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Early changes in CSF sTREM2 in dominantly inherited Alzheimer's disease occur after amyloid deposition and neuronal injury

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Abstract

Emerging evidence supports a role for innate immunity and microglia in Alzheimer's disease (AD) pathophysiology. However, no marker related to microglia has been included in the temporal evolution models of AD. TREM2 is a transmembrane protein involved in innate immunity and is

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Data and materials availability: Data and tissue generated by DIAN are available to qualified investigators according to DIAN's data and tissue sharing policies (www.dian-info.org/resourcedb/). The DIAN Genetics Core follows the NIH guidelines on the sharing of genetic material and associated data as stated in the Alzheimer's Disease Genetics Sharing Plan (www.nia.nih.gov/research/dn/alzheimers-disease-genetics-sharing-plan).

SUPPLEMENTARY MATERIALS

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Materials and Methods

selectively expressed by microglia and genetically linked to AD and other neurodegenerative disorders. Its ectodomain is released by proteolysis as a soluble variant (sTREM2) and can be detected in the cerebrospinal fluid (CSF). In patients with autosomal dominant AD, we tested how many years before the expected symptom onset did CSF sTREM2 increase in mutation carriers (MCs) compared to noncarriers (NCs). We also determined the temporal sequence of changes in CSF sTREM2 and markers for amyloid deposition and neurodegeneration as well as cognitive performance. We included 218 participants consisting of 127 MC and 91 NC siblings from the Dominantly Inherited Alzheimer Network. We observed that CSF sTREM2 increased in MCs compared to NCs 5 years before the expected symptom onset and this difference remained significant until 5 years after the expected symptom onset. Changes in CSF sTREM2 occurred after alterations were observed in markers for brain amyloidosis and neuronal injury. We propose that microglial activation occurs several years before the expected symptom onset, but after amyloidosis and neuronal injury have already occurred.

INTRODUCTION

Twenty-five years after the amyloid hypothesis was proposed (1), multiple evidence supports the notion that the pathogenic sequence of Alzheimer's disease (AD) is triggered by amyloid pathology, followed by neurofibrillary tangle degeneration and cognitive decline (2–5). However, inflammatory events and, specifically, activation of microglia have been observed in basically all neurodegenerative disorders including AD, suggesting that the immune response plays an important part in the pathological cascade of neurodegeneration (6). For AD, microglial involvement was already noted in the initial publication by Alois Alzheimer in 1907 (7). Since then, the role of microglia in AD pathology has been debated, and because of the lack of selective inflammatory markers, it is still unclear whether inflammation and microglial activation or β -amyloid ($A\beta$)– and tau-related abnormalities occur first in the cascade of pathological events. This is an essential question because its answer may have major consequences not only for the relevance of the amyloid cascade but also for therapeutic approaches. This debate was reinforced by the recent identification of AD-related gene variants in loci harboring immune-related genes (8–16). Among them, *TREM2* gene mutations are of particular interest, because these are associated with an increase in the risk for AD to a similar extent as that observed for the *APOE* $\epsilon 4$ allele (9, 14). *TREM2* encodes an innate immune receptor that is expressed on the surface of cells of the myeloid lineage, such as monocytes, macrophages, osteoclasts, or microglia, in the central nervous system (17). Further studies have also reported that mutations in the *TREM2* gene may also increase the risk for Parkinson's disease (18), frontotemporal dementia (FTD) (18–20), and amyotrophic lateral sclerosis (21). Homozygous loss-of-function mutations in *TREM2* cause Nasu-Hakola disease and FTD-like syndrome (22–24), rare neurodegenerative diseases with a predominant frontal lobe phenotype, but without amyloid or neurofibrillary tangle deposition. Moreover, systems biology studies have identified *TREM2* and its adaptor protein DAP12 as central hubs in AD pathogenesis (25, 26). Loss of *TREM2* also affects the outcome of traumatic brain injury, multiple sclerosis, and cuprizone-induced demyelination (27–29). Thus, *TREM2* plays a key role in a multitude of neurological disorders and may therefore not only hold a key for understanding disease progression but also be a new and unexpected therapeutic target, probably even for cases

who have already progressed to overt disease. Functionally, TREM2 signaling affects microglial phagocytosis, migration, and chemotaxis (30–32). Therefore, it is tempting to speculate that the underlying cellular mechanisms of TREM2 mutations, which lead to an increased risk for AD and neurodegeneration, may involve phagocytosis and/or inflammatory defects. TREM2 is shed by ADAM10 (32, 33), releasing soluble TREM2 (sTREM2) into the extracellular space. Certain mutations, such as the p.T66M *TREM2* mutation, prevent appropriate folding of the ectodomain and thus lead to the retention of the full-length immature protein within the endoplasmic reticulum (ER) (32). In vivo, sTREM2 is released into the cerebrospinal fluid (CSF), where it can be quantified by enzyme-linked immunosorbent assay (ELISA) and mass spectrometry (32, 34–38). Consistent with its retention within the ER, sTREM2 is almost undetectable in the CSF and plasma of patients carrying the homozygous p.T66M *TREM2* mutation (32, 38). Moreover, we recently demonstrated that the levels of CSF sTREM2 dynamically change during normal aging and during the progression of sporadic AD, where they peak at the early symptomatic stages of the disease (37).

