

Longitudinal increase of CSF soluble TREM2 is driven by early aggregation of A β 42 and associates with slower amyloid deposition and clinical decline in autosomal-dominant Alzheimer's disease

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Title page

Longitudinal increase of CSF soluble TREM2 is driven by early aggregation of A β ₄₂ and associates with slower amyloid deposition and clinical decline in autosomal-dominant Alzheimer's disease

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Abstract

Therapeutic modulation of TREM2-dependent microglial function provides an additional strategy to slow progression of Alzheimer disease (AD). Although studies on animal models suggest that TREM2 is protective, the trigger of increased TREM2 expression during disease progression and its clinical and pathological consequences in AD remain unclear. We measured longitudinally soluble TREM2 (sTREM2) as a surrogate marker for protective TREM2-signalling in cerebrospinal fluid (CSF) from participants in the Dominantly Inherited Alzheimer Network (DIAN) observational study. In mutation carriers (MC), the longitudinal sTREM2 increase followed the earliest aggregation of A β ₄₂ captured by CSF-A β ₄₂ decrease, but not yet by Pittsburgh compound-B Positron Emission Tomography (PiB-PET). Higher sTREM2 increase rates provided protection from A β -deposition, whereas lower rates enhanced p-tau increase associated with PiB-PET increase. Moreover, presymptomatic MC with high or low sTREM2 increase rates have opposite associations between CSF A β ₄₂ and PiB-PET longitudinal changes, suggesting that TREM2 modifies A β plaque deposition and compaction. Finally, higher sTREM2 increase rates protected from cortical shrinkage and cognitive decline. Our findings position the TREM2 response within the amyloid cascade right after the first pathological changes in A β ₄₂ aggregation, support ongoing efforts to develop TREM2 modulating therapies, and predict a very early window for therapeutic intervention.

1 Introduction

2 Recent advances in understanding the dynamic responses of microglia to pathological challenges such
3 as the deposition of insoluble proteins and cell death during neurodegeneration have increased our
4 knowledge of immune cell function in the brain and even allowed the development of novel
5 therapeutic approaches^{1,2}. Historically, microglia were primarily believed to pathologically contribute
6 to disease progression, *e.g.*, by promoting aberrant synaptic pruning and activation of the
7 inflammasome^{3,4}. However, single-cell sequencing technologies have identified dynamic microglial
8 populations, which sense their environment and trigger defensive responses to Alzheimer disease
9 (AD) pathology⁵. Moreover, large genome-wide association studies have identified a number of risk
10 variants for sporadic AD in genes expressed exclusively within microglia in the brain. Specifically,
11 analyses of the disease-associated variants in the triggering receptor expressed on myeloid cells 2
12 (TREM2) were extremely intriguing as they strongly suggest that loss-of-function mutations are
13 associated with an increased risk for late onset AD (LOAD)⁶⁻⁹. TREM2 loss of function locks
14 microglia in a homeostatic state and prevents the switch to disease-associated microglia (DAM)^{5,10}.
15 Since DAM facilitate lipid metabolism, efficiently remove amyloid β -peptide (A β) seeds, and form a
16 barrier around amyloid plaques, their protective activities are now explored for disease modifying
17 therapeutic strategies^{1,2}. In that regard, a variety of different TREM2-agonistic antibodies have been
18 generated, all triggering several protective microglial functions, by stabilizing and cross-linking
19 signalling-competent TREM2¹. Moreover, one of these antibodies already passed a phase-1 clinical
20 trial and multiple therapeutic antibodies will soon be tested for their efficacy in AD patients¹¹. It is,
21 therefore, of greatest importance to translate our knowledge on protective TREM2 functions from
22 animal models to AD patients. Quantitative analysis of soluble TREM2 (sTREM2) in cerebrospinal
23 fluid (CSF) as a surrogate marker for TREM2-dependent microglial-activation allows such
24 translational efforts. We have previously shown that cell-surface full-length TREM2 is shed by
25 proteases of the ADAM family, releasing soluble TREM2 (sTREM2) into biological fluids including
26 CSF^{9,12}. Since only cell-surface full-length TREM2 is capable to efficiently initiate downstream

1 signalling, and ADAM proteases cleave TREM2 preferentially on the plasma membrane^{9,12}, CSF
2 sTREM2 can be considered as a surrogate marker for TREM2 expression and signalling.

