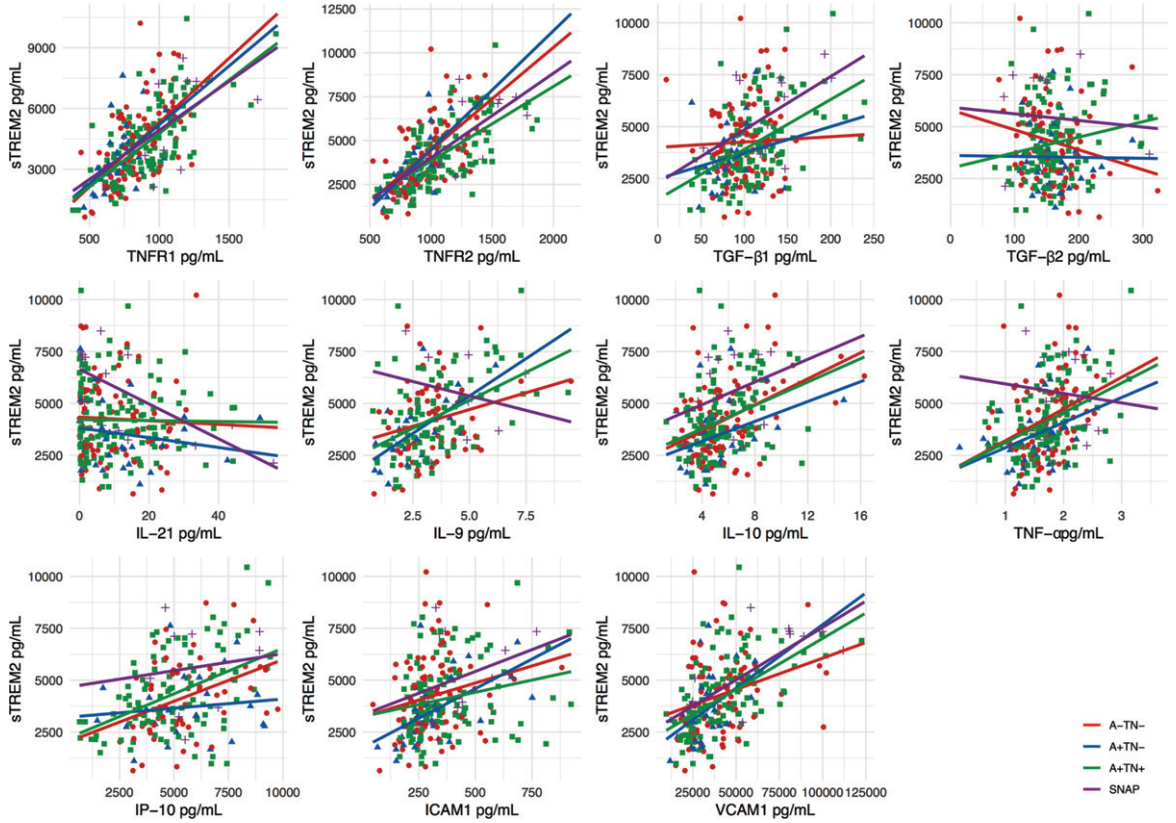

Fig. 2. Correlations of CSF inflammatory proteins with sTREM2 per ATN group.

concentrations in late versus early AD categories and controls, i.e., a strong dependence on tau pathology and neurodegeneration; most of these proteins have anti-inflammatory functions or are part of the TNF-α pathway. Based on their general pattern of change, we can divide the studied inflammatory proteins into three different categories, the first of which includes markers largely remaining unchanged as AD progresses, whereas the concentration of markers in the second category increases as tau pathology and neurodegeneration become relevant; the third group of markers includes proteins being increased in individuals with tau and/or neurodegeneration but no apparent fibrillar Aβ pathology (i.e., SNAP). 2) All TNF-α pathway associated CSF inflammatory proteins and a few proteins with anti-inflammatory functions (e.g., TGF-β, IL-9, and IL-10) show strong associations with sTREM2 (i.e., a marker related to microglial activation). 3) Pathway enrichment analyses for proteins associated with sTREM2 indicates that clusters related to immunoglobulin and cytokine production and cellular response to lipopolysaccharides, cytokines or steroid hormones play a central role.

