**CSF sTREM2 and Tau Work Together in Predicting Increased Temporal Lobe Atrophy in Older Adults**

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Submission Type: **Article**
Character count title: **91**
Word count Abstract:
Word count Introduction:
Word count text:
Number of references:
Number of tables:
Number of figures:
Nonstandard characters list:
Search terms**:** [36] cognitive ageing, [26] Alzheimer's disease, [120] MRI, [131] all immunology.

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**Financial disclosure statement**
The study was funded by the Norwegian Health Association, the South-Eastern Norway Regional Health Authorities, the Medical Student Research Program of Norway and the Olav Thon Foundation. The sponsors had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; and preparation, review, or approval of the manuscript.
N.B. Halaas, K. Henjum, S. Dakhil, A-V. Idland, L.N.G. Nilsson, D. Sederevicius, D. Vidal-Piñeiro, K. Walhovd, T.B. Wyller, L.O. Watne and A.M. Fjell report no disclosures. H. Zetterberg has served at scientific advisory boards for Roche Diagnostics, Wave, Samumed and CogRx, has given lectures sponsored by Alzecure and is a co-founder of Brain Biomarker Solutions in Gothenburg AB, a GU Ventures-based platform company at the University of Gothenburg (all unrelated to the submitted work).

## ABSTRACT:

*Objective:* To test the association between neuroinflammation, as indexed by soluble Triggering Receptor Expressed on Myeloid Cells 2 (sTREM2) concentrations in CSF, and brain atrophy in cognitively normal older adults with different Alzheimer's disease (AD) biomarker profiles.
*Methods:* Brain MRIs and CSF sTREM2, total tau (t-tau), phosphorylated tau (p-tau) and Aβ42 were analyzed in 115 cognitively normal older adults. MRIs were repeated after two (n= 95) and four (n= 62) years. CSF sampling was repeated after four years in 14 participants. Participants were classified according to the National Institute on Ageing and Alzheimer's Association (NIA-AA) framework, applying CSF measures (Aβ42 and p-tau) for A/T-classification. Virtual histology was performed based on the Allen Human Brain Atlas of gene expression.

*Results:* High baseline sTREM2 was associated with accelerated cortical thinning in the temporal cortex of the left hemisphere, as well as hippocampal atrophy bilaterally, independently of age and AD biomarkers. The relationship between sTREM2 and atrophy was significantly stronger in participants with a high level of p-tau (T+) compared to participants with normal p-tau or with amyloidosis (A+). sTREM2-related atrophy did generally not increase with biomarker positivity across the AD continuum (A-T- → A+T- → A+T+). Areas with sTREM2-related cortical thinning corresponded with areas of high microglial gene expression in virtual histology analyses.
*Conclusion*: Increased sTREM2 was associated with accelerated cortical and hippocampal atrophy in cognitively normal older participants. The findings suggest a link between neuroinflammation, neurodegeneration and amyloid-independent tauopathy.

## INTRODUCTION

Enhanced microglial responses exacerbate and contribute to the development of neurodegenerative disease (NDD) [1-5]. Triggering Receptor Expressed on Myeloid Cells 2 (TREM2) is a transmembrane receptor protein expressed predominantly by microglia in the CNS. A soluble fragment of TREM2 (sTREM2) is cleaved from the membrane-bound protein and released to CSF[2, 6]. Loss-of-function mutations in *TREM2* enhance the risk of NDDs, including Alzheimer's disease (AD)[2, 7, 8]. The effects of TREM2 expression on NDD are complex, probably depending on both microglial activation state and existing pathology at different disease stages [2, 8].

Frontotemporal cortices and hippocampi are commonly affected by atrophy in ageing[9]. The pathophysiological mechanisms of age-related neurodegeneration remain poorly understood. The association between sTREM2 and structural changes in the ageing brain has been explored in two studies. In a cross-sectional study, elevated CSF sTREM2 was related to increased gray matter volume and reduced diffusivity in the temporoparietal cortices and precuneus in mild cognitive impairment, suggesting brain swelling in relation to neuroinflammation[10]. In a longitudinal study, no association was found between high versus low baseline CSF sTREM2 and hippocampal atrophy along the AD continuum[11].