3 Cross-sectional studies have shown that CSF sTREM2 levels are elevated in late presymptomatic and
4 in early symptomatic stages both in sporadic and in autosomal-dominant AD (ADAD), and correlate
5 with tau pathology-related markers in CSF (total tau -t-tau- and phosphorylated tau on threonine 181 -
6 p-tau-), reinforcing the idea of TREM2-dependent microglial activation as a defensive response to AD
7 pathology¹³⁻¹⁶. Higher CSF sTREM2 levels at baseline are also associated with a slower hippocampal
8 shrinkage and slower memory decline in symptomatic phases of sporadic LOAD supporting the
9 potential beneficial role of TREM2¹⁷. However, cross-sectional CSF sTREM2 levels only give an
10 estimate of the microglial activation state at a single time-point and are influenced by a high
11 interindividual variability. Therefore, cross-sectional CSF sTREM2 levels do not represent the
12 dynamic TREM2-dependent microglial response during AD progression. In fact, longitudinal studies
13 are more accurate than cross-sectional studies to investigate pathological processes occurring in AD
14 as they have a higher power to discriminate the temporal changes and the dynamic relationships
15 between them¹⁸⁻²⁰. Moreover, based on longitudinal amyloid PET imaging studies and seeding
16 experiments in mice²¹, the beneficial effect of TREM2-dependent microglial functions is expected to
17 be most effective in the earliest stages of A β -deposition. Thus, any effects of TREM2 on disease
18 initiation and progression must be studied during an asymptomatic phase of patients known to
19 develop AD symptoms at later time points. In contrast to sporadic AD, ADAD has a predictable
20 clinical onset in each family and penetrance of the involved mutations is mostly complete²². That
21 allows to stage individuals relative to their expected year of symptom onset and enabled us to study
22 biomarker dynamics from a very early presymptomatic phase in a way that is not possible with
23 sporadic AD patients. The Dominantly Inherited Alzheimer Network (DIAN) observational study has
24 recruited a large number of participants from families suffering from ADAD, many of them with
25 longitudinal markers of A β -deposition, tau-related pathology, neuronal death and dysfunction, and
26 longitudinal cognitive evaluations^{23,24}. We studied the longitudinal change of CSF sTREM2
27 throughout the course of AD in the observational DIAN cohort, the factors triggering this change, and

the relationship between the dynamics of CSF sTREM2 and the dynamics of other biomarkers representing A β -accumulation and deposition, tau-pathology and brain structure, as well as the influence of the dynamics of CSF sTREM2 on cognitive decline. Our goal was to explore potential protective activities of TREM2 during the presymptomatic phase of the disease, to search for an interaction of sTREM2 rate of change with amyloid and tau pathology and to identify a window for therapeutic modulation of TREM2.

Results

Early A β changes drive CSF sTREM2. We measured sTREM2 in the longitudinal CSF samples from participants in the DIAN observational study. **Table 1** summarizes baseline characteristics of the participants. First, we assessed cross-sectionally the time point at which CSF sTREM2 levels significantly differ in mutation carriers (MC) from non-carriers (NC) according to the methods already described elsewhere¹⁹. We found that CSF sTREM2 levels started to be significantly higher in MC than in NC 21 years prior to the expected symptom onset (**Fig. 1a**). Interestingly, this time point is close to the point at which the cortical uptake of PiB-PET starts to be significantly higher in MC than NC (approximately at -22 estimated years from expected symptom onset (EYO))^{19,22}. Although, CSF sTREM2 levels were significantly higher in MC than in NC 21 years before the expected symptom onset, we could not find a time point at which the rate of CSF sTREM2 change was significantly different in MC from NC (**Fig. 1b**). We did not find any influence of sex, educational level, age, or EYO at baseline on the subsequent rate of CSF sTREM2 change studied by univariate Linear Mixed Effects (LME) models in MC and NC (**supplementary table 1**). The mutation status (NC vs MC) and the mutant gene involved did not significantly affect the rate of sTREM2 change (**supplementary table 1 and 2**).

