

ALZHEIMER'S DISEASE

Increased soluble TREM2 in cerebrospinal fluid is associated with reduced cognitive and clinical decline in Alzheimer's disease

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Loss of function of TREM2, a key receptor selectively expressed by microglia in the brain, contributes to the development of Alzheimer's disease (AD). We therefore examined whether soluble TREM2 (sTREM2) concentrations in cerebrospinal fluid (CSF) were associated with reduced rates of cognitive decline and clinical progression in subjects with AD or mild cognitive impairment (MCI). We measured sTREM2 in CSF samples from 385 elderly subjects, including cognitively normal controls, individuals with MCI, and subjects with AD dementia (follow-up period: mean, 4 years; range 1.5 to 11.5 years). In subjects with AD defined by evidence of CSF A β ₁₋₄₂ (amyloid β -peptide 1 to 42; A+) and CSF p-tau₁₈₁ (tau phosphorylated on amino acid residue 181; T+), higher sTREM2 concentrations in CSF at baseline were associated with attenuated decline in memory and cognition. When analyzed in clinical subgroups, an association between higher CSF sTREM2 concentrations and subsequent reduced memory decline was consistently observed in individuals with MCI or AD dementia, who were positive for CSF A β ₁₋₄₂ and CSF p-tau₁₈₁ (A+T+). Regarding clinical progression, a higher ratio of CSF sTREM2 to CSF p-tau₁₈₁ concentrations predicted slower conversion from cognitively normal to symptomatic stages or from MCI to AD dementia in the subjects who were positive for CSF A β ₁₋₄₂ and CSF p-tau₁₈₁. These results suggest that sTREM2 is associated with attenuated cognitive and clinical decline, a finding with important implications for future clinical trials targeting the innate immune response in AD.

INTRODUCTION

Evidence from genome-wide association studies demonstrated that alterations of the innate immune system play a crucial role in the development of AD dementia (1, 2). In particular, rare missense variants in the gene encoding the triggering receptor expressed on myeloid cells 2 (*TREM2*) are associated with a two- to threefold increase in the odds ratio of developing AD (3, 4). This is comparable to that for the $\epsilon 4$ allele of the gene encoding apolipoprotein E (ApoE), the strongest genetic risk factor for sporadic AD (5). TREM2 binds to the DNAX-activating protein of 12 kDa (DAP12), a receptor-

signaling complex within the brain that is exclusively expressed by microglia (6, 7). TREM2 signaling serves as a key regulator that allows microglia to switch from a homeostatic to a disease-associated state (8, 9). Disease-associated microglia are characterized by up-regulated gene clusters triggering essential microglial functions such as chemotaxis, phagocytosis, and other functions that together may modulate amyloid plaque seeding and compaction (10–16). However, despite the evidence that TREM2 enhances the scavenger function of microglia and that disease-associated *TREM2* variants are apparently loss-of-function mutations (13, 15), chronic microglial activation has been associated with the release of proinflammatory cytokines and may be detrimental (17). Therefore, an important question is whether TREM2-related microglial activation protects or exacerbates the development of dementia in patients with AD (18).

The translation of preclinical findings on TREM2 has recently been facilitated by the development of a soluble TREM2 (sTREM2) marker in cerebrospinal fluid (CSF) (13, 19–21). The extracellular domain of TREM2 protein is shed by ADAM10/17 cleavage and released in its soluble form into the interstitial fluid of the brain (13, 22, 23). Given that probably only full-length TREM2 expressed on the microglial surface is signaling competent and that TREM2 expression in microglia is greatly increased during neurodegeneration (9, 16), sTREM2 in biological fluids serves as a readout for TREM2-triggered microglial activity (21, 24–26). In cross-sectional studies of patients with late-onset AD, we and others have previously reported that CSF sTREM2 increases in a disease stage-dependent fashion (20, 21, 24, 25), reaching a peak during early symptomatic stages (21). This was confirmed in patients with autosomal dominant AD where we observed an increase in CSF sTREM2 about 5 years before the estimated onset of symptoms (20). A recent study assessed

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the association between CSF sTREM2 and regional gray matter volume in subjects with early-stage AD who had mild cognitive impairment (MCI) and CSF biomarker evidence of AD. The study reported that CSF sTREM2 was associated with higher gray matter volume in this group when controlled for CSF amyloid β -peptide ($A\beta$) and tau (27), suggesting protective effects. However, that study had a cross-sectional design and was not conclusive with regard to beneficial effects because higher gray matter volume might also have resulted from inflammation-related swelling and cognitive function was not investigated.

Here, we examined a large well-characterized sample of 385 elderly participants from the Alzheimer's Disease Neuroimaging Initiative (ADNI). We investigated whether baseline CSF sTREM2 concentrations were predictive of subsequent changes in hippocampal volume, cognitive changes, and clinical progression to MCI or AD dementia. Our primary hypothesis was that in subjects with biomarker evidence of AD pathology, higher CSF sTREM2 might be associated with a slower rate of decline in episodic memory or cognition during an observation period of up to 11.5 years (mean, 4 years). We tested our hypothesis in subjects classified as AD according to the AT/N framework recently proposed by the National Institute on Aging and Alzheimer's Association (NIA-AA) (28). Subjects were classified on the basis of biomarkers to determine abnormal deposition of $A\beta$ (A) and pathological tau (T), regardless of clinical symptoms. We focused on subjects with AD based on CSF marker evidence of $A\beta$ and tau pathology (A+T+). To assess whether any CSF sTREM2 effects varied by disease stage, we conducted an exploratory subanalysis of CSF sTREM2 concentrations in clinically defined subgroups of AD with mild cognitive impairment (A+T+) or AD with dementia (AD dementia A+T+) (29).

RESULTS

CSF sTREM2 concentrations are associated with reduced cognitive decline in AD

We assessed CSF sTREM2 concentrations in individuals who were cognitively normal (CN) with normal amounts of CSF $A\beta$ and tau (CN A-T-) and in patients defined as having AD according to their CSF $A\beta$ and tau concentrations (AD A+T+), regardless of their clinical symptoms (28). Characteristics of patients with AD are summarized in Table 1. In the AD A+T+ group, we found increased CSF sTREM2 concentrations ($P = 0.002$; Fig. 1A) and a decreased ratio of CSF sTREM2 to p-tau₁₈₁ ($P < 0.001$; Fig. 1B) compared to the cognitively normal control group (CN A-T-).

We next tested whether higher baseline CSF sTREM2 concentrations (used as a continuous measure) predicted attenuated future cognitive decline in the AD A+T+ group. To this end, we computed a linear mixed-effects regression analysis to test the interaction of CSF sTREM2 concentrations \times time on cognitive scores. Note that in all regression models, the variable "time" refers to the follow-up duration of the neuropsychological testing. CSF sTREM2 concentrations were assessed only at baseline. We controlled for concentrations of CSF $A\beta_{1-42}$ and CSF p-tau₁₈₁ among other confounding variables including clinical syndrome (CN, MCI, or dementia), age, gender, and education. Our rationale was that CSF sTREM2 concentrations would increase in association with greater neurodegeneration, potentially reflecting higher microglial activity in response to neurotoxic amyloid and tau pathology (8, 9). Thus, we addressed the pivotal question of whether higher CSF sTREM2 concentrations

Table 1. Subject characteristics for each group. Values are shown as mean, with SD in parentheses.