Here, we use CSF sTREM2 as a marker to investigate the temporal order of pathological events early during the disease and even before the onset of clinical symptoms. We evaluated CSF sTREM2 levels in cases with autosomal dominant AD (ADAD) enrolled within the Dominantly Inherited Alzheimer Network (DIAN) cohort (www.dian-info.org/de/). DIAN is a multicenter observational study that follows individuals at risk for carrying an ADAD mutation. The unique design of the DIAN project enabled us to investigate AD in its pre-clinical stages and to determine the trajectories of markers during the natural course of the disease. A seminal paper published in 2012 demonstrated that the first detectable pathological event in ADAD is the alteration of A β _{1–42} levels and the onset of brain amyloidosis [as measured by Pittsburgh compound B positron emission tomography (PIB-PET)], followed by increased tau, enhanced brain atrophy, and decreased glucose metabolism (2). Only after these preclinical changes did cognitive impairment and eventually dementia appear. A similar model of dynamic marker changes in sporadic AD has been presented by Jack and colleagues (3, 4). Although increasing evidence supports the involvement of innate immunity in AD pathogenesis (39–41), no marker related to innate immunity has been included in the temporal evolution models of AD. Here, we use the DIAN cohort to address the pivotal question of whether microglial activation precedes or follows changes in A β and tau.

RESULTS

Association of CSF sTREM2 with mutation status and age

We analyzed data from 218 participants in the DIAN cohort, including 127 mutation carriers (MCs) and 91 noncarriers (NCs). The characteristics of the participants are shown in Table 1. In the entire sample, CSF sTREM2 tended to be higher in males than in females ($F_{1,199} = 3.9$, $P = 0.051$) but was not affected by the *APOE* $\epsilon 4$ status ($F_{1,209} = 0.27$, $P = 0.603$; table S1). CSF sTREM2 was increased in MCs compared to NCs after adjusting for gender, age, and *APOE* $\epsilon 4$ status ($F_{1,201} = 4.6$, $P = 0.033$; Fig. 1A and table S1). Among MCs, CSF sTREM2 levels were not different between the three ADAD mutations (*PSEN1*, *PSEN2*, and

APP; $F_{2,36} = 2.2$, $P = 0.125$; Fig. 1B). Moreover, the amount of CSF sTREM2 was not associated with the clinical severity of mutation subtype, as measured by the mean age of symptom onset, which is specific for different mutations in the *PSEN1*, *PSEN2*, and *APP* genes. ($\beta = -0.159$, $SE = 0.097$, $P = 0.105$; Fig. 1C). However, CSF sTREM2 was positively associated with age in both MCs ($\beta = +0.526$, $SE = 0.075$, $P < 0.0001$) and NCs ($\beta = +0.330$, $SE = 0.109$, $P = 0.003$; Fig. 1D).

Early increase of CSF sTREM2 in ADAD patients

To study changes in CSF sTREM2 during the asymptomatic and symptomatic progression of AD, we used a linear mixed model including mutation status, estimated years from expected symptom onset (EYO; and its quadratic and cubic terms, and the interactions with mutation status), and gender as fixed effects and family affiliation as a random effect. The interactions of the quadratic and the cubic terms with mutation status were significant predictors of CSF sTREM2 ($P = 0.0009$ and $P = 0.008$, respectively; table S2), suggesting a nonlinear relationship between EYO and CSF sTREM2 that differed between MCs and NCs. On the basis of the estimated trajectory of CSF sTREM2 levels across EYOs, we compared the CSF sTREM2 levels at 5-year EYO intervals by *t* tests, as described by Bateman *et al.* (2). CSF sTREM2 started to be significantly increased in MCs compared to NCs at EYO = -5, and this difference remained significant until EYO = +5 years (Fig. 2, Table 2, and fig. S1). In more advanced stages (EYO > +5), differences did not reach statistical significance, which might, however, be due to the low number of participants in later EYOs (Fig. 2). To confirm that our results were not influenced by age, *APOE* $\epsilon 4$ status, or education, we also computed the estimates of CSF sTREM2 for each 5-year EYO adjusting by age (table S3), *APOE* $\epsilon 4$ status (table S4), and education (table S5), and the values were similar to those without including these covariables.