Although a recent study failed to show associations between sTREM2 and peripheral markers of inflammation in CSF [24], more sophisticated analyses of associations with pro- and anti-inflammatory proteins and their receptors and effectors have so far not been conducted in humans. Here we report positive correlations of CSF sTREM2 with TNFR1 and 2, TNF-α and its effectors ICAM1 and VCAM1 when considering the entire cohort, which suggests an interrelation between TNF-α mediated inflammatory pathways with sTREM2. With exception of ICAM1, this association was also present in the most severely affected AD subgroup (A+TN+) and to a lesser degree in A+TN- and controls. SNAP did not show any association between sTREM2 and components of the TNF-α pathways suggesting a different underlying pathomechanism in this potential non-AD group. TNF-α is one of the best described AD-related inflammatory CSF biomarkers; however, its association with AD pathology remains ambiguous with studies show-

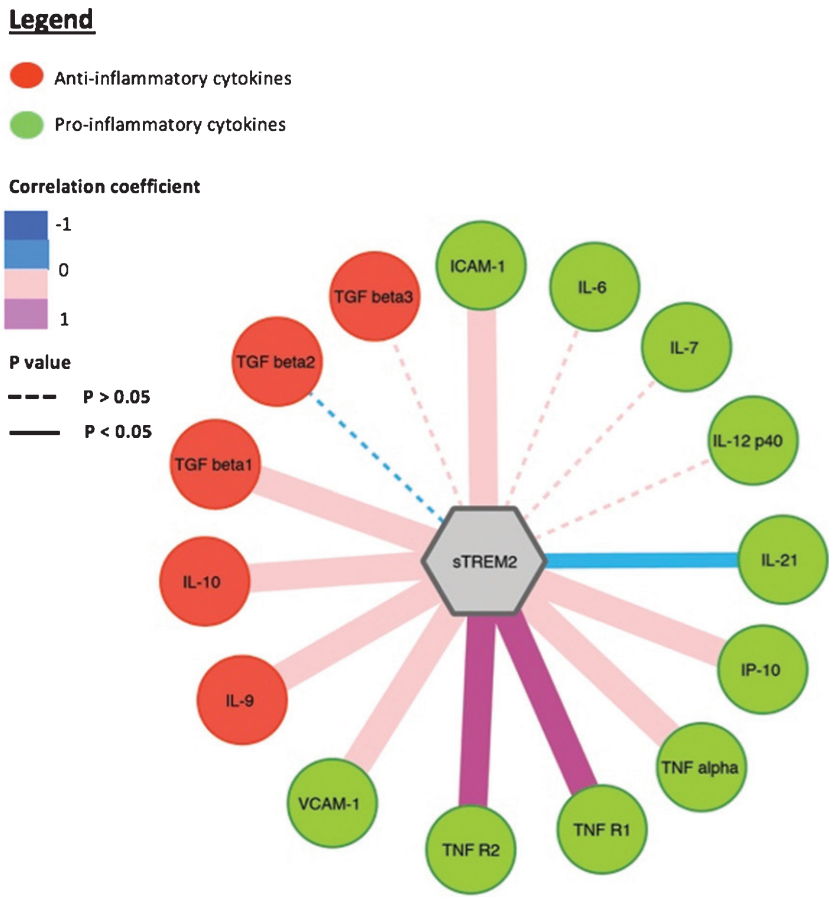

Fig. 3. Circular network graphs showing the correlations between inflammatory proteins and sTREM2. The correlation between sTREM2 with the anti- and pro-inflammatory cytokines is illustrated by the color of the edge (blue color scale = negatively correlated and pink color scale = positively correlated). Dashed edges show statistically non-significant correlation. The edge's width shows the statistical significance of the correlation (the wider the link the more statistically significant is the correlation). Nodes represent the cytokines where green corresponds to the pro-inflammatory proteins, red to the anti-inflammatory proteins and grey to sTREM2.

ing conflicting results [25]. The inhibitory TNF-α receptors soluble TNFR1 and 2 and the downstream TNF-α effectors ICAM1 and VCAM1 showed AD category dependent significant concentration differences in our study, implying a positive association with tau and neurodegeneration, with decreased concentrations in earlier disease categories when only Aβ deposition is present.