Next, we searched for the pathological factors influencing the longitudinal change of CSF sTREM2. To do so, we investigated whether baseline levels of A β -related, tau-related or structural MRI biomarkers available in DIAN participants were related to the subsequent rate of CSF sTREM2 change, both in MC and NC, using univariate LME models. For A β , we found that lower levels of

both, CSF A β ₄₂ and CSF A β ₄₂/A β ₄₀ at baseline independently predicted a subsequent higher rate of CSF sTREM2 increase in MC but not in NC (**Fig. 2a and supplementary table 3**). In contrast, we did not find any association between total cortical uptake in PiB-PET at baseline and the subsequent rate of CSF sTREM2 change (**Fig. 2b and supplementary table 3**). CSF p-tau, t-tau and structural MRI biomarkers at baseline showed no relationship to the subsequent longitudinal change of CSF sTREM2 in MC nor NC (**Fig. 2c and 2d and supplementary table 3**). Altogether, these results suggest that very early A β -pathological changes, even before fibrillary deposits are detectable by amyloid-PET imaging²⁵⁻²⁷, trigger the subsequent longitudinal increase of CSF sTREM2 in ADAD.

Higher sTREM2 increase rate slows A β -deposition. To further understand the relationship between the longitudinal dynamics of CSF sTREM2 and the evolution of AD pathology represented by the correspondent biomarkers, we studied whether the rate of CSF sTREM2 change was associated with the rate of change of A β -accumulation and tau-related pathology biomarkers in MC by bivariate LME models^{28,29}. We found that a higher rate of CSF sTREM2 increase was related to a slower decrease in CSF A β ₄₂ (lower rate of CSF A β ₄₂ decrease) in presymptomatic MC ($r = 0.56$, $p = 0.01$, **Fig. 3a**) and with a slower increase in the total cortical PIB-PET uptake in symptomatic MC ($r = -0.67$, $p = 0.006$, **Fig. 3b**). For tau-pathology related markers, we found a trend for an association between a higher rate of CSF sTREM2 increase and a higher rate of t-tau increase when considering all MC together, but not when stratifying by the clinical state ($r = 0.34$, $p = 0.08$, **Fig. 3c**). No association with the rate of p-tau change in MC nor in any MC subgroup was found (presymptomatic/ symptomatic MC, **Fig. 3d**).

Higher sTREM2 increase rate attenuates tau pathology. Although we did not find a clear connection between the rates of change of CSF sTREM2 and tau-related markers, we further explored the influence of the longitudinal sTREM2 change on the association between A β -related markers and CSF p-tau longitudinal changes. In presymptomatic MC, but not in symptomatic MC, the association between the rate of CSF p-tau change and the rate of change in the PiB-PET cortical mean differed by the rate of CSF sTREM2 change above or below the median ($\beta = -0.394$ (high sTREM2 change), $p = 0.005$ for the linear interaction) (**Fig. 4a and 4b; appendix 1, supplementary tables 6 and 7**). This suggests a slower p-tau increase rate relative to PiB-PET in presymptomatic MC with higher rates of

CSF sTREM2 increase. Regarding the rate of CSF A β ₄₂ change, its association with the rate of CSF p-tau change also differed by the rate of CSF sTREM2 change in symptomatic MC with CSF sTREM2 increases above or below the median ($\beta = -4.065$ (high sTREM2 change), $p = 0.027$ for the quadratic interaction) (**Fig. 4c and 4d, appendix 1, supplementary tables 6 and 7**). That indicates a slower p-tau increase related to the CSF A β ₄₂ change in symptomatic MC with a higher rate of CSF sTREM2 change. Taken together these results suggest that TREM2 activation attenuates the evolution of tau-related pathology in ADAD in an A β -dependent manner.