An increase in CSF sTREM2 follows initial changes in A β and tau

We compared the time course of alterations in CSF sTREM2 with changes in the temporal evolution of biochemical, neuroimaging, and cognitive markers along the range of -25 to +10 EYO (Table 2). We observed that levels of CSF sTREM2 followed A β deposition, as measured by PIB-PET imaging, a decrease in CSF A β_{1-42} , and an increase in CSF T-tau, but were concurrent with precuneus FDG-PET SUVR, hippocampus volume, and cognitive (MMSE) changes (Fig. 3 and Table 2). These findings demonstrate that, at least in ADAD patients, microglial activity (as measured by an increase in CSF sTREM2) did not precede but rather followed A β and tau and associated neurodegenerative changes, which is in line with the amyloid cascade hypothesis. Of note, similar results were obtained when linear (fig. S2A), quadratic (fig. S2B), and cubic models were used (fig. S2C; for details, see the Supplementary Materials).

CSF sTREM2 association with CSF core markers of AD

The associations between CSF sTREM2 and each of the core CSF markers of AD were tested in linear regression models adjusted for age, gender, and *APOE* $\epsilon 4$ status. In the pooled sample of subjects, higher levels of CSF sTREM2 were associated with higher levels of CSF T-tau ($\beta = +0.349$, $SE = 0.065$, $P < 0.0001$; Fig. 4A) and P-tau_{181P} ($\beta = +0.307$, $SE = 0.060$, $P < 0.0001$; Fig. 4B), but no association was found with A β_{1-42} ($\beta = -0.020$, $SE =$

0.065, $P = 0.764$; Fig. 4C). In NCs, higher CSF sTREM2 was associated with higher CSF T-tau, P-tau_{181P}, and A β ₁₋₄₂ (T-tau: $\beta = +0.371$, SE = 0.108, $P = 0.001$; P-tau_{181P}: $\beta = +0.360$, SE = 0.122, $P = 0.004$; A β ₁₋₄₂: $\beta = +0.313$, SE = 0.099, $P = 0.002$; Fig. 4, D to F). In MCs, higher CSF sTREM2 was associated with both higher CSF T-tau and P-tau_{181P} (T-tau: $\beta = +0.298$, SE = 0.088, $P = 0.001$; P-tau_{181P}: $\beta = +0.270$, SE = 0.079, $P = 0.001$; Fig. 4, G and H), but not A β ₁₋₄₂ ($\beta = -0.073$, SE = 0.088, $P = 0.409$; Fig. 4I). The sample contained some extreme values of T-tau and P-tau_{181P}; the analysis with and without these values yielded similar results.

CSF sTREM2 is selectively increased in patients with mild clinical symptoms

Next, we assessed the changes in CSF sTREM2 as a function of clinical stages (as measured using the CDR scale). We compared CSF sTREM2 levels between NCs ($n = 91$; CDR = 0, $n = 88$; CDR = 0.5, $n = 3$), asymptomatic MCs (CDR = 0, $n = 52$), MCs with very mild dementia (CDR = 0.5, $n = 51$), MCs with mild dementia (CDR = 1, $n = 16$), and MCs with moderate to severe dementia (CDR = 2, $n = 8$). CSF sTREM2 differed between groups ($F_{4,206} = 5.0$, $P = 0.001$) adjusted for gender, age, *APOE* $\epsilon 4$ status, and education. CSF sTREM2 was increased in MCs with CDR = 0.5 and with a CDR = 1 compared to NCs ($P = 0.006$ and $P = 0.044$, respectively) and to the MC group with CDR = 0 ($P = 0.003$ and $P = 0.021$, respectively; Fig. 5). MCs with CDR = 2 did not have significantly increased CSF sTREM2 compared to the rest of the groups, although this should be interpreted with care because of the low number of patients in this clinical stage.

DISCUSSION

Here, we have demonstrated that CSF sTREM2 increases early in the progression of ADAD, that is, before the expected onset of symptoms, but after amyloidosis and neuronal injury have already begun. This finding supports the amyloid cascade hypothesis and adds the first innate immunity marker to a temporal model of AD progression (see Fig. 3).

The strength of this study is the inclusion of a large and very well characterized cohort of people destined to develop AD. Moreover, studying ADAD has the advantage that the prevalence of other co-morbidities is lower than in sporadic AD, and hence, the changes in any marker can be mainly attributed to AD and not to other conditions. However, such analyses have their natural limitations. First, the changes of CSF sTREM2 through the course of the disease are analyzed in cross-sectional data. Second, more participants in the later stages of the disease (that is, EYO > +5) would be required for confirmation of the tendency of CSF sTREM2 to return to normal levels (Fig. 3). Third, the detection of markers in any study, not only in the one described here, depends on the relative sensitivity of the technique used to detect differences, which may not be equal between different markers (42). In regard to the sensitivity of sTREM2 detection, we have shown previously the potential of our ELISA assay to sensitively detect even minor changes in sTREM2 (32, 37). Fourth, ADAD and sporadic AD are different entities. However, both share major pathophysiological similarities, and both appear to be triggered by the amyloid cascade (43). Fifth, as we previously stated (32, 37), the overlapping values of CSF sTREM2 levels between patients and controls preclude its use as a diagnostic marker, but it provides a

marker to track microglial activity throughout the evolution of the disease. Finally, the results presented here need to be confirmed with an independent microglia-related marker (for example, PET tracer ^{18}F -GE180, a ligand of the 18-kDa translocator protein TSPO and a marker of activated microglia) (44, 45). Nonetheless, our previous results where we observed that TREM2 activity, as measured by phagocytic capacity, correlated with its surface transport and subsequent shedding support the notion that sTREM2 levels reflect microglia activation (32).