The National Institute on Ageing and Alzheimer' Association (NIA-AA) recently published a biomarker-based research classification of AD-related neuropathological changes [12]. Here we explored whether sTREM2 expression was associated with increased rates of age-related brain atrophy by relating CSF sTREM2 to cortical thinning and hippocampal volume loss in cognitively normal older adults classified according to the NIA-AA guidelines.

## METHODS

### *Sample*

172 patients undergoing elective gynecological (genital prolapse), urological (benign prostate hyperplasia, prostate cancer, or bladder tumor/cancer) and orthopedic (knee or hip replacement) surgery in spinal anesthesia, aged 65 years or older the year of inclusion, were

recruited to the COGNORM-study from 2012-2013 at Oslo University Hospital and Diakonhjemmet Hospital, Oslo, as previously described [13]. Dementia, previous stroke with sequelae, Parkinson's disease and other acknowledged or suspected brain disease likely to influence cognition were exclusion criteria. Participants were evaluated with a comprehensive battery of cognitive tests prior to surgery, comprising the Mini Mental Status Examination (MMSE) [14], Clock Drawing Test [15], Word List Memory Task [16], Trail Making Test A and B [17], Kendrick Object Learning Test [18] and verbal fluency (The Controlled Word Association Test, with the letters F, A and S, and Animal Naming) [19], yielding 11 test scores. Cognitive testing was repeated yearly, with a comparable test battery. To select cognitively normal participants at baseline, we excluded patients with 1) an MMSE score <28 and more than one other test with a score more than 1.5 SD below the mean normal value for age, sex, and educational level (n=3) and 2) suspected undiagnosed dementia with referral to a memory clinic at baseline (based on test scores and clinical data, n=6).
CSF at baseline was collected in 155 participants at the onset of anesthesia prior to administration of the anesthetic agent. 14 participants underwent a second lumbar puncture with CSF sampling after a mean of 4.5 years. Magnetic resonance imaging (MRI) was performed at baseline after surgery in 128 participants, with a mean time between CSF collection and MRI acquisition of 60 days. MRI were repeated twice with a mean follow up time of 2.1 (1.7-2.8 years, SD = 0.22) and 4.3 (3.9-4.7, SD = 0.23) years.
The final sample consisted of 115 cognitively normal participants with sTREM2 and MRIs at baseline, 95 with two MRIs and 62 with three MRIs. Of these, two participants lacked tau and Aβ42 CSF measures (see below).

***CSF Sampling and biochemical analyses***
CSF was collected in polypropylene tubes, centrifuged, aliquoted and stored at – 80° C, as described elsewhere[13]. Samples for sTREM2 and AD biomarker analysis were sent on dry ice to the laboratories without information about clinical data (masked data). CSF AD biomarkers, CSF Aβ42, phosphorylated tau (p-tau) and total tau (t-tau), were determined using INNOTEST enzyme-linked immunosorbent assays (ELISA; Fujirebio, Ghent, Belgium) at Sahlgrenska University Hospital (Mölndal, Sweden) by board-certified laboratory technicians, as previously described[13]. CSF sTREM2 was assayed by a sandwich ELISA at Oslo University Hospital, as previously described [20].

***Biomarker profiling: The AT-framework***
Participants were classified according to the AT-framework [21] by applying CSF measures with the following cut-off values: A+ <Aβ42 530 pg/ml <A- and T+ > p-tau 60 pg/ml> T- as established for the laboratory [22]. 30 participants were A+ and 83 A-, 48 T+ and 65 T-. Along the proposed AD spectrum, 50 had the A-T- biomarker profile ("normal AD biomarkers"), 15 had A+T- ("AD pathological change") and 15 had A+T+ ("AD"). In addition, 33 corresponded to the A-T+ profile ("Non-AD pathological change").