The relationship between CSF A β ₄₂ and PiB-PET longitudinal changes is modified by the

sTREM2 increase rate. Our results indicate a seemingly discrepant relationships between the CSF sTREM2 dynamics and the longitudinal changes in CSF A β ₄₂ and the cortical uptake in PiB-PET. That could reflect putative effects of TREM2-activation on both, clearance of early A β ₄₂-seeds and compaction of A β -plaques^{21,30-34}, which may affect the equilibrium of CSF A β ₄₂ and PiB-PET cortical uptake. To explore this possibility, we assessed whether the rate of CSF sTREM2 change influenced the relationship between the rates of change of CSF A β ₄₂ and PiB cortical uptake. Interestingly, the quadratic model predicting the rate of CSF A β ₄₂ change by the rate of PiB-PET uptake change significantly improved when including the binary rate of CSF sTREM2 change and its interaction with PiB-PET cortical mean, especially in presymptomatic MC (multiple r-squared = 0.51 (vs. 0.15), adjusted r-squared = 0.45 (vs. 0.09), **appendix 1, supplementary tables 8 and 9**). The association between CSF A β ₄₂ and the PiB PET cortical mean rates of change differed significantly in the groups with high (above median) versus low (below median) rate of CSF sTREM2 change ($\beta = 0.932$ (high sTREM2 change), $p = 0.004$, for the linear interaction; $\beta = -6.082$ (high sTREM2 change), $p < 0.001$, for the quadratic interaction) (**Fig. 4e and 4f**). In presymptomatic MC with a high rate of CSF sTREM2 change, CSF A β ₄₂ decreased with the increase of PIB-PET cortical uptake, whereas those with a low rate of CSF sTREM2 change showed an opposite relation. In symptomatic MC, this interaction effect was not significant, maybe due to the low number of participants in this group. These results suggest an influence of CSF sTREM2 dynamics on the relationship between CSF A β ₄₂

and PIB-PET longitudinal changes in early stages of the disease, which may be in line with the well-described role of TREM2 in clearance and compaction of A β -aggregates^{21,30,32,34,35}.

Higher sTREM2 increase rate protects from cortical shrinkage in the precuneus. A higher rate of sTREM2 increase was associated with slower cortical shrinkage in the precuneus of the presymptomatic MC ($r = 0.46$, $p = 0.04$; **Fig. 5a**). We also found a trend for an association between higher sTREM2 increase rate and slower cortical shrinkage in the precuneus in the case of symptomatic MC (**Fig. 5a and 5b**). Although we did not find any association between the rate of sTREM2 change and the rate of hippocampal shrinkage in presymptomatic nor symptomatic MC (**Fig. 5c and 5d**), we observed that a higher CSF sTREM2 level at baseline was associated with slower hippocampal shrinkage in line with our previous results¹⁷ (**supplementary table 4**).

Higher sTREM2 increase rate is associated with slower cognitive decline. Finally, we studied the influence of the longitudinal dynamics of CSF sTREM2 on cognitive decline. To do so, we used again a bivariate LME model assessing the association between the rate of CSF sTREM2 change and the rate of cognitive decline, measured by a cognitive composite³⁶. We found a strong association between a higher rate of CSF sTREM2 increase and a slower cognitive decline in presymptomatic MC ($r = 0.62$, $p = 0.003$, **Fig. 5e and 5f**). This finding is in line with the effects of a higher rate of sTREM2 increase on CSF A β_{42} and slower cortical shrinkage in the precuneus in the presymptomatic MC. On the other hand, we did not find any significant association between the rate of CSF sTREM2 change and the rate of cognitive decline in symptomatic MC ($r = -0.07$, $p = 0.79$, **Fig. 5e**). Thus, these findings indicate an early beneficial effect on cognition of the TREM2-dependent microglial response.

Discussion

Although major advances have been made in the past to develop amyloid-lowering therapeutic strategies, their clinical outcome remained disappointing. Clinically, secretase inhibitors caused major side effects probably due to the inhibition of proteolytic processing of physiologically required substrates^{37,38}. Anti-A β immunotherapeutic approaches efficiently removed A β -plaques³⁹, but had so