Although it is well established that microglial activation and neuroinflammation frequently accompany the early development of amyloid and tau pathology (39–41, 46), the temporal evolution of glia-related marker changes in AD has not been addressed. For decades, there was an intensive discussion about which comes first, inflammation triggering amyloid deposition or neuronal injury, or vice versa (47, 48). The unique design of the DIAN study allowed us to evaluate the dynamic changes of CSF sTREM2 and determine the temporal order of these changes compared to changes in markers of amyloid deposition and neurodegeneration and measures of cognitive performance. CSF sTREM2 levels start to significantly increase in MCs 5 years before the expected onset of symptoms and remain high until 5 years after the expected onset of symptoms. Thus, changes in CSF sTREM2 occur later than alterations in brain amyloidosis and neuronal injury biomarkers, which occur at least 15 years before the expected symptom onset (Fig. 3 and Table 2). CSF sTREM2 changes are concurrent with the first signs of cerebral hypometabolism, hippocampal atrophy, and cognitive impairment (as measured by MMSE), which all become significant 5 years before the expected onset of the symptoms. Together, these findings support the notion that microglia involvement in AD is a relatively early event, which, however, follows changes in amyloid deposition and neuronal injury. This is consistent with the amyloid cascade hypothesis, which states that amyloid is the driving force of AD pathogenesis (5, 49). This may also explain why we and others do not observe clear associations between CSF $\text{A}\beta_{1-42}$ and CSF sTREM2 in AD (35–38). Our results show that the CSF sTREM2 increase occurs after amyloidosis, when markers of neuronal injury have already become abnormal; thus, CSF $\text{A}\beta_{1-42}$ and CSF sTREM2 change at two different stages of AD and do not evolve simultaneously. Instead, CSF sTREM2 changes are closely associated with CSF T-tau and P-tau_{181p}, which are markers of neurodegeneration subsequent to the development of $\text{A}\beta$ deposition. Thus, the increase of CSF sTREM2 may reflect an acute response to early and probably still subtle neuronal injury. These findings are in line with studies proposing that TREM2 recognizes apoptotic neurons and mediates their phagocytosis (50, 51). Detailed studies in large and well-defined cohorts using PET imaging techniques may be required to further support and extend the temporal evolution of disease progression proposed in our study. Currently available results of microglia PET imaging in AD are mixed and hampered by small sample sizes (52, 53). Nevertheless, a recent study using a second-generation TSPO PET tracer suggests microglial activation as early as the presymptomatic stages and further increasing in the prodromal stage of sporadic AD (54). These findings are in general agreement with our conclusions. In contrast, available data about astroglial activation in a small group of ADAD subjects using the astrocyte-specific PET tracer [^{11}C]deuterium-L-deprenyl suggest a different course

regarding changes in astrogliosis, with an initial peak at the presymptomatic stage and a subsequent decline (55, 56).

Increases in CSF sTREM2 plateaued in later symptomatic stages in MCs, although other markers of neurodegeneration, neuronal death, and dysfunction (for example, hippocampal atrophy, CSF T-tau, and FDG-PET hypometabolism) continued to increase. This observation may be explained by microglial senescence, which occurs during aging and progresses to a dystrophic pathological stage. Dystrophic but not activated microglia are abundant in later stages of AD (57, 58). The current results are consistent with our previous observation in sporadic AD dementia patients, who had lower CSF sTREM2 compared to patients in the mild cognitive impairment (MCI) stage, despite having higher CSF T-tau (37). Notably, Fagan *et al.* (59) described that CSF markers of neurodegeneration (T-tau, P-tau_{181P}, and VILIP-1) decreased in symptomatic ADAD patients in a study including longitudinal CSF samples, a finding that was not observed in a previous cross-sectional study from the same group (2). It can be speculated that CSF sTREM2 may also follow a descending trajectory in later stages, in parallel to that observed with neuronal injury markers. Results from neuropathological studies suggest that the amyloid burden needs to exceed a certain threshold to trigger a glial response, but once this response is initiated, it is less dependent on amyloid plaques and more closely related to neurofibrillary degeneration (60, 61). The fact that microglial activation follows amyloidosis and neuronal injury does not exclude the possibility that dysfunctional microglia may in turn contribute to subsequent amyloidosis and neuronal injury, for example, by reduced amyloid plaque clearance (32) or reduced protection from amyloid plaque growth (62, 63).