	CN A-T-	All A+T+	MCI A+T+	AD dementia A+T+
Number	100	285	184	66
Age in years	72.8 (5.36)	73.53 (7.39)	72.9 (7.11)	73.6 (8.51)
Gender (F/M)	45/55	126/159	77/107	32/34
Years of education	16.1 (2.80)	15.91 (2.88)	16.1 (2.81)	15.2 (3.11)*
Clinical follow-up in years	4.82 (2.40)	3.69 (2.00)*	4.05 (1.94)*	2.18 (0.473)*
ADNI-MEM score	1.11 (0.58)	-0.15 (0.74)*	-0.07 (0.57)*	-0.89 (0.5)*
ADAS13 score	9.1 (4.32)	20.19 (8.53)*	18.67 (6.32)*	29.66 (6.83)*
$A\beta_{1-42}$ (pg/ml)	1527 (311)	639.62 (172.89)*	657 (167)*	552 (166)*
p-tau ₁₈₁ (pg/ml)	16.4 (2.95)	37.76 (13.12)*	38.0 (13.5)*	39.9 (13.5)*
Total tau (pg/ml)	186.6 (33.3)	370.9 (115.2)*	374.6 (120.1)*	386.9 (113.5)*
CSF sTREM2 (pg/ml)	3762 (1841)	4540 (2422)*	4452 (2518)*	4608 (2201)*

*Significantly ($P < 0.05$) different from cognitively normal controls (CN A-T-) via post hoc Tukey test.

at a given stage of AD pathology (measured by CSF $A\beta_{1-42}$ and CSF p-tau₁₈₁) were associated with less decline in memory and global cognition. Results of the regression analyses including the effect size d and P values from both parametric and nonparametric tests are displayed in Table 2. Specifically, for the prediction of the decline in episodic memory as measured by the composite score ADNI-Memory (MEM) (30), we observed a significant interaction effect of time \times CSF sTREM2 concentration ($\beta = 0.071$, $P = 0.001$), where higher baseline CSF sTREM2 concentrations were associated with a slower decline in ADNI-MEM scores over time (Fig. 2A). For changes in global cognition as measured by the 13-tasks version of the AD assessment scale (ADAS13), higher CSF sTREM2 concentrations were associated with a slower increase in ADAS13 scores ($\beta = -0.076$, $P = 0.034$; Fig. 2B), suggesting a potential beneficial effect of CSF sTREM2 concentrations on cognition, although that P value missed the Bonferroni corrected threshold $P < 0.0125$.

Next, we repeated the regression analyses using CSF sTREM2 and p-tau₁₈₁ concentrations as indicators of an increase in CSF sTREM2 relative to tau pathology and as predictors of cognitive decline. Similar to the results for CSF sTREM2, the interaction of CSF sTREM2 and p-tau₁₈₁ \times time was significant for both ADNI-MEM scores ($\beta = 0.088$, $P < 0.001$; Fig. 2C) and ADAS13 scores ($\beta = -0.102$, $P = 0.002$; Fig. 2D). Higher baseline concentrations of CSF sTREM2 and p-tau₁₈₁ were associated with slower rates of worsening performance on the ADNI-MEM and ADAS13 tests. All of these analyses survived the Bonferroni-corrected significance threshold ($P < 0.0125$). No significant effects of CSF sTREM2 concentrations or the ratio of

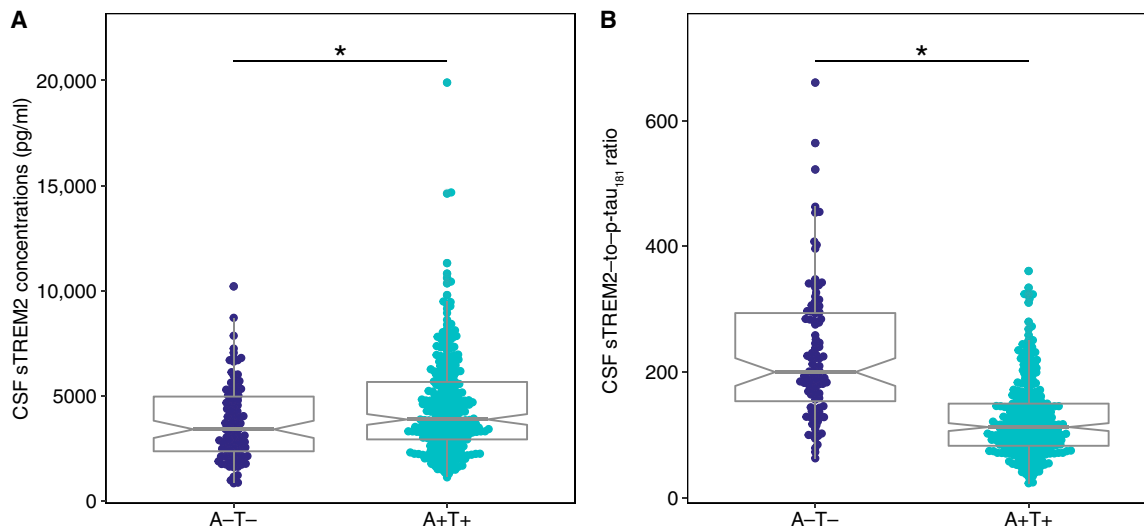


Fig. 1. Baseline values of CSF sTREM2 concentrations and CSF sTREM2-to-p-tau₁₈₁ ratio. Box plots show CSF sTREM2 concentrations (A) and the ratio of concentrations of CSF sTREM2 to p-tau₁₈₁ (B). CSF samples were obtained from 100 cognitively normal control subjects (CN), who were negative for CSF A β ₁₋₄₂ and CSF p-tau₁₈₁ (A-T-). CSF samples were also obtained from 285 subjects who were positive for CSF A β ₁₋₄₂ and CSF p-tau₁₈₁ (A+T+), including those who were cognitively normal or who had MCI or AD dementia. For both measurements, the group differences tested by ANCOVA were statistically significant, which remained after removal of the three outliers from the A+T+ group. Outliers were defined according to the Tukey criterion (66). * $P < 0.01$.

CSF sTREM2 to p-tau₁₈₁ on the rate of cognitive change were found in the CN control group (CN A-T-, $P < 0.05$). Summary statistics of the regression analyses including 95% confidence intervals and standardized effect sizes of interaction terms are shown in Table 2. In addition, we tested the predictive effects of CSF sTREM2 concentrations when controlling additionally for markers of neurodegeneration (the N category within the AT/N system) including CSF total tau concentrations and hippocampal volume measured by magnetic resonance imaging (MRI) in addition to the other covariates (CSF p-tau₁₈₁, CSF A β ₁₋₄₂, age, gender, and education). The results of these analyses were congruent with our main results, regardless of the additional controls for CSF total tau concentrations (table S1) or hippocampal volume (table S2).

Effect of CSF sTREM2 on cognitive changes in clinically defined subgroups of A+T+ subjects

To assess whether the association between CSF sTREM2 concentrations and subsequent cognitive changes varied by clinical stage of AD, we performed subanalyses in the clinical subgroups of A+T+ subjects. These A+T+ subjects included those with MCI and AD dementia defined according to NIA-AA research criteria (28). The cognitively normal A+T+ subgroup yielded only 30 subjects and thus was not included in the subanalysis. CSF sTREM2 concentrations were significantly increased in the MCI A+T+ subgroup and the AD dementia A+T+ subgroup compared to cognitively normal subjects who were A-T- (CN A-T-). The ratio of CSF sTREM2 to p-tau₁₈₁ was significantly reduced in the MCI A+T+ and AD dementia A+T+ subgroups compared to the CN A-T- group ($P < 0.05$; fig. S1). For the ADNI-MEM score, the interaction of CSF sTREM2 concentrations \times time was significant in both the MCI A+T+ subgroup ($\beta = 0.071$, $P = 0.011$; fig. S3A) and the AD dementia A+T+ group ($\beta = 0.050$, $P = 0.033$; fig. S3B). For the ADAS13 score, the interaction effect did not reach significance in the MCI A+T+ subgroup ($\beta = -0.076$, $P = 0.096$) or AD dementia A+T+ subgroup ($\beta = -0.047$, $P = 0.384$). This may reflect the smaller effect size for

the ADAS13 score (Table 2), which required a larger sample size for reliable detection. For the interaction of the CSF sTREM2 to p-tau₁₈₁ \times time, we found significant effects for the ADNI-MEM score in both the MCI A+T+ subgroup ($\beta = 0.092$, $P < 0.001$) and AD A+T+ group ($\beta = 0.059$, $P = 0.009$). For the ADAS13 score, the CSF sTREM2 to p-tau₁₈₁ ratio \times time interaction was significant in the MCI A+T+ subgroup ($\beta = -0.105$, $P = 0.012$) but not in the AD dementia A+T+ subgroup (Table 2). These results remained consistent when additionally controlling for baseline CSF total tau concentrations (table S1) or hippocampal volume (as a marker of neurodegeneration, N; table S2).