far mostly minute and inconsistent effects on cognitive and functional outcome. Furthermore, the recent approval for Aducanumab has not been exempt of controversy^{40,41}. Thus, intense efforts are on the way to identify new therapeutic targets. It is now widely accepted that TREM2-dependent microglial functions are required to limit initiation and progression of AD pathology in animal models of amyloidosis. Agonistic antibodies were developed with the goal to increase TREM2-dependent protective functions^{1,2}. They promote TREM2 expression, increase clustering of microglia around amyloid plaques, reduce amyloid plaque load and neuritic dystrophies, and even improve cognition in mouse models^{1,2}. It is therefore of greatest importance, to prove that TREM2-dependent protective functions observed in mouse models and cultured human microglia also occur in AD patients and to define a therapeutic window, when elevation of TREM2 activity is most efficacious. The only way to study that is using a biomarker approach⁴². Considering sTREM2 as a surrogate marker for cell-surface signaling-competent TREM2^{9,13-15,17}, we studied its longitudinal changes in CSF during disease development in the longitudinal DIAN cohort, the largest and best characterized ADAD cohort^{19,22,24}. The predictable nature of ADAD allowed us to determine the relationship between the longitudinal dynamics of CSF sTREM2 and other longitudinal biomarkers associated with progression of AD pathology, along with the longitudinal cognitive evolution from the very early preclinical phases, decades before the first symptom, until a late phase with fully developed AD symptomatology. Correlating the concentrations of CSF sTREM2 with pathological measures, such as A β - and tau-related changes, as well as hippocampal and precuneus shrinking, and cognition allowed us to draw conclusions on a potentially protective function of TREM2 in human brains and may also predict an optimal time window for pharmacological intervention.

Interestingly, we found that lower baseline levels of CSF A β ₄₂, but not higher baseline cortical uptake of PIB-tracer nor markers for tau-related pathology or neuronal death, were associated with a higher increase in CSF sTREM2. This finding suggests that very early A β -related pathology, even before A β -deposits are detectable by A β -PET imaging, drives sTREM2 generation. In line with that, a very early A β aggregation stage, before seeds are detectable by histology, has been recently described in animal models, and suggested to play a key role as a target for disease-modifying drugs⁴³. Further

1 supporting the relationship between CSF sTREM2 increase and the early A β -pathological changes,
2 CSF sTREM2 levels begin to be significantly higher in MC 21 years before the expected symptom
3 onset, close to the time-point where the longitudinal change of CSF A β ₄₂ and A β -PET imaging starts
4 to diverge in MC compared to NC^{19,22}. Thus, microglia may be able to extremely sensitively monitor
5 and respond to the slightest A β related pathological challenges in the human brain. Using a different
6 analytical approach, we previously reported that CSF sTREM2 levels at baseline were higher in MC
7 than in NC in a time window of five years before and after the expected symptom onset¹³. The
8 difference between these findings may be due to a higher discriminative power of our current
9 method^{19,22} detecting differences in the extremes of the EYO time line, where the number of included
10 participants is low.

11 Our current work also shows that a higher sTREM2 increase rate is associated with a slower decrease
12 in CSF A β ₄₂ in presymptomatic MC, and a slower increase in total cortical PIB-PET uptake in
13 symptomatic MC. This association agrees with our previous results in symptomatic LOAD⁴⁴.
14 Moreover, our current results show differential associations between the rate of CSF sTREM2 change
15 and CSF A β ₄₂ or PiB-PET cortical uptake in different clinical phases. That supports a potential dual
16 protective effect in line with previous findings in animal models, aimed to reduce plaque-associated
17 toxicity^{21,35}. In the early presymptomatic stages, the association between higher sTREM2 increase
18 rates and a slower CSF A β ₄₂ decrease may be related to microglia clustering around the smallest
19 amyloid seeds, limiting their growth and spreading²¹. At later stages during the symptomatic phase of
20 the disease, when plaques are fully developed, a protective function of microglia may be carried out
21 by their barrier function and their ability to compact amyloid plaques^{21,30,32,34,35}. Microglia may
22 contribute to amyloid plaque compaction by secreting ApoE, which drives A β aggregation directly
23 within the plaque²¹. Additionally, we found that in MC with a high sTREM2 increase rate, a higher
24 increase rate in the PIB-PET uptake is related to a higher decrease rate in the CSF A β ₄₂ levels,
25 probably reflecting the sequestration of A β ₄₂ in dense core amyloid plaques in an attempt to reduce
26 the most toxic amyloid aggregates as recently suggested by Huang *et al*³⁵. The opposite is found in
27 MC with lower sTREM2 increase rates, possibly reflecting a failure of microglia in compacting A β -