Finally, our findings are also relevant to the design of secondary prevention clinical trials. CSF sTREM2 may be useful to monitor the efficacy of drugs targeting inflammation. Thus, CSF sTREM2 may not only be a marker of microglial activity in response to neuronal injury but also serve as a therapeutic marker. In this regard, it will be interesting to follow changes in sTREM2 during anti-A β immunotherapeutic studies.

MATERIALS AND METHODS

Study design

We studied individuals carrying an ADAD mutation (*PSEN1*, *PSEN2*, or *APP*) and their NC siblings included in the DIAN. DIAN [National Institute on Aging (U19 AG032438); R.J.B., principal investigator] was initiated in 2008. The main goal of DIAN is to develop a registry of families with known ADAD mutations and investigate the pathophysiological changes that occur in the different stages of AD (2, 64). The study is coordinated from Washington University, with participation by 15 other centers worldwide. We measured CSF sTREM2 for 221 participants, but 3 of them had no clinical information and were excluded from the analysis. Thus, the study sample consisted of a total of 218 participants, 127 MCs and 91 NCs. The sample size was calculated while taking into account the effect size of the difference in CSF sTREM2 between healthy controls, MCI, and AD patients that we previously described (32, 37) to reach an 80% power and a 5% significance.

DIAN study participants undergo a comprehensive clinical and neuropsychological evaluation that has been described elsewhere (2, 65). EYO was calculated as the difference between the participant's age at evaluation and the age of parental symptom onset (2). The presence of an ADAD mutation and the *APOE* genotype was determined using standard methods described elsewhere (66). CSF collection followed standard procedures (59, 67), and the measurements of CSF A β ₁₋₄₂, T-tau, and P-tau_{181P} were performed using the Luminex bead-based multiplexed xMAP technology (INNO-BIA AlzBio3, Innogenetics) (59).

Measurement of sTREM2

CSF sTREM2 was measured by an ELISA previously established by our group using the MSD platform (see the Supplementary Materials) (32). CSF samples were randomly distributed across plates and measured in triplicate. The operator was blinded to the clinical information. Two dedicated CSF samples (ISs) consisting of pooled CSF were loaded onto all plates. To account for the interplate variability, all the measurements were expressed in relation to the IS with the highest sTREM2 concentration. The absolute values (ng/ml) are reported in Table 1, and the estimated absolute levels of CSF sTREM2 at different 5-year intervals of EYO are shown in fig. S1 and table S6; the results are similar to those for relative CSF sTREM2. The mean intraplate coefficient of variation (CV) was 3.1% (all triplicate measures had a CV of <15%), and the interplate CV for each of the IS was 11.0 and 7.4%.

Statistical analysis

Comparisons of demographic, clinical, and biochemical data between NCs and MCs were performed by Pearson's χ^2 tests, *t* tests, or Mann-Whitney tests, as appropriate. CSF sTREM2 values calculated by interpolation on the standard curve and normalized to an IS were subsequently square root-transformed to approach Gaussian normal distribution, where no significant deviation from normal was found after transformation (Shapiro-Wilk test, *P* = 0.165). All subsequent analyses were done with the transformed CSF sTREM2 values.

To investigate the effect of mutation status (MCs versus NCs) on CSF sTREM2, we performed a linear mixed model regression analysis including the mutation status, age, gender, and *APOE* ϵ 4 as independent fixed effects and family affiliation as a random intercept effect. *APOE* genotype was dichotomized into *APOE* ϵ 4 carrier and noncarrier groups. In additional linear regression models, we compared the levels of CSF sTREM2 between mutation subtypes including *PSEN1*, *PSEN2*, and *APP* mutations. The association between CSF sTREM2 levels and mean age of symptom onset caused by individual mutations on *PSEN1*, *PSEN2*, and *APP* genes [defined as the published mean age of symptom onset of a particular mutation (68)] was tested in a linear model adjusted for gender and *APOE* ϵ 4 status.

To test how CSF sTREM2 and other biochemical, cognitive, and imaging markers change as a function of EYO (see Fig. 3 and Table 2), we used a similar approach to that previously published by Bateman *et al.* (2) for the sake of comparability of the current findings. That is, a linear mixed model with mutation status, EYO (and its interaction with mutation status),