***MRI acquisition and processing***
T1-weighted MPRAGE 3D images were acquired with a 1.5 T Siemens Avanto scanner using a 12-channel head coil (TR=2400 ms, TE=3.79ms, Field of View=240mm, slice

thickness=1.20mm, pixel size=1.25x1.25mm). Images were processed with the longitudinal stream in FreeSurfer 6.0 (FS) (https://surfer.nmr.mgh.harvard.edu), described elsewhere [23-26], generating maps of cortical thickness and hippocampal volume. Since FS is an almost fully automated processing tool, manual editing was not performed to avoid introducing errors. Maps were smoothed using a circularly symmetric Gaussian kernel with a full width at half maximum of 15 mm [27] before being entered into statistical analyses.

***Virtual histology***

We used a "virtual histology" approach, described elsewhere[28, 29], to test how anatomical differences in TREM2-related cortical thinning related to inter-regional gene expression profiles associated with specific cell types, estimated ex-vivo [30]. The analysis provides information on the specific types of cells possibly involved in TREM2-related thinning and thus facilitates a neurobiological interpretation. Gene-expression data were obtained in ex-vivo brains from the Allen Human Brain Atlas (Allen Institute for Brain Science; http://www.brain-map.org [30]), matched to the 34 regions of the Desikan/Killiany Atlas [31] by use of MNI152 coordinates.

For each gene, the consistency of the inter-regional expression profile was evaluated with the mean Spearman correlation between each of the donor's profiles and the median profile of that gene. In addition, we obtained gene expression data from the BrainSpan atlas (www.brainspan.org). We selected data from 9 donors (age > 12) across 11 cortical regions homologous to the Desikan/Killiany Atlas. Next, a 2-step procedure was applied to remove genes with inconsistent regional expression profiles. First, we selected genes showing consistent expression profiles across the 34 regions in the 6 donors included in the Allen Human Brain Atlas (i.e., donor-to-median correlation rho > 0.446 [28]). Second, we compared the (mean) profiles of gene expression between the Allen and the BrainSpan atlases across the 11 homologous cortical regions. We used a critical threshold of r=0.52 between both profiles, corresponding to one-side $p<0.05$). From the 20737 genes profiled in the Allen Human Brain Atlas, a panel of 2511 genes was retained as showing consistent gene-expression regional profiles.

A list of genes expressed in specific cell types was obtained from Zeisel et al [32]. The list of cell-specific genes was intersected with the panel of genes with consistent profiles (n=2511) after which the following number of genes per cell-type remains: S1 pyramidal neurons (n=73), CA1 pyramidal neurons (n=103), interneurons (n=100), astrocytes (n=54), microglia (n=48), oligodendrocytes (n=60), ependymal (n=84), endothelial (n=57), and mural (n=25).

***Statistical analysis***

Spatiotemporal linear mixed effects (ST-LME) models were run to test the relationship between cortical thickness change and baseline levels of sTREM2 by a matlab add-on to FreeSurfer [33, 34]. Thickness at each vertex and time point was used as dependent variable, with random intercept, and time from baseline, sTREM2 and the time × sTREM2 interaction as predictors of interest, with age and sex as nuisance covariates in all analyses. For volumetric analyses (hippocampus), intracranial volume was used as an additional covariate. All continuous predictor variables were z-transformed before being entered in the models. Separate models were run with Aβ42, Aβ42 × sTREM2, total-tau, total-tau × sTREM2, and

Aβ42 and total-tau, as additional covariates. Surface results were tested against an empirical null distribution of maximum cluster size across 10 000 iterations using Z Monte Carlo simulations, synthesized with a cluster-forming threshold of $p < 0.01$ (two-sided), yielding results corrected for multiple comparisons across space. Thickness values for each participant and time point from the clusters surviving statistical correction were extracted and used for post-hoc analyses.

Post hoc analyses included generalized additive mixed models (GAMM) with the same variables as above, to visualize change trajectories and test interactions, run in R (https://www.r-project.org*)* using Rstudio *(*www.rstudio.com) IDE with the package "mgcv" [35]. GAMMs were also run to test relationship between sTREM2 and hippocampal volume and memory performance over time. To test whether the participants' biomarker profile affected sTREM2-related atrophy, GAMMs with sTREM2×time by biomarker-profile as an additional 3-way tensor interaction were run, including also all main effects and lower order interactions in the same models.