1 plaques. Additionally, CSF A β ₄₂ and A β -PET imaging are suggested to measure different aspects of
2 A β -pathology as, so far, no relationship was found between their longitudinal changes²⁵. We now
3 solved this problem by the introduction of CSF sTREM2 in their relationship. Thus, we obtained a
4 relatively accurate model to predict the CSF A β ₄₂ changes by the PiB-PET changes. This highlights
5 the important role of TREM2 in amyloid-plaque metabolism, and points to CSF sTREM2 as a
6 relevant marker for a better interpretation of the A β -pathology related markers and their dynamics in a
7 clinical setting.

8 Regarding tau-related AD pathology, we could not detect any significant association between the
9 baseline levels of tau-related markers and the subsequent longitudinal sTREM2 change in CSF and
10 neither between the rate of CSF sTREM2 change and the dynamics of CSF t-tau and p-tau. Previous
11 cross-sectional studies in both sporadic and genetic AD clinical cohorts have shown a strong
12 correlation between CSF sTREM2 and CSF t-tau and p-tau¹³⁻¹⁶. We interpret the strong cross-
13 sectional correlation between CSF sTREM2 and tau-related markers as the static view of AD
14 evolution where both markers are sequentially higher, reflecting the disease progression, as a result of
15 A β deposition. Albeit the lack of evidence of a direct relationship between the dynamics of CSF
16 sTREM2 and tau-related markers, we found that the rate of CSF sTREM2 increase influenced the
17 association between A β -pathology markers and CSF p-tau dynamics. A higher rate of CSF sTREM2
18 increase attenuated the rate of p-tau increase related with the PiB-PET increase rate in the
19 presymptomatic MC and with the rate of CSF A β ₄₂ change in symptomatic MC. Nevertheless,
20 difficulties with the interpretation of the rates of change of tau-related CSF markers have been already
21 described. An unexpected longitudinal decrease in CSF p-tau was found in the DIAN cohort before
22 the symptom onset, being associated with its sequestration in neurofibrillary tangles, while the rate of
23 t-tau change remained stable along the entire disease evolution¹⁹. On the other hand, studies in
24 sporadic AD have shown an increasing rate of change in tau-related CSF markers⁴⁵. Although we
25 should interpret our current results with caution, our data support a protective role of TREM2
26 functions on tau-related pathology dependent on A β -pathology, which is in line with recent results in
27 mouse models⁴⁶.

Protective effects of TREM2 on A β and tau related pathology might lead as a consequence to reduced cortical thinning. Accordingly, we found a clear relationship between a higher sTREM2 increase rate and slower cortical shrinkage in the precuneus in presymptomatic MC and a trend for an association in symptomatic MC. However, we could not detect an association between the longitudinal sTREM2 change and the longitudinal evolution of hippocampal volume. The striking association between a higher rate of increase in CSF sTREM2 and slower cortical shrinkage in the precuneus with a lack of association in the hippocampus may not occur by chance. The precuneus is the first region affected by A β accumulation in ADAD followed by a decrease in cortical FDG-PET signal and subsequent cortical shrinkage²². This canonical sequence is not followed by the hippocampal region, where the atrophy is the main event, not following a significant increase in amyloid deposition²². That suggests that the beneficial effect of TREM2 follows a regional pattern, being more evident in early phases in those brain areas with a higher amyloid accumulation rate. That is also in line with the triggering of TREM2 protective functions by the early A β -pathological changes. However, we have already shown a protective role of higher CSF sTREM2 levels at baseline on the hippocampal shrinkage in symptomatic phases of sporadic LOAD¹⁷, and we replicate that here in ADAD.