To assess whether the effect of a higher CSF sTREM2 to p-tau₁₈₁ ratio on cognitive decline was as large as that of the core AD biomarkers CSF A β ₁₋₄₂ and p-tau₁₈₁, we computed Cohen's effect sizes d for the effects of CSF concentrations of sTREM2, CSF A β ₁₋₄₂, CSF p-tau₁₈₁ on cognitive changes using the same regression equations as described above. The effect size d is a variance-standardized effect size measure where by convention $d = 0.2$, $d = 0.5$, and $d = 0.8$ are defined as "small," "medium," and "large," respectively (31). The results for the different groups are shown in Fig. 3. With regard to predicting changes in the ADNI-MEM score, the effect sizes were $d = 0.43$ for CSF sTREM2, $d = 0.27$ for CSF A β ₁₋₄₂, and $d = 0.47$ for CSF p-tau₁₈₁. With regard to predicting changes in the ADAS13 score, the effect sizes were $d = 0.28$ for CSF sTREM2, $d = 0.33$ for CSF A β ₁₋₄₂, and $d = 0.45$ for CSF p-tau₁₈₁. Thus, regarding the change in episodic memory, the beneficial effect of higher CSF sTREM2 concentrations was of small to medium size and comparable in size to the detrimental effect of CSF p-tau₁₈₁ concentrations on cognitive changes.

Higher CSF sTREM2 concentrations and CSF sTREM2 to p-tau₁₈₁ ratios are associated with slower hippocampal atrophy

To assess whether higher CSF sTREM2 concentrations were associated with slower neurodegeneration, we used longitudinal hippocampal

Table 2. Regression analyses for the effects of CSF sTREM2 concentrations on the rate of change in the ADNI-MEM and ADAS-cog scores and in hippocampal volume. All models are controlled for age, gender, education, CSF p-tau₁₈₁ × time, CSF-Aβ₁₋₄₂ × time, CSF sTREM2, CSF Aβ₁₋₄₂, CSF p-tau₁₈₁ (fixed effects) plus random slope (time) and intercept. Regular *P* values were assessed using Satterthwaite's method. For nonparametric confirmation of results, *P* values were further assessed using permutation testing with 1000 iterations (*P*-perm.). *B*, regression coefficient; *CI*, confidence interval; *T* value, *t* test statistic; Cohen's *d*, effect size *d*; *P*-perm., *P* value from permutation test.

ADNI-MEM	Interaction term	B (SE)	95% CI	T value	Cohen's d	P	P-perm.
CN A-T-	CSF sTREM2 × time	0.036 (0.046)	-0.052-0.125	<1	NA	0.429	0.395
	CSF sTREM2/p-tau × time	0.043 (0.043)	-0.042-0.127	<1	NA	0.331	0.312
All A+T+	CSF sTREM2 × time	0.071 (0.022)	0.028-0.113	3.258	0.43	0.001	0.020
	CSF sTREM2/p-tau × time	0.088 (0.020)	0.049-0.128	4.354	0.55	<0.001	<0.001
MCI A+T+	CSF sTREM2 × time	0.071 (0.028)	0.017-0.125	2.581	0.41	0.011	0.046
	CSF sTREM2/p-tau × time	0.092 (0.025)	0.043-0.141	3.663	0.57	<0.001	0.004
AD dementia A+T+	CSF sTREM2 × time	0.050 (0.023)	0.006-0.094	2.194	0.62	0.033	0.027
	CSF sTREM2/p-tau × time	0.059 (0.022)	0.016-0.103	2.671	0.73	0.009	0.006
ADAS13	Interaction term	B (SE)	95% CI	T value	Cohen's d	P	P-perm.
CN A-T-	CSF sTREM2 × time	0.087 (0.047)	-0.006-0.179	1.849	NA	0.074	0.084
	CSF sTREM2/p-tau × time	0.070 (0.043)	-0.015-0.157	1.626	NA	0.115	0.151
All A+T+	CSF sTREM2 × time	-0.076 (0.035)	-0.145--0.007	-2.137	0.28	0.034	0.045
	CSF sTREM2/p-tau × time	-0.102 (0.033)	-0.167--0.037	-3.078	0.38	0.002	0.023
MCI A+T+	CSF sTREM2 × time	-0.076 (0.046)	-0.165-0.013	-1.672	NA	0.096	0.205
	CSF sTREM2/p-tau × time	-0.105 (0.042)	-0.186--0.024	-2.530	0.39	0.012	0.033
AD dementia A+T+	CSF sTREM2 × time	-0.047 (0.054)	-0.151-0.056	<1	NA	0.384	0.352
	CSF sTREM2/p-tau × time	-0.076 (0.054)	-0.181-0.029	-1.402	NA	0.166	0.137
Hippocampal volume	Interaction term	B (SE)	95% CI	T value	Cohen's d	P	P-perm.
CN A-T-	CSF sTREM2 × time	-0.010 (0.013)	-0.046-0.016	<1	NA	0.445	0.382
	CSF sTREM2/p-tau × time	-0.007 (0.013)	-0.032-0.018	<1	NA	0.606	0.552
All A+T+	CSF sTREM2 × time	0.023 (0.010)	0.004-0.043	2.318	0.39	0.022	0.024
	CSF sTREM2/p-tau × time	0.023 (0.009)	0.005-0.041	2.533	0.41	0.012	0.024
MCI A+T+	CSF sTREM2 × time	0.034 (0.013)	0.008-0.060	2.563	0.49	0.012	0.010
	CSF sTREM2/p-tau × time	0.030 (0.012)	0.007-0.053	2.580	0.49	0.011	0.010
AD dementia A+T+	CSF sTREM2 × time	-0.004 (0.023)	-0.047-0.040	-0.159	NA	0.874	0.888
	CSF sTREM2/p-tau × time	0.0001 (0.001)	-0.0001-0.0003	<1	NA	0.999	0.999

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volumetric data acquired by MRI for subjects who had a minimum of 2 years of MRI follow-up. We used hippocampal volume as a marker of gray matter atrophy given that hippocampus atrophy is a hallmark of AD, with hippocampal volume declining throughout the course of AD (32). Using the same linear mixed models as described above, we found a significant CSF sTREM2 concentration \times time interaction in the A+T+ subgroup with longitudinal MRI ($n = 184$) such that subjects with higher CSF sTREM2 concentrations ($\beta = 0.023$, $P = 0.022$; Fig. 4A) or a higher CSF sTREM2 to p-tau₁₈₁ ratio ($\beta = 0.023$, $P = 0.012$; Fig. 4B) showed slower hippocampal atrophy rates over time. Clinical subgroup-specific analysis in the A+T+ subjects yielded a significant interaction for MCI A+T+ subjects (CSF sTREM2 \times time: $\beta = 0.034$, $P = 0.012$; fig. S4A; CSF sTREM2-to-p-tau₁₈₁ ratio \times time $\beta = 0.030$, $P = 0.011$; fig. S4B) but not for

AD dementia A+T+ subjects (CSF sTREM2 \times time $\beta = -0.004$, $P = 0.874$; CSF sTREM2-to-p-tau₁₈₁ ratio \times time $\beta = 0.0001$, $P = 0.999$). These exploratory analyses did not survive Bonferroni correction though ($P < 0.008$). No significant interaction effects were detected in the cognitively normal control A-T- group, neither for CSF sTREM2 concentrations \times time ($\beta = -0.012$, $P = 0.390$) nor CSF sTREM2-to-p-tau₁₈₁ ratio \times time ($\beta = -0.007$, $P = 0.756$). Model statistics are summarized in Table 2. In addition, controlling all analyses for CSF total tau concentrations yielded consistent results (table S1).

A higher CSF sTREM2-to-p-tau₁₈₁ concentration ratio is associated with slower clinical progression

Last, we assessed whether higher baseline CSF sTREM2 concentrations are predictive of the time to conversion to a worse clinical stage (MCI or AD dementia). We included all subjects of the A+T+ group who did not have dementia at baseline, that is, who were cognitively normal or who had MCI. Using Cox regression, we tested CSF sTREM2 concentrations (controlled for A β , p-tau₁₈₁, age, gender, and education) as a predictor of conversion from cognitively normal to MCI or AD dementia or from MCI to AD dementia. For CSF sTREM2 concentrations, we did not detect significant effects on clinical progression ($\beta = -0.113$, $P = 0.143$). However, when testing the ratio of CSF sTREM2 to p-tau₁₈₁ concentration as a predictor, we found significantly slower clinical progression in subjects with a higher CSF sTREM2-to-p-tau₁₈₁ ratio ($\beta = -0.486$, $P = 0.005$; Fig. 5). Thus, a higher CSF sTREM2-to-p-tau₁₈₁ ratio was associated with a lower risk to clinically progress for up to 11.5 years.