To test the association between sTREM2-related thinning and the cell-type expression profiles, the vertex-wise parameter estimates (Beta-coefficients) of the left cortical surface from the sTREM2 - cortical thinning analysis were summarized into the 34 regions of interest (ROIs) and multiplied by -1 so that positive values corresponded to more atrophy with higher sTREM2. A Pearson's correlation was performed between the Beta-coefficients and the profile of gene expression for each marker gene across the regions. We used the average expression-thinning correlation for each panel of genes (each cell-type) under the assumption that if a cell-type underlies steeper thinning with high sTREM2 levels, the mean of the expression-thinning correlation for the genes in the cell-type panel will differ from that of a random set of genes [29]. For each cell-type panel, we obtained the empirical null distribution of the test statistic by iteratively (n=10.000) selecting a random number corresponding to the number of genes included in the cell-type panel, calculating their expression-thinning correlation coefficients and mean average value. Based on the empirical null distribution, we obtained two-sided p-values for each cell-type panel, which was further False Discovery Rate (FDR)-adjusted for multiple testing (n=9 cell-type panels). For visualization, we used density estimation to calculate the empirical distributions of the expression-thinning correlations for each of the 9 cell-types, the empirical average of these correlations as well as the 2.5% and 97.5% critical values of the empirical null distributions. An overview of R scripts and data files is available at the repository.

***Standard protocol approvals, registrations and patient consents***

The study was conducted in accordance with the Declaration of Helsinki and approved by the Regional Committee for Ethics in Medical Research in Norway (REK, 2011/2052). All participants provided written informed consent.

***Data availability statement***

Data are available upon reasonable request to the authors, given adherence to ethical protocols.

**RESULTS**

*Population demographics*

Age correlated positively with increased sTREM2 levels both in cross-sectional and longitudinal analysis (mean annual increase of sTREM2 of approximately 10 %). Population demographics are described in Table 1.

*[Insert table 1 about here]*

*Relationship between sTREM2 and brain atrophy*

Higher levels of sTREM2 were related to increased rates of cortical thinning in three clusters located in the lateral and inferior left hemisphere temporal cortex, covering 1578, 830 and 753 $mm^2$ (Figure 1A).

*[Insert Figure 1 about here]*

Post hoc GAMMs were run for each cluster and the time × sTREM2 interactions visualized by contour plots (Figure 2A). These revealed that the time × sTREM2 interaction on cortical thickness was driven by participants with high sTREM2 levels, especially those 1 SD or more above the mean. Thus, for illustrative purposes we dichotomized the sample by $Z \geq 1$ (32 observations) vs. $Z < 1$ (240 observations), and estimated change-slopes in each group (see Figure 2B-C). As can be seen, high sTREM2 participants showed highly significant (all p's < 0.0005) close to linear thinning in all 3 clusters. In contrast, despite higher power, participants with a normal sTREM2 level showed marginally significant thinning in cluster 2 only ($p = 0.04$).

*[Insert Figure 2 about here]*

Additional ST-LME's were run including Aβ42 and/ or total-tau as additional covariates. The effects of sTREM2 on thickness change were not reduced by regressing out Aβ42 levels (see Figure 1B). The total spatial extension of sTREM2 effects with Aβ42 regressed out was 3405 $mm^2$, compared to 3161 $mm^2$ in the original model where Aβ42 was not included. The Aβ42 × sTREM2 interaction term was not significantly related to thickness. The analyses were repeated with both Aβ42 and total-tau as covariates (Figure 1C). This influenced the spatial extension of the effects, reducing the significant effect area with 41.3% compared to the initial model. In a separate analysis, we found that the total-tau × sTREM2 interaction term was not significantly related to thickness. Post hoc analyses showed that sTREM2 correlated positively with p-tau ($\rho=0.51$, $p<0.001$) and t-tau ($\rho=0.52$, $p<0.001$), but not with Aβ42 ($\rho=0.11$, $p=0.25$). Participants with a high sTREM2 concentration (> 1 SD over mean) had a significantly higher concentration of p-tau and t-tau ($p<0.001$) than participants with a normal sTREM2 concentration.