We found a very striking association between a higher rate of CSF sTREM2 increase and a slower cognitive decline in the presymptomatic AD stage. This result is consistent with the association observed between a higher rate of CSF sTREM2 increase and a slower pathological progression in the presymptomatic phase of the disease, represented by a slower decrease in CSF A β ₄₂ and slower cortical shrinkage in the precuneus, highlighting the association between the A β -pathological process, the neurodegeneration process and the consequent cognitive decline. We reported earlier that higher CSF sTREM2 levels at baseline exert a beneficial effect on cognition, specifically on memory domains and not on general cognitive composites, in symptomatic sporadic LOAD¹⁷. The current results did not show a significant effect of the CSF sTREM2 levels at baseline on cognition, probably because the cognitive composite we used in our current work is not specific for the memory domain and also because our study is focused on the presymptomatic AD phase, including a limited number of symptomatic participants. Taken together our results suggest that a higher rate of CSF sTREM2

1 increase slows A β -deposition and precuneus shrinkage, having a clear clinical readout via its strong
2 association with a slower cognitive decline in a presymptomatic AD stage. The beneficial effect of
3 TREM2-functions may continue in symptomatic stages by slowing hippocampal shrinkage in patients
4 with highest CSF sTREM2 levels.

5 The main limitation of our study is that this is an observational cohort, thus any causative
6 relationships must be interpreted with caution. Moreover, we used a variety of biomarkers, which are
7 indirect measures for studying pathological processes. Furthermore, the study of the relationship
8 between the longitudinal changes of tau-related markers and CSF sTREM2 was restricted to CSF
9 markers, with no available tau-imaging. Finally, CSF sTREM2 is a surrogate of TREM2 signaling and
10 expression of the entire brain, allowing no regional conclusions. Our recently developed TREM2
11 reporter mouse will allow us to address this pivotal question. Our study has important strengths. This
12 is the first study assessing longitudinally CSF sTREM2 changes through an extensive period from
13 very early presymptomatic AD stages until a late symptomatic stage. We report a comprehensive and
14 complete set of highly consistent findings including the biological triggers of the increase of CSF
15 sTREM2 and the effects on A β deposition, tau-related pathology, brain structure and cognition.

16 Our findings have major implications for the future design of clinical studies and the interpretation of
17 A β -related pathology markers. Based on the extremely early response of microglia to amyloid
18 deposition, microglial modulating drugs should be given as early as possible and probably even in
19 combination with anti-A β antibodies. Moreover, it is heavily discussed, where within the amyloid
20 cascade microglia should be placed. Based on the results of our longitudinal study, increase of CSF
21 sTREM2 and, therefore, the induction of microglial TREM2 activity may be placed right after the
22 earliest deposition of amyloid plaques, probably immediately after or even during the seeding process.
23 Taken together, our findings support the development of TREM2 modulating therapeutics to slow
24 initiation and progression of AD.

Methods

Study design, participants and procedures

The Dominantly Inherited Alzheimer Network (DIAN) observational study is a well-described longitudinal and international study, launched in 2008, and recruiting individuals from families carrying mutations in the *presenilin 1 (PS1)*, *presenilin 2 (PS2)* and *amyloid precursor protein (APP)* genes from 17 sites distributed in USA, Argentina, UK, Germany, Spain, and Australia. This study is supervised by the institutional review board at Washington University (St. Louis, MO, USA), which provided human studies approval. Participants or their caregivers provided written informed consent in accordance with their local institutional review board. Asymptomatic individuals were followed with a 3 year-interval until 3 years after their parental age at onset, when the follow-ups become annual. Symptomatic participants were followed annually. The estimated years from expected symptom onset (EYO) was calculated as the participant's current age relative to parental age at first progressive cognitive decline for each visit per asymptomatic participant²⁴. In the case of symptomatic participants, the EYO was calculated as the participant's current age relative to the participant's age at symptom onset.

Participants in the study underwent longitudinally a comprehensive clinical and neuropsychological evaluation, CSF collection and analysis, neuroimaging and genetic characterization in a follow-up pattern and following the methods already described²²⁻²⁴. Dementia status was determined by the Clinical Dementia Rating (CDR)⁴⁷. The genetic characterization of the autosomal dominant Alzheimer disease mutations and the Apolipoprotein E (*APOE*) genotyping was performed according to the methods already described²⁴. Clinical evaluators were blinded to mutation status of participants. Regarding biomarker measurements, CSF was obtained in the morning by lumbar puncture and followed the pre-analytical processing described elsewhere⁴⁸. Amyloid β -peptide₁₋₄₂ ($A\beta_{42}$), Amyloid β -peptide₁₋₄₀ ($A\beta_{40}$), total tau (t-tau), and tau phosphorylated at threonine 181 (p-tau) were measured by immuno-assay using the LUMIPULSE platform. Samples were run in duplicates and those measurements with a coefficient of variation (CV) $\geq 25\%$ were excluded.