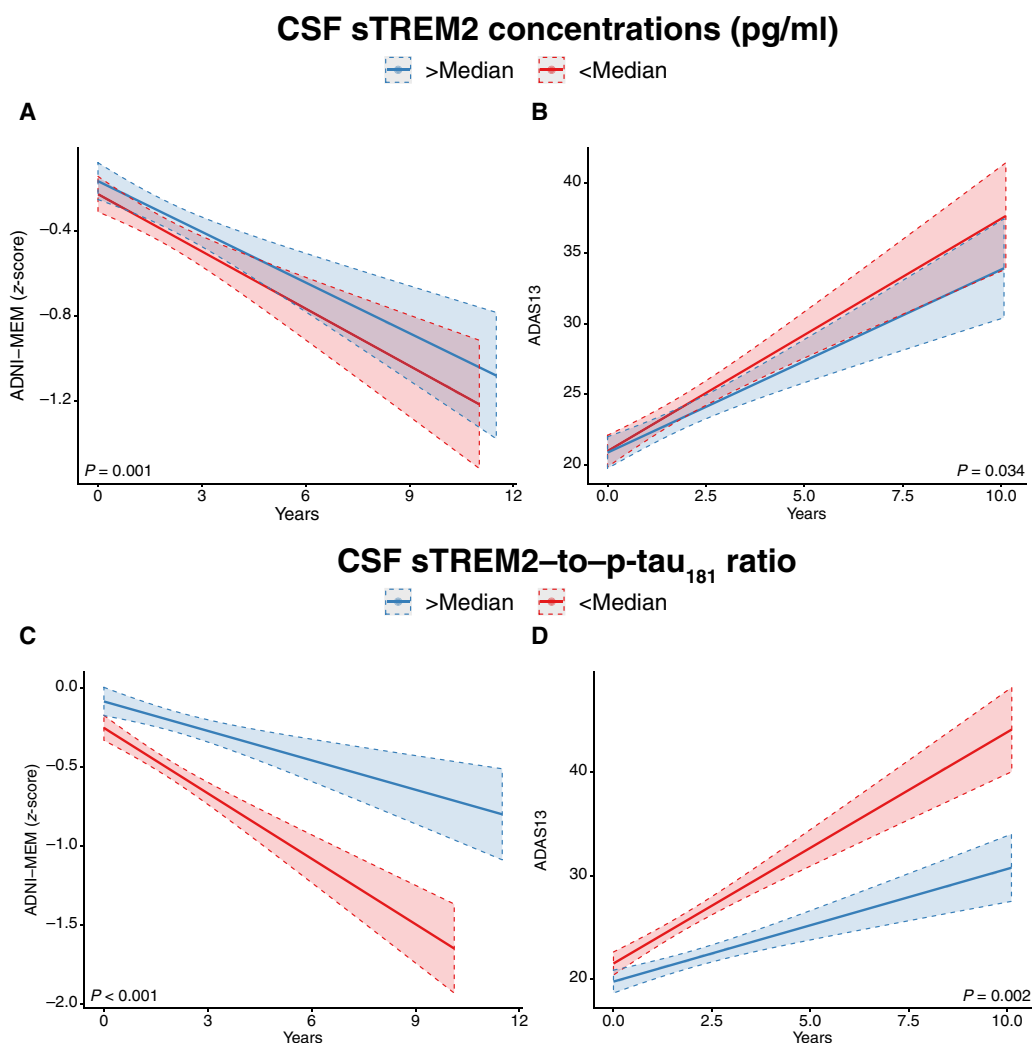


Fig. 2. Effect of CSF sTREM2- and CSF sTREM2-to-p-tau₁₈₁ ratio on changes in cognition. Regression plots show the change in episodic memory (A and C) and global cognition (B and D) as a function of CSF sTREM2 concentrations (top) or the CSF sTREM2-to-p-tau₁₈₁ ratio (bottom) at different time points during cognitive follow-up in all subjects who were positive for CSF A β ₁₋₄₂ and CSF p-tau₁₈₁ (A+T+). For illustration purposes, the regression lines are shown for subjects with high versus low values of CSF sTREM2 or the CSF sTREM2-to-p-tau₁₈₁ ratio (split at the median). In all regression analyses, CSF sTREM2 concentrations and CSF sTREM2-to-p-tau₁₈₁ ratios were included as continuous measures. The full regression models included the interaction term of CSF sTREM2 (or CSF sTREM2-to-p-tau₁₈₁ ratio) \times time, controlled for CSF p-tau₁₈₁ \times time, and CSF A β ₁₋₄₂ \times time interactions among other potentially confounding variables.

DISCUSSION

Here, we assessed whether baseline concentrations of CSF sTREM2 were associated with a slower or faster rate of cognitive decline and clinical worsening in AD. Our results show that higher CSF sTREM2 concentrations at baseline predicted a reduced rate of subsequent cognitive decline in subjects with a CSF biomarker profile of AD (A+T+ group), regardless of the clinical stage. Higher CSF sTREM2 concentrations were associated with less decline in both episodic

memory and global cognition. For our secondary outcome parameters, higher CSF sTREM2 concentrations showed a trend of an association with attenuated decline in hippocampal volume. Furthermore, a higher CSF sTREM2-to-p-tau₁₈₁ ratio predicted a slower rate of

conversion from cognitively normal to MCI or AD dementia. Given that sTREM2 detected in the CSF likely reflects cerebral TREM2 expression and the amount of signaling-competent TREM2 on the surface of activated microglia, these results suggest that higher TREM2-related microglial function may be predictive of slower disease progression at the symptomatic stage of AD. Our results suggest that higher CSF sTREM2 is not only associated with less cognitive decline but may also be associated with neuroprotective effects as suggested by slower hippocampal volume changes, although the analyses of hippocampal volume changes were exploratory in nature and need to be confirmed in future studies. We further caution that although the current study examined the association between CSF sTREM2 and subsequent cognitive changes, a causative effect between microglial TREM2 function and clinical outcome is not necessarily implied. Nonetheless, our results demonstrate that in patients with biomarker evidence of underlying AD, higher CSF sTREM2 concentrations predict a more benign disease course in AD. A protective role of TREM2-related processes is also in line with the finding that several TREM2 variants are loss-of-function mutations (33). In the case of the TREM2 pT66M variant or complete loss of TREM2, microglia are prevented from adopting a disease-associated state, which would trigger phagocytosis, migration, chemotaxis, and microglial proliferation (10, 13, 15, 34).

Our clinical findings are based on a large sample of well-characterized subjects with biomarker evidence of AD pathology. For staging of AD, we used an AD biomarker-informed classification system in line with recently proposed NIA-AA research diagnostic guidelines for AD (28). Consistent with previous findings (35, 36), we showed that abnormal CSF concentrations of tau and Aβ were predictive of faster cognitive decline at each disease stage. In contrast, increases in CSF sTREM2 concentrations in A+T+ subjects were associated

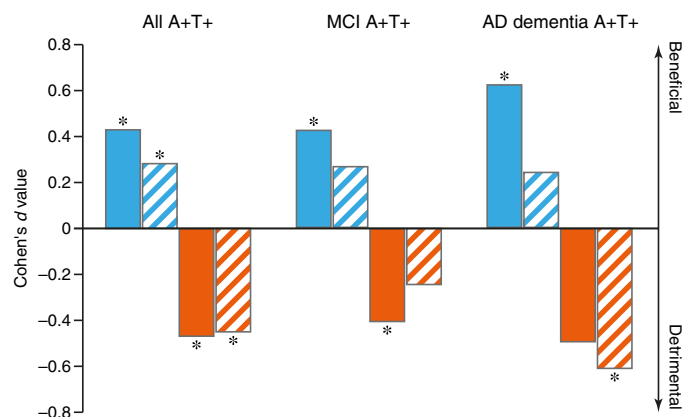


Fig. 3. Effect sizes for CSF sTREM2 and CSF p-tau₁₈₁. Bars show the effect size (Cohen's *d* value) of the association between CSF sTREM2 concentrations (blue) or CSF p-tau₁₈₁ concentrations (orange) on the rate of change in episodic memory (assessed by the ADNI-MEM score; solid bars) or global cognition (assessed by the ADAS13 score; striped bars). The effect sizes derived from linear mixed-effects analyses are shown for all subjects who were positive for CSF Aβ₁₋₄₂ and CSF p-tau₁₈₁ (A+T+) at different clinical stages (MCI, *n* = 184; AD dementia, *n* = 66) and after the data were pooled (*n* = 285). A positive *d* value means that higher CSF concentrations were associated with slower cognitive decline (less decrease in the ADNI MEM score and less increase in the ADAS13 score). A negative *d* value means that higher CSF concentrations were associated with worse cognitive decline as determined by mixed-effects regression analyses. **P* < 0.05.