gender (fixed effects), and family affiliation (random effect) was computed for each of the markers and cognitive variables. Consistent with the approach by Bateman *et al.* (2), we performed a polynomial regression analysis including EYO quadratic (EYO^2) and cubic (EYO^3) terms and their interactions with mutation status. For each outcome variable, the model that best fitted the data was determined by forward selection of the predictors, and the final model was chosen on the basis of the Akaike information criterion (AIC; a lower AIC indicates a better fit; see table S7). To ensure that the results were not driven by extreme values of EYO, the analyses were repeated, this time excluding participants with $EYO > +20$ (two NCs and one MC). The resulting models were similar to those calculated including these three participants. To determine differences in markers and clinical measurements between MCs and NCs at different EYOs, we computed the estimated levels of each variable at each 5-year interval of EYO on the basis of the established regression models. Group differences in the estimated values between MCs and NCs for each 5-year EYO interval were determined by *t* tests, as done by Bateman *et al.* (2). Consistent with the approach applied in previous DIAN studies (2, 59), comparisons between MCs and NCs were restricted at EYO ranging from -25 to $+10$, because of the low number of subjects at more extreme values of EYO (see Fig. 2). To assure that the sequence of marker changes was not dependent on our model selection procedure, we plotted the marker curves for each marker, with the model order (first, second, or third order) held constant across marker models (fig. S2). Table S8 depicts for each model order the earliest time point (EYO) at which a particular marker became different between MCs and NCs. The sequential order of marker changes remained consistent across different fixed model orders, confirming the robustness of our findings.

To plot the progression of CSF sTREM2 throughout the disease in the context of the other biomarkers and cognitive measures, we followed an approach first presented by Bateman *et al.* (2), that is, for each variable, the predicted difference between MCs and NCs at each EYO generated by the same final linear mixed effects model described above was divided by the SD of the respective variable within the pooled sample so that the scale of all variables was standardized (Fig. 3 and fig. S2). These figures were built with the SAS software (SAS Institute).

We compared CSF sTREM2 levels between NCs and MCs in different clinical stages (determined by global CDR) in a linear mixed model regression analysis adjusted for age, gender, *APOE* $\epsilon 4$ status, and education as independent fixed effects and family affiliation as random intercept. Individuals falling into $CDR = 2$ to $CDR = 3$ were grouped together because of the low number of subjects in these stages. Post hoc tests were used for pair-wise comparisons of CSF sTREM2 levels between CDR groups, using Bonferroni correction.

The association between CSF sTREM2 and the CSF core markers for AD (T-tau, P-tau_{181P}, and $A\beta_{1-42}$) was studied with a linear model adjusted for age, gender, and *APOE* $\epsilon 4$ status. The analysis was performed in the whole sample of subjects and when stratifying for the mutation status. The standardized regression coefficients (β) are reported. To rule out that the associations were driven by extreme values, we performed the analysis both including or excluding outliers (defined as AD CSF core markers 3 SDs below or above the group mean), and the analysis yielded similar results.

Statistical analysis was performed in SPSS IBM, version 20.0, statistical software and the freely available statistical software R (<http://www.r-project.org/>). All tests were two-tailed, with a significance level of $\alpha = 0.05$.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1. Association of CSF sTREM2 with mutation status and age

(A) Comparison of CSF sTREM2 between NCs and MCs in the DIAN cohort. (B) Comparison of CSF sTREM2 in MC participants carrying a *PSEN1*, *PSEN2*, or *APP* mutation. *PSEN2* MCs (black dots) were merged with *PSEN1* MCs because of the low number of subjects. ns, nonsignificant. (C) Regression plot showing CSF sTREM2 as a function of the mean age of symptom onset for each individual mutation. (D) Regression plot showing CSF sTREM2 as a function of age in the NC and MC groups. The blue or red bars in (A) and (B) represent the mean and the 95% confidence interval (CI). Group comparisons were assessed by a linear mixed model adjusted for age, gender, and *APOE* $\epsilon 4$ status (fixed effects) and family affiliation (random effect). The solid lines in (C) and (D) indicate the regression line for each of the groups calculated by a linear model adjusted for gender and *APOE* $\epsilon 4$ status. The dashed lines indicate the 95% CI. The standardized regression coefficients (β) and the *P* values are also shown. In graph (D), the individual values are not shown to protect participants' confidentiality.

Fig. 2. CSF sTREM2 at specific EYOs

Estimated mean levels of CSF sTREM2 for NCs and MCs at successive 5-year intervals of EYO, based on the regression model that best fitted the data (see table S7 and “Statistical analysis”). The amount of CSF sTREM2 is expressed relative to an IS and has been square root–transformed (sqrt) to approach a Gaussian distribution. The error bars represent the SE. CSF sTREM2 was higher in MCs than in NCs from EYO = −5 to EYO = +5. ** $P < 0.01$; *** $P < 0.001$. The number (n) of NC and MC individuals studied for each EYO interval is tabulated below the x axis.

Fig. 3. Cross-sectional comparison of CSF sTREM2, cognitive, structural, metabolic, and biochemical changes as a function of EYO

The graph represents the standardized differences between MCs and NCs as a function of EYO generated by the linear mixed effects model that best fit each marker (table S7). The order of differences suggests that the increase in CSF sTREM2 is preceded by changes in brain amyloidosis (that is, decreased CSF A β ₁₋₄₂ and fibrillar A β deposition) and alteration in brain injury markers (that is, CSF T-tau). Yet, CSF sTREM2 changes are concurrent with the first signs of cerebral hypometabolism, hippocampal atrophy, and clinical impairment. Individual data points are not displayed to prevent disclosure of mutation status. The shaded area indicates the EYO interval (from -5 to +5), in which CSF sTREM2 is increased in MCs compared to NCs.