Finally, GAMMs were run testing the effect of sTREM2 on hippocampal atrophy. For both left ($F = 3.97$, $p < 0.05$) and right ($F = 3.13$, $p < 0.02$) hippocampus, higher baseline levels of sTREM2 were related to higher atrophy rates (Figure 3). We performed post hoc tests

dividing participants in groups of "high" vs. "normal" sTREM2, based on either a mean split or above/ below 1SD over the mean. The group analyses revealed no significant interactions with interval on hippocampus volume (all p's > 0.34), demonstrating that dichotomizing sTREM2 yields reduced sensitivity, and that sTREM2 is best treated as a continuous variable.

*[Insert Figure 3 about here]*

*Biomarker profiling*

GAMMs were run to test whether the sTREM2-associated atrophy differed as a function of AT biomarker profile, with the following contrasts (sample size in parenthesis).

A+ vs. A-: For no regions was the sTREM2-related atrophy higher in the A+ group, while it was slightly higher in the A- group for Cluster 2 ($F = 3.57$, $p = 0.020$) and Cluster 3 ($F = 4.14$, $p = 0.043$).

T+ vs. T-: sTREM2-related atrophy was higher for all regions for the T+ group, and the difference was significant for Cluster 2 ($F = 7.78$, $p = 0.006$) and both hippocampi (left: $F = 7.40$, $p = 0.007$, right: $F = 7.44$, $p = 0.001$).

A+/T- vs. A-/T+: For all regions, sTREM2 was more strongly related to atrophy in the A-/T+ group, with the differences being significant for Cluster 2 ($F = 7.90$, $p = 0.006$) and right hippocampus ($F = 8.67$, $p = 0.002$).

A+/T+ vs. A-/T-: The sTREM2 × interval relationship differed between groups for left hippocampus only, where it was significantly stronger in A+/T+ (Left: $F = 6.83$, $p = 0.01$).

*Longitudinal sTREM2 analyses*

Longitudinal sTREM2 data were available for a smaller subset of participants (n = 14, mean interval 4.27 years, SD = 0.24). sTREM2 increased significantly (Baseline: 7.54, SD = 3.87; Follow-up 10.64, SD = 5.98, $t = 4.04$, $p = 0.001$) and correlated strongly between time points ($r=0.92$, $p<4.00E^{-06}$). Notably all samples displayed an increase. Change in sTREM2 also correlated with both baseline ($r = 0.56$, $p=0.035$) and follow-up ($r = 0.85$, $p=0.0001$) values. Acknowledging the small sample, tentative GAMMS were run, testing the relationship between sTREM2 changes (Follow-up – Baseline values) and hippocampal atrophy. For both left and right hippocampus, a significant sTREM2 change × time interaction was found (Left: $F = 21.61$, $p < 4.64e^{-05}$; Right: $F = 11.72$, $p<0.002$), showing more atrophy in participants with more increase in sTREM2.

Similar analyses were run for the three cortical clusters identified in Figure 1. We did not see any significant relationships between thinning and sTREM2 increases (all p's >0.10), likely due to low statistical power.

*Relationship between sTREM2 and memory change*

GAMMs were run with memory score as dependent variable, with 515 observations spread over 5 time-points, age as predictor, controlling for sex, with time point as an additional covariate to control for retest effects (Figure 4). Age had a linear negative effect on memory ($t=-4.7$, $p=3.46e-06$). Adding baseline sTREM2 as an additional predictor, there was no significant main effect of sTREM2 levels on memory score ($F = 1.91$, $p = 0.17$), nor a significant interaction between sTREM2 and interval ($F = 1.59$, $p = 0.21$).

*[Insert figure 4 about here]*

*Virtual histology*

The sTREM2-related cortical thinning profile was correlated with the expression profiles of cell-specific genes. The results are shown in Figure 5 and Table 2. The average correlation for the CA1-pyramidal neurons ($p< 0.001$) and microglia ($p=0.03$) cell-types significantly differed from the empirical null distributions (FDR-corrected <0.05). Both distributions were shifted towards positive coefficients, i.e. the cortical regions associated with steeper sTREM2-related cortical thinning showed higher expression of CA1-pyramidal and microglia-specific genes.