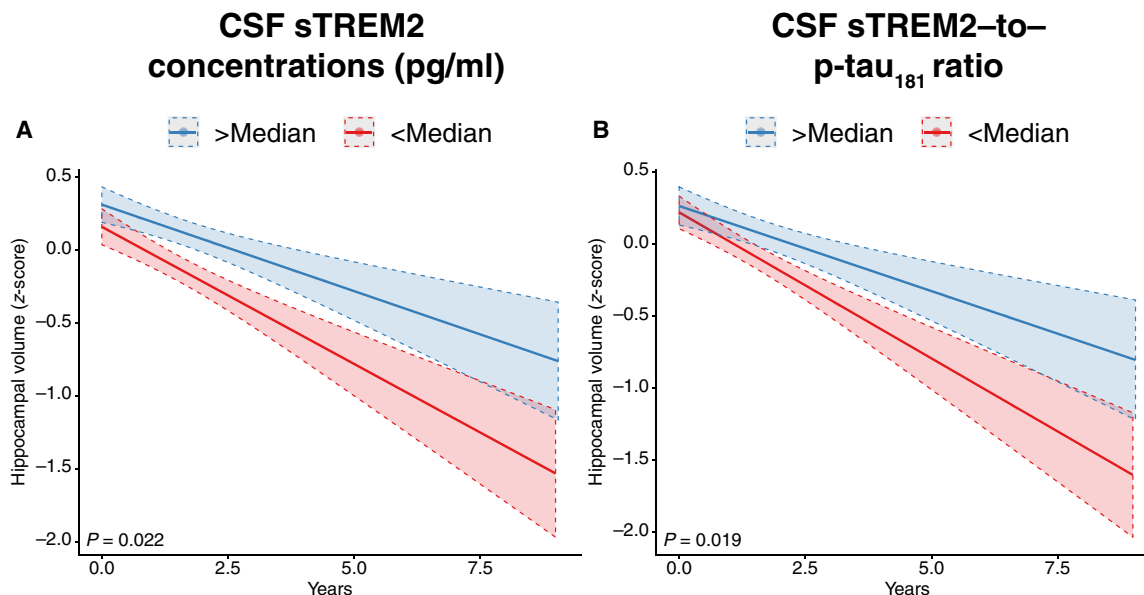


Fig. 4. Effect of CSF sTREM2- and CSF sTREM2-to-p-tau₁₈₁ ratio on changes in hippocampal volume. Regression plot shows the change in bilateral hippocampal volume measured by MRI as a function of CSF sTREM2 concentrations at different time points during follow-up (A) and CSF sTREM2-to-p-tau₁₈₁ ratios (B) for subjects who were positive for CSF Aβ₁₋₄₂ and CSF p-tau₁₈₁ (A+T+) and for whom longitudinal hippocampal volume data were available (*n* = 285). For illustration purposes, the regression lines are shown for subjects with high versus low values for CSF sTREM2- or CSF sTREM2-to-p-tau₁₈₁ ratios (split at the median). All regression analyses included the interaction term of time × CSF sTREM2 concentrations (or CSF sTREM2-to-p-tau₁₈₁ ratios) as continuous measures and controlled for the interactions of CSF p-tau₁₈₁ × time and CSF Aβ₁₋₄₂ × time among other potentially confounding variables.

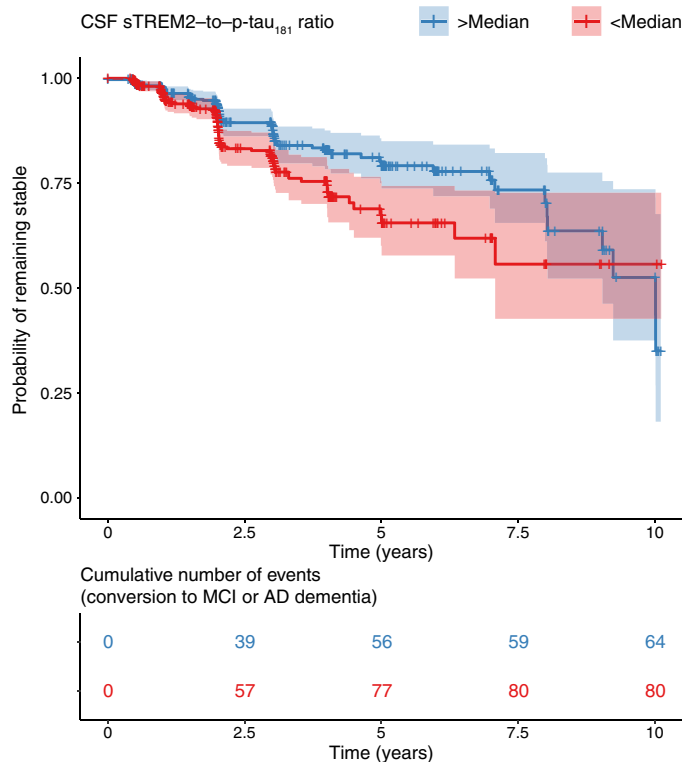


Fig. 5. Survival plot for conversion to MCI or AD dementia. (Top) The Cox regression-derived survival plot shows that a higher ratio of the concentrations of CSF sTREM2 to p-tau₁₈₁ (split by median) was associated with a higher probability of remaining clinically stable over time in the A+T+ group. (Bottom) The table indicates the number of subjects in the high and low CSF sTREM2-to-p-tau₁₈₁ ratio subgroups at successive follow-up time points.

with more benign subsequent cognitive changes. The effect size of higher CSF sTREM2 concentrations as a predictor of reduced cognitive decline was comparable to that of higher CSF p-tau₁₈₁ concentrations as a predictor of worse cognitive decline, suggesting that CSF sTREM2 concentrations may be relevant to future disease progression. The clinical relevance of altered CSF sTREM2 concentrations is supported by our finding of an association of a higher CSF sTREM2-to-p-tau₁₈₁ ratio with attenuated rate of conversion from cognitively normal or MCI to AD dementia. The association between higher CSF sTREM2 and an attenuated rate of clinical worsening may be seen as counterintuitive considering that an increase in CSF sTREM2 concentrations is associated with higher CSF p-tau₁₈₁ and total tau concentrations (20, 21, 24, 25). Mechanistically, it is likely that in response to amyloid pathology and neurofibrillary tangle degeneration (reflected by increased CSF p-tau₁₈₁), microglial activation may be triggered by enhanced expression and surface localization of TREM2 (reflected by increased CSF sTREM2 concentrations). Thus, without controlling for AD pathology, increased CSF sTREM2 concentrations may simply reflect the presence of AD and correlate negatively with cognitive performance (37). The key question is whether stronger TREM2-related microglial activation at a given stage of AD pathology could be beneficial for slowing subsequent neurodegeneration and clinical progression. Therefore, we sought in our analyses to test the predictive value of higher CSF sTREM2 concentrations on future disease progression (cognitive, clinical, and hippocampal volume), and we controlled for biomarkers of AD pathology including

CSF A β ₁₋₄₂ and p-tau₁₈₁. Our results show that the higher the concentration of CSF sTREM2 for a given degree of neurofibrillary tangle degeneration (as measured by p-tau₁₈₁), the better the clinical outcome.