Fig. 4. Association between CSF sTREM2 and CSF core markers of AD

Scatter plots representing the associations of CSF sTREM2 with each of the CSF core markers of AD (T-tau, P-tau_{181P}, and A β_{1-42}) in all participants [green; (A) to (C)], in NCs [blue; (D) to (F)], and in MCs [red; (G) to (I)]. The solid lines indicate the regression line for each of the groups calculated by a linear model adjusted for age, gender, and *APOE* $\epsilon 4$ status. The dashed lines depict the 95% CI. The standardized regression coefficients (β) and the *P* values are also shown. The sample contained some outliers (defined as 3 SDs below or above the group mean) of the CSF core markers of AD (2 T-tau and 1 P-tau_{181P} value). To exclude that the associations were not driven by these extreme values, we performed the analysis with and without these values. The graphs are represented without the outliers.

Fig. 5. CSF sTREM2 and clinical stages

CSF sTREM2 in NCs and MCs at different clinical stages, as defined by the CDR score (0, cognitively normal; 0.5, very mild; 1, mild; 2, moderate; 3, severe dementia). Participants with a CDR ≥ 2 were grouped together because of the low number of subjects in these groups. The blue or red bars represent the mean and the 95% CI. Group differences were assessed by a linear mixed model adjusted for age, gender, *APOE* $\epsilon 4$ status, and education (fixed effects) and family affiliation (random effect).

Table 1
Characteristics of participants

Data are expressed as number of patients and percentage (%), mean and SD, or median and interquartile range. Pearson's χ^2 test was used for the group comparisons of categorical variables, *t* test was used to compare continuous variables, and Mann-Whitney test was used to compare ordinal variables. IS, internal standard; MMSE, Mini-Mental State Examination; T-tau, total tau; P-tau_{181P}, tau phosphorylated at Thr¹⁸¹.

	MCs (<i>n</i> = 127)	NCs (<i>n</i> = 91)	P (group effect)
Age (years)	40.4 (11)	39.5 (11)	0.556
Females, no. (%)	63 (50)	49 (54)	0.537
<i>APOE</i> ε4 carriers, no. (%)	36 (28)	31 (34)	0.367
Participant EYO (years)	−6.1 (10)	−7.5 (12)	0.350
MMSE	28 (24–30)	29 (28–30)	<0.0001 *
Cognitive status, no. (%) [†]			<0.0001 *
CDR = 0	52 (41)	88 (97)	
CDR = 0.5	51 (40)	3 (3)	
CDR = 1	16 (13)	0	
CDR = 2 to 3	8 (6)	0	
Family mutations, no. (%) [‡]			0.040 *
<i>PSEN1</i>	97 (76)	55 (60)	
<i>PSEN2</i>	11 (9)	12 (13)	
<i>APP</i>	19 (15)	24 (26)	
CSF biomarkers			
Aβ _{1–42} (pg/ml)	315 (155)	430 (136)	<0.0001 *
T-tau (pg/ml)	133.0 (98.3)	60.5 (27.6)	<0.0001 *
P-tau _{181P} (pg/ml)	71.3 (42.0)	30.5 (10.3)	<0.0001 *
CSF sTREM2 [§]			
Relative to an IS	0.590 (0.290)	0.480 (0.219)	0.033 *
Absolute (ng/ml)	3.561 (1.661)	2.824 (1.292)	0.004 *

* Significant differences.

[†] Cognitive status was defined by the clinical dementia rating (CDR) score (0, cognitively normal; 0.5, very mild; 1, mild; 2, moderate; 3, severe dementia).

[‡] In NC participants, the mutation present in their family is shown.

[§] CSF sTREM2 levels were square root-transformed and assessed by a linear mixed model adjusted for age, gender, and *APOE* ε4 status (fixed effects) and family affiliation (random effect). The *P* values indicated in the last column refer to the group effects in these tests.

Table 2
Markers and clinical estimates in MCs and NCs as a function of EYO

Mean estimated levels of biomarkers, neuroimaging, and cognitive variables for each group and selected EYO. Estimates were obtained by a linear mixed model including mutation status, EYO (or higher-order terms and their interactions with mutation status), and gender as fixed effects and family affiliation as random effect. For each EYO, the group difference, 95% CI, and *P* value for the two-sample independent *t* test of each variable are reported. Differences are calculated from unrounded values. CSF sTREM2 is expressed relative to an IS and square root-transformed to approach a Gaussian distribution. Given that CSF sTREM2 levels are affected by age, we repeated the regression analysis introducing age as a covariate, and the pattern of group differences remained the same (table S3). Adjusting by *APOE*ε4 (table S4) or education (table S5) also yielded similar results. FDG, fluorodeoxyglucose; PVE, partial volume effect; SUVR, standardized uptake value ratio.