*[Insert Figure 5 and table 2 about here]*

## DISCUSSION

In this study we evaluated the association between CSF sTREM2 and longitudinal changes on structural MRI in cognitively normal older adults. We showed that 1) a high baseline level of sTREM2 was associated with accelerated cortical thinning in the lateral and inferior temporal cortex in the left hemisphere in areas with high microglial expression, 2) high baseline sTREM2 and greater longitudinal increases of sTREM2 were both associated with greater loss of hippocampal volume over time, 3) the association between sTREM2 and atrophy was significantly stronger and mainly present in participants with high levels of p-tau and 4) baseline sTREM2 was not related to cognitive decline, suggesting that any clinical detrimental effect of sTREM2-associated atrophy was not detectable by longitudinal memory testing.

*Increased CSF sTREM2 predicts cerebral atrophy*

High levels of sTREM2 were associated with accelerated cortical thinning and greater loss of hippocampal volume over time. Contrariwise, a recent study on participants along the AD-continuum, dichotomizing participants based on high or low sTREM2 levels, revealed no association between sTREM2 and longitudinal hippocampal volume loss over a four-year period[11]. In post hoc analyses, we found that a small group of participants with high levels of sTREM2 (>1 SD above the mean sTREM2) was revealed to account for most of the association between sTREM2 and cortical thinning, but not between sTREM2 and hippocampal volume. Our analyses show that important information is lost when sTREM2 is defined as a categorical variable, which may explain the divergent findings.

Microglial activation has traditionally been perceived as a secondary process to tissue damage/loss in healthy brains, firstly as a housekeeping function[3, 5]. However, the activities of microglia and particularly the TREM2 receptor are more complex than previously thought[2, 8]. A study in aged mice showed that phagocytosis of apoptotic neurons could induce subtypes of microglia with a reduced ability for homeostatic control and increased expression of neurodegeneration-related genes through APOE-TREM2-dependent pathways[36]. Also, a

recent study on tauopathy in mice reported that TREM2-deficiency led to less microglial activation and atrophy in the temporal and piriform cortices, without affecting tau deposition/levels[37]. This suggests that an initially beneficial activation of microglia may become detrimental following a phenotypic shift in aged or NDD-related microglia, leading to a TREM2-mediated exacerbation of atrophy directly or through secondary noxious effects. Microglial TREM2 expression differs between brain regions in older individuals and the areas we found most affected by sTREM2-associated cortical thinning were found to correspond with known areas of high microglial expression through virtual histology[38]. These areas are among the regions known to undergo accelerated thinning in cognitively normal older adults [9]. As mentioned, elevated CSF sTREM2 has been linked to findings suggestive of brain edema in mild cognitive impairment in temporal areas[10], indicating that sTREM2 may be involved in neuroinflammatory regulation in affected areas in early neurodegeneration. Moreover, high baseline levels of sTREM2 were associated with higher increases of sTREM2 over time, hypothetically indicating a phenotypical shift in microglial sTREM2-expression. Taken together these findings strengthen the plausibility of sTREM2-mediated atrophy. In likelihood, neurodegeneration and neuroinflammation have reciprocal effects, each having the capability to bring about the other at different stages of neurodegeneration, potentially perpetuating a vicious cycle of deterioration.

*sTREM2-related atrophy is associated with high levels of p-Tau*

Pathological changes in AD are known to arise decades before symptomatic onset of the disease[39], with increased CSF t-tau and p-tau reflecting neurodegeneration and neuroaxonal tangle formation[40]. Although the cascade of events leading to AD-pathological changes is still discussed, one hypothetical sequence of events promotes that amyloidosis, tangle formation and neurodegeneration occurs prior to microglial activation [4]. A biomarker-based research framework of AD has recently been proposed, featuring the A/T/N-classification[12]. Categorizing participants in A/T-groups, we found that biomarker positivity along the AD-continuum (A-T- → A+T- → A+T+) did not increase sTREM2-related atrophy. Specifically, there appeared to be an amyloid-independent association between microglial sTREM2 -expression and cortical thinning. Aβ-independent sTREM2 expression has been described in several studies, supporting these findings[11, 41]. While the association between sTREM2 and cortical thinning remained significant after correction for CSF t-tau, the spatial extent of the clusters was reduced by 41 % after correction for t-tau concentrations, denoting shared variance between t-tau, cortical thinning and sTREM2. CSF t-tau has been shown to correlate with hippocampal atrophy and grey matter degeneration on structural MRI[42]. A positive correlation between CSF t-/p-tau and sTREM2 levels has previously been demonstrated in cognitively normal older adults[20].