Our findings are consistent with those of a recent study where higher CSF sTREM2 concentrations were associated with higher cortical gray matter integrity at the MCI stage (27), suggesting a potentially beneficial function of the TREM2-related immune response, particularly in the symptomatic phase of AD. Our results are also in general agreement with previous findings reporting increased microglial activation (based on PET imaging) in cognitively normal or MCI subjects who were amyloid positive on PET (38, 39) and a correlation between higher microglial activation on PET and higher tau on PET (40). Greater numbers of microglia based on PET imaging were associated with a slower subsequent cognitive decline in subjects with MCI (38). This is also in line with the observation that microglial-PET signals increase during physiological aging of wild-type mice, reflecting the accumulation of activated microglia around sites of neuronal damage. Notably, no such increase in microglial-PET signals nor in clustering of microglia during physiological aging has been observed upon loss of TREM2 function (13). Together, these results suggest a protective effect of greater microglial activation during disease progression in AD. The protective effects of higher TREM2 function have also been observed in transgenic mouse models of AD (16, 41, 42). A number of AD pathology studies in transgenic mouse models suggest enhanced amyloid plaque seeding and reduced amyloid plaque compaction combined with increased plaque-associated dystrophic neurites in the absence of TREM2 function (11, 16, 43). Increased TREM2 expression resulted in a reduction in dystrophic neurites in a transgenic mouse model of AD (44), a finding in line with our observation of the association between higher TREM2 concentrations and attenuated disease progression in human subjects.

For tauopathies, several studies have reported beneficial effects of higher TREM2 function, which was associated with reduced neuronal loss and improved spatial memory in the PS19 transgenic mouse model of tauopathy (45–49). However, a recent study reported beneficial effects of TREM2 deficiency in a transgenic mouse model of tauopathy (50). Differences between the studies may be explained by proinflammatory cytokine release, which was decreased in TREM2-deficient transgenic tauopathy mice (50) but was up-regulated or unaffected in those studies that reported protective effects of TREM2 (45–49). Thus, TREM2-related release of proinflammatory cytokines may play an important role in the beneficial effects of TREM2-triggered microglial activity in the presence of tau pathology (47, 51). In addition, a recent study reported that selective TREM2 haploinsufficiency but not a complete loss of TREM2 increased tau pathology, which may suggest that some compensatory mechanisms occurred after the complete loss of TREM2 function (52). The findings regarding the effect of TREM2 in animal models of amyloid and tau pathology are heterogeneous, with both beneficial and detrimental effects being reported. Disease stage may be a moderating factor for TREM2-associated microglial activity (16, 53, 54), a factor that could also account for some inconsistencies observed in human subjects (39, 55). However, disease stage is a broad descriptive category; the key molecular events that are associated with TREM2 and that determine beneficial versus detrimental microglial activity remain to be established. It is tempting to speculate that any beneficial effects of increased TREM2 expression are associated with enhanced TREM2-mediated signaling. TREM2-triggered microglial activity facilitates

chemotaxis and clustering of microglia around amyloid plaques, engulfment of amyloid seeds (16), compaction of amyloid plaques, removal of cellular debris (10, 15), and myelin repair (56) and potentially may also reduce pathological tau and neuroinflammation (48). Thus, increased TREM2 signaling may facilitate a protective response to neuronal injury. Studying the association between CSF sTREM2 concentrations and pro- and anti-inflammatory markers in future studies in patients with AD will be instrumental for understanding the reduced cognitive and clinical worsening associated with CSF sTREM2 concentrations (51, 54).

For the interpretation of the current study, some caveats should be considered. First, the current study included mostly subjects with dementia symptoms, but only a few subjects showed biomarker evidence of AD pathology and were still cognitively normal. Thus, it is unclear whether the current findings are also applicable to the pre-symptomatic stage of AD. Second, CSF sTREM2 concentrations are not a direct measure of TREM2-related microglial activity. Although TREM2 is cleaved by the secretase ADAM10 and thus may be released into the interstitial fluid in the brain, the relationship between sTREM2 in the CSF and higher microglial expression of TREM2 remains to be investigated. Third, although TREM2 is a key driver for switching microglia from a homeostatic to a disease-associated state, TREM2 receptor signaling acts in concert with a multitude of other microglia receptors, which were not assessed in the current study. Last, a causative effect between microglial TREM2 function and clinical outcome is not necessarily implied by our findings.

Together, we show that higher CSF sTREM2 concentrations are associated with a protective effect for cognitive changes at a similar magnitude to that found for the adverse effects of primary AD pathologies assessed by CSF $A\beta_{1-42}$ and p-tau₁₈₁ concentrations. Our findings have important implications for therapeutic attempts that aim to modulate microglial function, suggesting that stimulating TREM2-mediated microglial activity may be most beneficial within the early symptomatic phase of AD. From a clinical point of view, CSF sTREM2 concentrations could serve as a target engagement marker for treatments aimed at modulating microglial function. Despite the fact that the current study is observational in nature and therefore cause-effect conclusions cannot be drawn, our results support the notion that the modification of TREM2 function is a valuable target in AD. Our results suggest that drugs that up-regulate TREM2-related microglial activity may benefit patients with AD. Furthermore, our data indicate that the early symptomatic disease stage of AD is a preferred time window for therapeutic microglial modulation.

MATERIALS AND METHODS

Study design

In the current study, we examined cross-sectional CSF measurements of sTREM2, $A\beta_{1-42}$, and p-tau₁₈₁ concentrations as predictors of longitudinally assessed changes in cognition, clinical symptoms, and hippocampal volume. A total of 385 subjects were included from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu). ADNI was launched in 2003 as a public-private partnership, with the primary goal to assess serial neuroimaging and biological markers for the prediction of disease progression in AD. The subjects studied herein were a subgroup of $n = 1035$ participants for whom CSF sTREM2 samples at any time point were

available. Beyond the inclusion and exclusion criteria of ADNI, the following additional selection criteria were applied for the current study: (i) availability of baseline CSF sTREM2 values, (ii) no rare variants in the *TREM2* gene, (iii) minimum of 1-year follow-up duration for neuropsychological or clinical testing to avoid strong censoring effects (4), CSF A+T+ profile or CSF A-T- profile for cognitively normal individuals (see fig. S2 for flow chart of subject selection). Application of these inclusion criteria led to the selection of $n = 385$ subjects, who did not differ in terms of age, gender, or education, or mini mental state exam (MMSE) score from the entire sample of 1035 subjects, suggesting that no selection bias was evident. All subjects were recruited between the years 2005 and 2013. Clinical diagnosis of amnesic MCI was defined according to the Mayo Clinic criteria (57). Neuropsychological and clinical testing was done annually over a period of up to 11.5 years (mean, 4 years; range, 1.5 to 11.5). AD dementia diagnosis was accomplished according to standard clinical criteria as described previously (58). The study was approved by the Institutional Review Board (IRB) of all participating centers in ADNI, as well as our local IRB at Ludwig-Maximilians University. All study participants (or their relatives) provided written informed consent.

Following the recently proposed NIA-AA guidelines (28), AD was defined as a biological construct and not by clinical symptoms. These criteria require both evidence of amyloid deposition (A+), defined in this study as abnormal values of CSF $A\beta_{1-42}$, and of tau pathology (T+), defined in this study as abnormal values of CSF p-tau₁₈₁ (28). On the basis of previously established cutoff points (59), the criterion for pathological CSF $A\beta_{1-42}$ was defined as $A\beta_{1-42} < 976.6$ pg/ml, and for pathological p-tau₁₈₁, it was defined as p-tau₁₈₁ > 21.8 pg/ml. In contrast, we applied the traditional syndromal categories (CN, MCI, and AD dementia) for the clinical staging of AD (28).

CSF sTREM2 measurements were done at the laboratory of C. Haass at the DZNE Munich using ELISA with minor modifications to the procedure described previously (13, 21). The CSF concentrations of $A\beta_{1-42}$ and p-tau₁₈₁ were assessed by the ADNI biomarker core team at the University of Pennsylvania using the electrochemiluminescence immunoassays Elecsys.