Differences in TH7 pathways linked to T-cell activation have been described previously [16]. T-cell activation dependent groups of inflammatory proteins were analyzed here, considering TH1, TH2, and TH17 (T-helper cell) responses in different AD categories. For the TH1 and TH2-pathway responses, we revealed no significant alterations in relation to the underlying AD pathophysiology, and no significant group differences were found for IL-10, IL-7, and IL-12p40. However, group comparisons showed significant alterations in inflammatory protein concentrations associated with T-helper cell activation. TGF-β1 and 2 have been demonstrated to play an essential role in TH17 T-helper cell differentiation [26]. We found concentration differences in TGF-β1 and 2 between early and later AD categories suggesting a strong underlying association with tau pathology and neurodegeneration. IL-9, an effector cytokine produced by TH9 lymphocytes, was significantly increased in SNAP, suggesting that along with alterations in TH17, TH9 lymphocyte-related pathways may also be upregulated in SNAP phenotypes. In contrast, we found the interferon gamma induced IP-10 increased in AD categories, in which mainly Aβ pathology is present, but reduced to normal levels in more severe disease categories with elevated CSF tau and neurodegeneration present. This result suggests that IP-10 is an early inflammatory marker

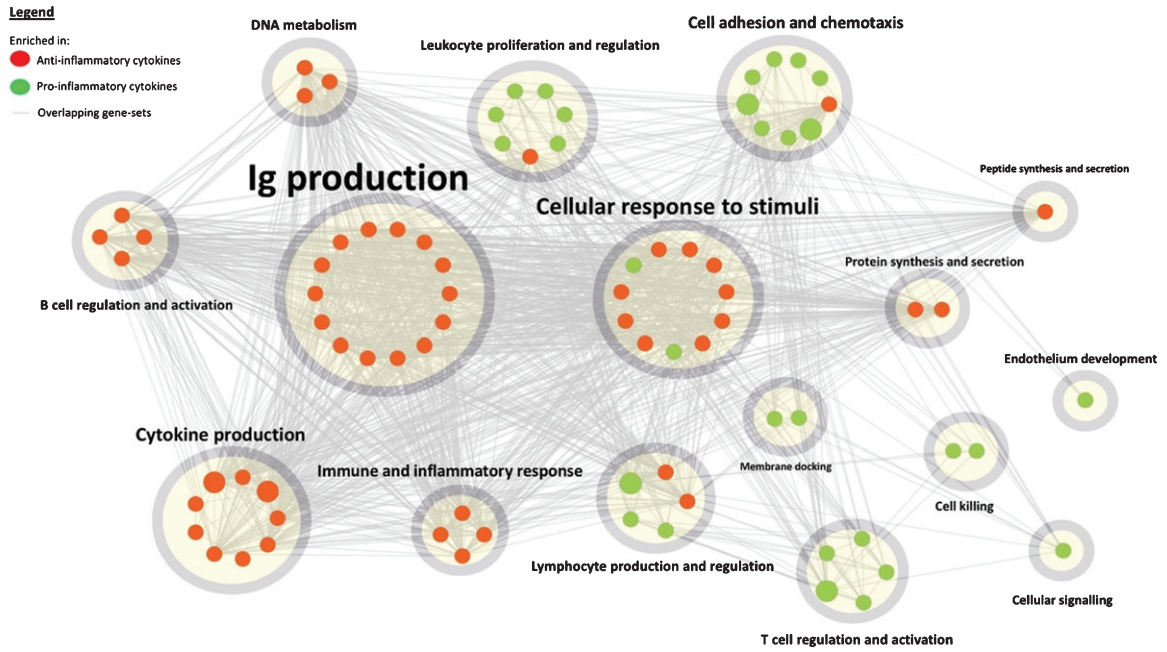

Fig. 4. Network map illustrating the significantly enriched pathways in GO:BP for the anti- and pro-inflammatory proteins with significant correlation with sTREM2. Nodes represent enriched pathways for each protein group (anti-inflammatory proteins in red, pro-inflammatory proteins in green and both groups in blue). The node's size is proportional to the number of gene-sets. The individual node's labels have been removed for clarity and can be found in the Supplementary Figure 1. Edges between the nodes illustrate the overlap between the gene-sets. The edge's width shows the amount of similarity between two connected gene-sets. Clusters were manually identified and labelled according to the parent group in which the biological pathways belong. $p \leq 0.005$ and FDR = 5%. Ig, immunoglobulin.

in disease progression, a feature already described in a previous investigation [21].