Considering that the association between sTREM2 and atrophy partly depended on concentrations of CSF p-tau, and the fact that participants with high sTREM2-values (> 1 SD over mean) had significantly higher concentrations of p-tau than participants with low sTREM2, one might postulate that prior neuronal injury associated with AD-related tangle formations in the brain may have initiated increased sTREM2 expression. At large, research

examining the pathophysiological relationship between (s)TREM2 and tau phosphorylation is scarce and the role of sTREM2 in tauopathy is still unsettled. A study evaluating the sequence of pathological changes in dominantly inherited AD, demonstrated that increases in CSF t-tau and p-tau were closely associated with, but prior to, increases in CSF sTREM2, supporting that tauopathy may stimulate microglial sTREM2 expression[43]. On the other hand, a study visualizing microglia in relation to neurofibrillary structures in healthy and neurodegenerative human brain tissue supported that senescent (dystrophic) microglia were associated with and probably preceded tau pathology[44]. Studies of tauopathy in mice are inconclusive, as some studies suggest a detrimental effect of TREM2-deficiency [45, 46], while others suggest that TREM2-deficiency may be protective[37, 47] of neurodegenerative changes and/or tau-pathology. The inconsistent findings suggest that (s)TREM2 may have a shifting role when NDDS progress, and tauopathy and microglial activation may have reciprocal effects. Our data establishes an association between neurodegeneration, tauopathy and sTREM2 expression in microglia, but do not permit us to determine causality. Increased knowledge concerning molecular mechanisms connecting atrophy and sTREM2-associated microglial activity in humans is needed to further interpret causal relations.

*Primary age-related tauopathy and Suspected Non-Alzheimer disease Pathophysiology*

Primary age-related tauopathy (PART) refers to neurofibrillary tangles that are highly prevalent in autopsies of older brains in the absence of Aβ-accumulation and separated from AD. PART typically affects the medial temporal lobe and habitually progresses no further than the limbic Braak-stages (III-IV, including hippocampal affection) [48]. PART is suspected to be one of the etiologies underlying a positive biomarker profile for tauopathy without abnormal Aβ-levels (A-/N+), Suspected Non-Alzheimer disease Pathophysiology (SNAP), due to observed overlapping anatomical distribution of neurodegenerative changes in these conditions, especially in the medial temporal lobe[49]. In cognitively normal older individuals, the proportion of SNAP increases with age and is consistently found to reach 1/4 in the oldest old. In the study population, 29 % of individuals had SNAP-profiles (median age 71) (table 1). PART may be associated with cognitive impairment and individuals with SNAP have an increased risk of progression to cognitive decline compared to biomarker-negative individuals. Nevertheless, both PART and SNAP appear to have less impact on cognition than AD[48, 49]. In this study the observed atrophy affected the temporal lobe (albeit not medially) and hippocampi and was related to tauopathy independently of abnormal amyloid levels. Furthermore, sTREM2-related atrophy was not associated with cognitive decline. In sum, we postulate that the observed sTREM2-related atrophy may reflect PART as measured by SNAP. However, without visualization of tau-pathology, the association between SNAP and PART remains uncertain. Utilizing Tau-PET could test the hypothesis further and help elucidate a possible relationship between sTREM2, atrophy and tau-pathology.

*Limitations*

One weakness of our study is the lack of a good measure for participants' cerebrovascular disease load, as cerebrovascular disease may have shared variance with the degrees of atrophy

and immunostimulation. We have a small study population at baseline, but substantial longitudinal data over several years strengthens our ability to detect change.

## CONCLUSION

We found that high levels of microglial sTREM2 expression predicted accelerated cortical thinning and hippocampal volume loss. The association was amyloid-independent and partly related to tau phosphorylation, suggesting a possible link between PART or age-associated-atrophy and neuroinflammation.