Measurements of CSF sTREM2, $A\beta_{1-42}$, total tau, and p-tau₁₈₁

The CSF sTREM2 assay is based on the MSD platform and has been comprehensively described previously (13, 20, 21). A detailed description of the CSF sTREM2 measurements in the ADNI samples, as well as the original data, can be downloaded from the LONI Image and Data Archive (<https://ida.loni.usc.edu>). Briefly, Streptavidin-coated 96-well plates (MSD Streptavidin Gold Plates, cat. no. L15SA) were first blocked overnight at 4°C in blocking buffer [3% bovine serum albumin (BSA) and 0.05% Tween 20 in PBS (pH 7.4)]. They were next incubated with the capture antibody (0.25 µg/ml; R&D Systems, cat. no. BAF1828), diluted in the antibody buffer [1% BSA and 0.05% Tween 20 in PBS (pH 7.4)] during 1 hour at room temperature, and subsequently washed four times with washing buffer (0.05% Tween 20 in PBS). Next, the recombinant human TREM2 protein (standard curve; Hölzel Diagnostika, cat. no. 11084-H08H), the blanks, the CSF samples (duplicates; dilution factor: 4), and four internal standards (IS) were diluted in assay buffer [0.25% BSA and 0.05% Tween 20 in PBS (pH 7.4)] supplemented with protease inhibitors (Sigma; cat. no. P8340) and incubated for 2 hours at RT. Plates were again washed three times and incubated for 1 hour at

RT with the detection antibody (1 $\mu\text{g/ml}$; Santa Cruz Biotechnology, B-3, cat. no. sc373828). After three additional washing steps, plates were incubated with SULFO-tag conjugated secondary antibody (0.5 $\mu\text{g/ml}$; MSD, cat. no. R32AC) for 1 hour in the dark at RT, washed three times with wash buffer and two times with PBS. The electrochemical signal was developed with MSD Read buffer T (cat. no. R-92TC), and the light emission was measured using the MESO QuickPlex SQ 120. The mean intraplate CV was 3.1% (all duplicate measures had a CV < 15%) and the interplate CV for each of the IS were 11.4, 12.2, 10.5, and 7.1%. The CSF sTREM2 values used herein are those corrected on the basis of values of the four IS that were loaded on all plates (variable “MSD_sTREM2CORRECTED” in the ADNI database).

The CSF core biomarker measurements were performed by the ADNI biomarker core team at the University of Pennsylvania, using the electrochemiluminescence immunoassays Elecsys $\text{A}\beta_{1-42}$, p-tau₁₈₁, and total tau on a fully automated Elecsys cobas e 601 instrument and a single lot of reagents for each of the three measured biomarkers (provided in UPENNBIOBK9.csv file available in the ADNI databank). The analyte measuring ranges (lower technical limit to upper technical limit) of these assays are as follows: 200 to 1700 pg/ml for Elecsys $\text{A}\beta_{1-42}$, 80 to 1300 pg/ml for total tau CSF, and 8 to 120 pg/ml for p-tau₁₈₁ CSF immunoassays.

Neuropsychological assessment

Annual assessment of neuropsychological tests was performed. We included a priori two measures of cognitive performance. Given that episodic memory is the cognitive domain primarily affected in early stages and by definition in amnesic MCI, we used the composite score of episodic memory called ADNI-MEM, which integrates memory scores of the Rey Auditory Verbal Learning Test, ADAS, as well as the Wechsler Logical Memory I and II and the word recall of the MMSE as previously described (30). The median follow-up time of neuropsychological and clinical testing was 4 years (range, 1.45 to 11.5 years). The Alzheimer’s Disease Assessment Scale Cognition 13-item scale (ADAS13) (60) was included to assess global cognition.

Measurement of hippocampal volume by MRI

Freesurfer-based (version 5.1) assessments of hippocampal volume were done on 3T MPRAGE MRI scans by the ADNI imaging core at UCSF (61) and were for the current study downloaded from the ADNI database (<http://adni.loni.usc.edu/>). For the longitudinal hippocampus volume measurement, images were processed using the Freesurfer-based longitudinal image processing framework (62). Briefly, for each subject, a median image is computed via robust registration of a subject’s images from all longitudinal time points to arrive at an unbiased subject-specific template. The subject-specific template informs the initialization of later algorithms including the surface reconstruction, nonlinear spatial normalization to atlas space, and parcellation to treat images from all time points in the same way and thus avoid a bias stemming from temporal order. All images undergo a series of preprocessing steps including intensity normalization, removal of nonbrain voxels, affine registration to Talairach space before the segmentation of subcortical white matter and subcortical nuclei, and a second intensity normalization. Subsequently, surface reconstruction was performed through a number of steps for all images, followed by nonlinear registration of the individual surface models to a spherical atlas and automated parcellation of the brain regions (62). Thus, the hippocampus volume for each time point and subject was

extracted and adjusted for intracranial volume using linear regression, following previous recommendations (63). The individual rate of change in hippocampus volume was subsequently modeled in mixed-effect regression analysis (see “Statistical Analysis” section below). We caution that although the ADNI study protocol instructs that subject-specific baseline and follow-up images be acquired on the same scanner model, hardware and software upgrades could not be tracked in the ADNI data base. Such changes may introduce measurement variability but are unlikely to systematically bias the analysis of CSF sTREM2 as a predictor of hippocampal volume changes. Detailed descriptions of the longitudinal Freesurfer-based imaging pipelines applied to the ADNI data can be found online (<http://adni.loni.usc.edu/>) and in previous publications (64).

Statistical analysis

To test whether higher CSF sTREM2 was associated with slower cognitive decline in subjects with AD pathology, we conducted linear mixed-effects regression models in the pooled sample of A+T+ subjects regardless of cognitive status (28). The dependent variables were either ADNI-MEM or ADAS13. The fixed effects included CSF sTREM \times time + CSF $\text{A}\beta_{1-42}$ \times time + CSF p-tau₁₈₁ \times time + CSF sTREM2 + CSF $\text{A}\beta_{1-42}$ + CSF p-tau₁₈₁ + clinical syndrome (CN, MCI, or dementia) + age + gender + years of education. Random effects included time and intercept. The variable time was defined as the follow-up duration of the neuropsychological testing in years. Note that we controlled in each analysis for the effects of CSF $\text{A}\beta_{1-42}$ \times time and CSF p-tau₁₈₁ \times time (in addition to the simple main effects of each variable), given that we were interested in whether higher concentrations of CSF sTREM2 relative to AD pathology (as assessed by CSF $\text{A}\beta_{1-42}$ and p-tau₁₈₁) were beneficial. As an alternative way to assess CSF sTREM2 concentrations relative to primary AD pathology, we used the ratio of CSF sTREM2 to p-tau₁₈₁. Thus, a stronger increase of CSF sTREM2 relative to the increase in CSF p-tau₁₈₁ would result in a larger ratio, whereas a weaker CSF sTREM2 increase relative to that of CSF p-tau₁₈₁ would result in a smaller ratio. Ratio indices such as CSF p-tau₁₈₁/ $\text{A}\beta_{1-42}$ have been previously found to be more predictive of AD than the linear combination of each term, and thus, ratio indices may represent biologically relevant quantities (65, 66). Therefore, we repeated the linear mixed-effects regression analyses on the rate of change of hippocampus volume or cognition (ADNI-MEM and ADAS13), this time using the novel ratio index of CSF sTREM2/p-tau₁₈₁ instead of CSF sTREM2 as the predictor. We applied a Bonferroni-corrected significance threshold ($P < 0.0125$ accounting for four tests) to test our primary hypothesis in the pooled A+T+ group, i.e., higher CSF sTREM2 or CSF sTREM2/p-tau₁₈₁ ratio is associated with slower rate of decline in cognition (as measured by ADNI-MEM or ADAS13). To ensure that our analyses were not confounded by potentially violated normality assumptions, we additionally derived two-sided P values from nonparametric permutation testing for all above listed analyses. Specifically, the values of CSF sTREM2 or sTREM2/p-tau₁₈₁ were permuted 1000 times among the subjects to determine a distribution of the CSF sTREM2 \times time or sTREM2/p-tau₁₈₁ \times time coefficient under the null hypothesis. Two-sided permutation P values were derived by assessing the percentage of permuted coefficients (i.e., absolute) that surpassed the absolute of the observed coefficient. For exploratory reasons, we further tested whether our results were robust when controlling for biomarker of neurodegeneration including CSF total tau or, alternatively, hippocampus volume. To this end,

we repeated all models, this time also controlling for baseline CSF total tau levels or hippocampus volume, in addition to the other covariates (CSF p-tau₁₈₁, CSF Aβ₁₋₄₂, age, gender, and education). Next, we computed the effect size *d* for the effect of baseline CSF biomarkers on cognitive changes (31). Cohen's effect size *d* was estimated from the linear mixed models using the lme.dscore command of the EMAtools R package (see <https://cran.r-project.org/web/packages/EMAtools/EMAtools.pdf>).