The results of our study are in line with recent findings in animal models and cell-culture showing that TREM2 leads to activation of a pro-inflammatory response, including IL-1β, IL-6, TNF, and IL-10 [27]. A possible TREM2 mediated pro-inflammatory TNF-α response might induce ICAM1 and VCAM1 expression as a consequence of inflammatory triggers ultimately leading to alterations of the BBB [28] and promotion of neurodegeneration in AD [29]. Previous studies suggest that the TREM2 activated pro-inflammatory response also leads to vascular endothelial dysfunction with consecutive BBB alterations known to play a crucial role in AD [19, 20]. The interplay between neuroinflammation and vascular dysfunction and the underlying molecular mechanism should be assessed in future studies.

Interestingly, TGF-β1, a trophic factor with neuroprotective functions, suppressing cytotoxic microglial activation [30] shows significant correlations with sTREM2 only in the most advanced AD category. Correlations with IL-10, a cytokine with predominant negative autocrine functions in microglia [31], are only found to be correlated with sTREM2 in controls. IP-10, a biomarker known to be associated with tau pathology [32], was correlated with sTREM2 only in the A+TN+subgroup.

To better understand the underlying functional pathobiology, we performed a pathway enrichment analysis of the inflammatory proteins significantly correlated with sTREM2. Pathways enriched for genes coding anti-inflammatory proteins were related to immune and inflammatory responses to stimuli as well as cytokine production, confirming findings from previous studies highlighting the ability of these proteins to regulate inflammatory processes in AD [33]. The biological meaning of the enriched pathways related to the activation and regulation of B cells and the production of immunoglobulins in the context of AD is not yet clear because evidence for the role of B cells in AD is sparse; however, a recent study showed that B cells are able to stimulate the formation of Aβ plaques in a cellular model [34].

In our study, pro-inflammatory proteins significantly correlated with sTREM2 enriched pathways related to neuronal loss, cell adhesion, and chemo-

taxis. Microglial migration to sites of Aβ deposits is an essential step in the initiation of inflammatory processes eventually resulting in neurodegeneration [35]. The presence of pathways related to cell death supports the results obtained in a study on AD brain tissue showing patches of ICAM1 in senile plaques [36]. The enrichment of the endothelium development pathway is in line with growing evidence from the literature suggesting that endothelial dysfunction participates in the development of AD [37]. Interestingly, TREM2 has recently been associated with interactions between endothelial cells and microglia in an AD mouse model [38].

A few limitations of the study must be acknowledged. The ADNI cohort is mainly white, educated, middle class and without any major comorbidities, thus, it would be important to repeat such a study with a more widely represented, larger demographic also considering relevant comorbid conditions (e.g., vascular disease). Furthermore, the standardization of biomarker cut-offs is still limited, and the results therefore often vary between the different laboratories; however, the current values have been used in several ADNI papers, and they appear to show reasonably good validity for the purposes of our study. Furthermore, our analyses only consider cross-sectional biomarker measurements and longitudinal analyses are warranted. While we studied a number of different inflammatory proteins associated with AD, other relevant markers where not addressed in this study; this includes YKL-40, consistently increased in AD cases versus controls and associated with CSF Aβ and tau levels [39–41]. There was no histopathological verification of the clinical diagnoses, but the ADNI cohort is enriched on purpose with probable pre-dementia AD cases, evidenced by the first published autopsy reports [42]. Finally, there is some missing data, especially for the marker TGF-β, which limits the number of included cases for certain correlations.

To conclude, our work provides further intriguing evidence in support of sTREM2 in CSF as a marker of neuroinflammation across the spectrum of early clinical AD, being linked statistically and biologically to established markers of neuroinflammation related to AD. Moving forward, future studies should use biomarker information to further categorize clinical AD category (e.g., MCI) and to explore the biological mechanisms underlying the dynamic relations between sTREM2 and other markers of inflammation relevant in the context of neurodegenerative processes. The data presented in this associative investigation could lay a foundation for future mechanistic studies. The recent finding that higher sTREM2 levels in CSF are potentially associated with reduced cognitive deterioration and hippocampal atrophy over time in AD may have implications for future clinical trials targeting the innate immune response to neurodegeneration [43]. This finding should also be explored and replicated in independent datasets.

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SUPPLEMENTARY MATERIAL

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