## Appendix 1

| Name | Location | Role | Contribution |
|---|---|---|---|
| N.B. Halaas, MD | University of Oslo | Author | Data collection. Interpretation of the data. Preparation of manuscript. |
| K. Henjum | Oslo University Hospital | Author | Analyses of sTREM2 in CSF. Interpretation of the data and revision of manuscript. |
| K. Blennow, MD, PhD | University of Gothenburg | Author | Analyses of Aβ-42 and tau in CSF. Interpretation of the data and revision of manuscript. |

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|---|---|---|---|
| Shams Dakhil | University of Oslo | Author | Data collection. Revision of manuscript. |
| A-V. Idland, MD | University of Oslo | Author | Initiation and design of the COGNORM-study.<br>Data collection.<br>Revision of manuscript. |
| L. Nilsson, PhD | University of Oslo | Author | Analyses of sTREM2 in CSF. Interpretation of the data and revision of manuscript. |
| D. Sederevicius | University of Oslo | Author | Statistical analyses and interpretation of data. Revision of manuscript. |
| D. Vidal-Piñeiro | University of Oslo | Author | Virtual histology analyses and interpretation of data. Revision of manuscript. |
| Kristine Walhovd | University of Oslo | Author | Interpretation of data. Revision of manuscript. |

| | | | |
|---|---|---|---|
| T.B.Wyller, MD, PhD | University of Oslo | Author | Initiation and design of the COGNORM-study. Interpretation of the data and revision of manuscript. |
| H. Zetterberg MD, PhD | University of Gothenburg | Author | Analyses of Aβ-42 and tau in CSF. Interpretation of the data and revision of manuscript. |
| L.O. Watne, MD, PhD | Oslo University Hospital | Author | Initiation and design of the COGNORM-study. Interpretation of the data and statistical analyses. Preparation and revision of manuscript. |
| A.M. Fjell | University of Oslo | Author | Initiation and design of the COGNORM-study. Statistical analyses and MRI interpretations. Interpretation of data and statistical analyses. |

| | |
|---|---|
| | Preparation and revision of manuscript. |

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## TABLES AND FIGURE LEGEND

**Table 1. Population characteristics at baseline**

**Table 2 Virtual histology results.**

**Figure 1 Relationship between sTREM2 and cortical thinning**
Panel A: Relationship between baseline levels of sTREM2 on cortical thinning. Significant clusters surviving corrections for multiple comparisons are shown in blue. Panel B: Same analysis as in A, with levels of Aβ42 regressed out. Panel C: Aβ42 and total-tau regressed out.

**Figure 2 Interactions between sTREM2 and time**
Panel A: Interactions between sTREM2 and cortical thickness over time in the three significant clusters. The contour plots illustrate how cortical thickness varies as a function of interval since baseline (time) and sTREM2 levels. As can be seen, blue colors, denoting thinner cortex, are seen for participants with high levels of sTREM2 over time. Panel B and C: Spaghetti plots illustrating the relationship between thickness change as a function of time in participants with normal ($z < 1$, red markers, Panel B) or high ($z \geq 1$, blue markers, Panel C) levels of sTREM2. The shaded region around the fit lines denotes +/-2 SE.

**Figure 3 Relationship between sTREM2 and hippocampal atrophy**
Panel A: Contour and spaghetti plot illustrating how left hippocampal volume varies as a function of interval since baseline and sTREM2 levels. Panel B: Right hippocampal volume.

**Figure 4 Relationships between sTREM2 and memory change**
Contour (Panel A) and spaghetti plots (Panel B) illustrating the relationship between interval since baseline and change in memory score as a function of baseline sTREM2 levels. The shaded region around the fit lines denotes +/-2 SE.

**Figure 5 Virtual histology**
Each plot corresponds to one of the 9 different cell type panels and shows the distribution of the expression – sTREM2-related thinning correlations for cell-specific genes. The x-axis represents the Pearson's correlation between sTREM2-related thinning and expression profiles for a given set of cell-specific marker genes while the y-axis indicates the probability density for the correlations across the cell-specific genes. The vertical edges of the shaded gray box indicate the unadjusted 2.5% and 97.5%-critical values obtained from the empirical null distribution of the average expression-thinning correlation.