EYO								
	-25	-20	-15	-10	-5	0	+5	+10
CSF sTREM2 (relative to IS, square root-transformed)								
NCs	0.66	0.67	0.68	0.68	0.70	0.72	0.77	0.84
MCs	0.59	0.60	0.66	0.74	0.82	0.89	0.91	0.87
Difference	-0.08	-0.07	-0.02	0.05	0.12	0.16	0.14	0.04
95% CI	-0.20 to 0.05	-0.17 to 0.02	-0.11 to 0.07	-0.03 to 0.14	0.04-0.21	0.07-0.25	0.05-0.24	-0.10 to 0.17
P	0.220	0.132	0.660	0.215	0.005*	0.0006*	0.004*	0.594
Aβ deposition in the precuneus (PIB-PET binding potential, PVE-corrected)								
NCs	0.21	0.26	0.29	0.32	0.35	0.36	0.36	0.35
MCs	0.39	0.52	0.73	1.02	1.41	1.87	2.43	3.07
Difference	0.19	0.26	0.43	0.70	1.06	1.52	2.07	2.72
95% CI	-0.43 to 0.81	-0.21 to 0.74	-0.01 to 0.88	0.24-1.15	0.61-1.51	1.08-1.96	1.61-2.53	2.12-3.32
P	0.551	0.276	0.057	0.003*	<0.0001*	<0.0001*	<0.0001*	<0.0001*
CSF Aβ ₁₋₄₂ (pg/ml)								
NCs	460	458	456	453	451	449	446	444
MCs	475	437	400	363	325	288	250	213
Difference	14	-21	-56	-91	-126	-161	-196	-231
95% CI	-76 to 105	-100 to 59	-127 to 15	-158 to -24	-193 to -59	-233 to -89	-276 to -116	-322 to -140
P	0.754	0.610	0.125	0.008*	0.0003*	<0.0001*	<0.0001*	<0.0001*

EYO								
	-25	-20	-15	-10	-5	0	+5	+10
CSF T-tau (pg/ml)								
NCs	56.3	59.5	62.6	65.8	68.9	72.1	75.2	78.4
MCs	57.0	79.2	101.5	123.7	146.0	168.2	190.5	212.7
Difference	0.6	19.7	38.9	58.0	77.1	96.2	115.3	134.4
95% CI	-45.5 to 46.7	-20.6 to 60.1	2.8-74.9	24.3-91.6	43.5-110.7	60.2-132.1	75.1-155.5	88.5-180.3
P	0.978	0.336	0.035 *	0.0008 *	<0.0001 *	<0.0001 *	<0.0001 *	<0.0001 *
Glucose metabolism in the precuneus (FDG-PET SUVR, PVE-corrected)								
NCs	1.96	1.95	1.94	1.93	1.92	1.91	1.90	1.89
MCs	2.00	1.94	1.88	1.83	1.77	1.72	1.66	1.60
Difference	0.04	-0.01	-0.06	-0.10	-0.15	-0.19	-0.24	-0.28
95% CI	-0.12 to 0.19	-0.14 to 0.12	-0.17 to 0.06	-0.21 to 0.01	-0.26 to -0.04	-0.31 to -0.08	-0.37 to -0.11	-0.44 to -0.13
P	0.632	0.892	0.358	0.072	0.009 *	0.002 *	0.001 *	0.0003 *
Total hippocampal volume (mm ³)								
NCs	9902	9653	9462	9327	9248	9227	9261	9353
MCs	9924	9701	9419	9076	8674	8211	7689	7107
Difference	22	48	-43	-251	-575	-1015	-1572	-2245
95% CI	-729 to 773	-524 to 620	-578 to 491	-796 to 295	-1117 to -32	-1546 to -484	-2153 to -991	-3028 to -1463
P	0.953	0.869	0.873	0.366	0.038 *	0.0002 *	<0.0001 *	<0.0001 *
MMSE score (no.)								
NCs	29.3	29.3	29.2	29.2	29.2	29.2	29.2	29.2
MCs	29.4	29.2	28.5	27.5	26.0	24.0	21.6	18.8
Difference	0.0	-0.1	-0.7	-1.7	-3.2	-5.2	-7.5	-10.4
95% CI	-3.3 to 3.4	-2.7 to 2.47	-3.1 to 1.7	-4.2 to 0.7	-5.7 to -0.9	-7.5 to -2.8	-10.1 to -5.0	-13.9 to -6.9
P	0.984	0.933	0.563	0.159	0.009 *	<0.0001 *	<0.0001 *	<0.0001 *

* Significant differences.