Using the same linear mixed model approach as described above, we assessed in a secondary exploratory analysis whether higher CSF sTREM2 levels were associated with slower progression of neurodegeneration, as assessed via longitudinally assessed hippocampal volume, using analogous regression analyses as described above. These exploratory analyses were all tested at $P < 0.05$ uncorrected. For predicting hippocampal volume changes, a total of six separate regression analyses were performed including either CSF sTREM2 or CSF sTREM2/p-tau₁₈₁ as predictors in either the pooled sample or the MCI A+T+ and AD dementia A+T+ groups, rendering a Bonferroni-corrected threshold of $P < 0.008$ as the more stringent significance threshold.

Next, we tested disease stage-specific effects of CSF sTREM2 on cognitive changes. We repeated the linear mixed-effects regression analyses for CSF sTREM2 and CSF sTREM2/p-tau₁₈₁ stratified by diagnostic group including MCI A+T+ and AD dementia A+T+. For these exploratory analyses, we used $P < 0.05$ as the significance criterion.

Last, we tested whether higher CSF sTREM2 is associated with the conversion rate from MCI to AD. To this end, we used Cox Regression analyses, with CSF sTREM2 or the CSF sTREM2/p-tau₁₈₁ ratio as main predictor, controlled for age, gender, Aβ₁₋₄₂, and CSF p-tau₁₈₁.

SUPPLEMENTARY MATERIALS

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Fig. S1. CSF sTREM2 and CSF sTREM2 to p-tau₁₈₁ ratio per group.

Fig. S2. Selection of the final subject group.

Fig. S3. Effect of CSF sTREM2 on changes in cognition in clinical subgroups.

Fig. S4. Effect of CSF sTREM2 measurements on hippocampal changes in MCI A+T+.

Table S1. Regression analyses with additional control for CSF total tau.

Table S2. Regression analyses with additional control for hippocampus volume.

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Acknowledgments: We thank J. L. Molinuevo, J. D. Gispert, and A.-L. Boulesteix for their helpful comments. **Funding:** C.C. is supported by grants from the NIH (R01AG044546, P01AG003991, RF1AG053303, R01AG058501, and U01AG058922). This study was supported by the Deutsche Forschungsgemeinschaft (DFG) within the framework of the Munich Cluster for Systems Neurology (EXC 1010 SyNergy), a DFG funded Koselleck Project (HA1737/16-1 to C.H.), the Cure Alzheimer's Fund (to C.H.), and the Association for Frontotemporal Degeneration (FTD-biomarker award to C.H.). M.S.-C. receives funding from the European Union's Horizon 2020 Research and Innovation Program under the Marie Skłodowska-Curie action grant agreement #752310. Data collection and sharing for this project were funded by the ADNI (NIH grant no. U01 AG024904 and Department of Defense award no. W81XWH-12-2-0012). The investigators within the ADNI contributed to the design and implementation of ADNI or provided data but did not participate in analysis or writing of this study. 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. ADNI is funded by the National Institute on Aging, the National Institute of Biomedical Imaging and Bioengineering, and through generous contributions from the following: AbbVie, Alzheimer's Association, Alzheimer's Drug Discovery Foundation, Araclon Biotech, BioClinica Inc., Biogen, Bristol-Myers Squibb Company, CereSpir Inc., Cogstate, Eisai Inc., Elan Pharmaceuticals Inc., Eli Lilly and

Company, EuroImmun, F. Hoffmann–La Roche Ltd. and its affiliated company Genentech Inc., Fujirebio, GE Healthcare, IXICO Ltd., Janssen Alzheimer Immunotherapy Research and Development LLC., Johnson & Johnson Pharmaceutical Research & Development LLC., Lumosity, Lundbeck, Merck & Co. Inc., Meso Scale Diagnostics LLC., NeuroRx Research, Neurotrack Technologies, Novartis Pharmaceuticals Corporation, Pfizer Inc., Piramal Imaging, Servier, Takeda Pharmaceutical Company, and Transition Therapeutics. The Canadian Institutes of Health Research is providing funds to support ADNI clinical sites in Canada. Private sector contributions are facilitated by the Foundation for the NIH (www.fnih.org). The grantee organization is the Northern California Institute for Research and Education, and the study is coordinated by the Alzheimer's Therapeutic Research Institute at the University of Southern California. ADNI data are disseminated by the Laboratory for Neuroimaging at the University of Southern California. **Author contributions:** M.E. supervised the study, contributed to the data analysis, and wrote the manuscript. N.F. analyzed the data and contributed to writing of the manuscript. M.S.-C. performed the CSF sTREM2 measurements and contributed to writing of the manuscript. E.M.-R. helped with the ELISA measurement of sTREM2. M.A.A.C. retrieved data from ADNI and analyzed the data. G.K. provided critical revision of the manuscript. L.P. contributed to the analysis of CSF sTREM2 samples. C.C. provided critical revision of the manuscript. Y.D. contributed to the analysis of CSF sTREM2. M.D. provided critical revision of the manuscript. J.Q.T. provided critical revision of the manuscript and contributed to the collection and analysis of CSF samples. L.M.S. provided critical revision of the manuscript and contributed to the collection and analysis of CSF samples. M.W.W. designed the ADNI study and provided critical revision of the manuscript. C.H. supervised the study, measured CSF sTREM2 concentrations, and contributed to drafting the manuscript. **Competing interests:** C.H. is co-inventor on pending patent no. EP16180844 entitled "TREM2 cleavage modulators and uses thereof". C.H. is an official collaboration partner of Denali Therapeutics and a chief consultant for ISAR Biosciences. C.C. receives research support from Biogen, Eisai, Alektor, and Paragon and is a member of the advisory board of ADx Healthcare, Halia Therapeutics, and Vivid Genomics. M.W. has served on Advisory Boards for Eli Lilly, Cerecin/Accera, Roche, Alzheon Inc., Merck Sharp & Dohme Corp., Nestle/Nestec, PCORI/PPRN, Dolby Family Ventures, Brain Health Registry, and ADNI. He has consulted or has been a speaker for Cerecin/Accera Inc., Alzheimer's Drug Discovery Foundation, Merck, BioClinica, Eli Lilly, Nestle/Nestec, Roche, Genentech, Lynch Group GLC, Health & Wellness Partners, and Bionest Partners. He holds stock options in Alzheon Inc. **Competing interests:** The other authors declare that they have no competing interests. **Data and materials Availability:** All data associated with this study are in the main text or the Supplementary Materials. All ADNI data including CSF TREM2 samples are available through the ADNI Data Archive. Interested scientists may apply for access via the ADNI-LONI website (<http://adni.loni.usc.edu/>).

Submitted 4 October 2018
Resubmitted 24 December 2018
Accepted 27 April 2019
Published 28 August 2019
10.1126/scitranslmed.aav6221

Citation: M. Ewers, N. Franzmeier, M. Suárez-Calvet, E. Morenas-Rodríguez, M. A. A. Caballero, G. Kleinberger, L. Piccio, C. Cruchaga, Y. Deming, M. Dichgans, J. Q. Trojanowski, L. M. Shaw, M. W. Weiner, C. Haass, for the Alzheimer's Disease Neuroimaging Initiative, Increased soluble TREM2 in cerebrospinal fluid is associated with reduced cognitive and clinical decline in Alzheimer's disease. *Sci. Transl. Med.* **11**, eaav6221 (2019).

Increased soluble TREM2 in cerebrospinal fluid is associated with reduced cognitive and clinical decline in Alzheimer's disease

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Sci Transl Med 11, eaav6221.
DOI: 10.1126/scitranslmed.aav6221

Boosting TREM2 in AD

Alzheimer's disease (AD) is a devastating neurodegenerative disorder, and clinical trials using anti-amyloid treatments have failed. New therapeutic targets are therefore required. Recent genetic studies have revealed a major role for microglia in disease progression. Loss-of-function mutations in TREM2 dramatically increase the risk for late onset AD, therefore boosting that TREM2 function may be a useful therapeutic strategy. Ewers *et al.* examined whether increased TREM2 is protective in patients with AD. They found that higher concentrations of soluble TREM2 in cerebrospinal fluid were associated with reduced memory decline and hippocampal shrinkage in patients with AD. Thus, therapeutic strategies that boost TREM2 signaling may be beneficial in AD.

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