MRI was performed using the Alzheimer disease Neuroimaging Initiative (ADNI) protocol⁴⁹, by a 3T scanner with regular quality control assessments. T1-weighted images were acquired for all participants. Volumetric segmentation and cortical surface reconstruction were done as described elsewhere²². In our study we analysed longitudinally cortical thickness in the precuneus, and hippocampal volume. Hippocampal volume was corrected for intracranial volume as already described²². Cortical thickness and hippocampal volume measurements were averaged across hemispheres. Amyloid imaging was done using the 11C-Pittsburgh Compound B (11C-PiB) as already described²². For the longitudinal analysis we used the total cortical A β uptake in the PIB-PET corrected by a Regional Spread Function (RSF) as it demonstrated a better sensitivity to longitudinal changes⁵⁰.

All participants with genetic, clinical, CSF and neuroimaging longitudinal data that passed the quality control from the 14th data freeze were included for sTREM2 quantification in CSF. As usually done in DIAN observational studies^{22,51}, families carrying the APP E693G (Dutch) mutation were excluded from the statistical analysis. These mutations often present with predominant cerebral amyloid angiopathy and diffuse A β plaques with little neurofibrillary tangle pathology⁵².

Quantitative sTREM2 determination in CSF

To avoid detection of sTREM2 variants, which are created by alternative splicing and are not surrogate markers for cell surface signalling competent TREM2 (**supplementary Fig. 2a**), we developed a novel MSD-based immunoassay using a novel neo-epitope specific antibody (1H3), which allows the selective detection of ADAM10/17 cleaved sTREM2 (**supplementary Fig. 2 and 3, supplementary table 5**). We measured 682 CSF samples from 261 participants in duplicates, distributed randomly across 19 plates and measured within three weeks. All longitudinal samples coming from the same participant were measured in the same plate. Duplicate measures had a CV < 15%. Three internal standard (IS) were included in all plates and measured in duplicates (CSF samples from clinical routine obtained in the Neurology Department of the University of Munich). The mean intraplate CV% was 4.8% and the interplate CV% was 8.4%, calculated as the mean between interplate CV% from the three included IS. To account for the interplate variability of the

measurements, the sTREM2 concentrations were adjusted according to the method already described for the ADNI cohort¹⁵.

Statistical analysis

The main goals were (1) to determine which factors were related with the longitudinal change of CSF sTREM2 and (2) to study the effect of the longitudinal change of CSF sTREM2 on AD evolution. For the first, we analysed the relationship between longitudinal CSF sTREM2 as outcome and baseline markers for A β accumulation (CSF A β ₄₂, ratio CSF A β ₄₂/A β ₄₀ and total cortical PIB-PET uptake), tau-related pathology (CSF t-tau and p-tau) and brain structure (cortical thickness in the precuneus, hippocampal volume) as predictor variables. The second outcome was assessed by analysing the correlation between the longitudinal change of CSF sTREM2 and the longitudinal change of CSF A β ₄₂, total cortical PIB-PET uptake, CSF t-tau and p-tau, cortical thickness in the precuneus, hippocampal volume, cortical FDG-PET uptake in the precuneus and cognition as measured by a cognitive composite already describe elsewhere³⁶. In brief, this cognitive composite comprises the following tests: DIAN Word List Test, Logical Memory delayed recall, Digit Symbol Coding test (total score), and Minimental Status Examination (MMSE). As secondary outcomes, we analysed the relationship between baseline CSF sTREM2, as predictor, and the longitudinal change of CSF A β ₄₂, total cortical PIB-PET uptake, CSF t-tau and p-tau, cortical thickness in the precuneus, hippocampal volume, and cortical FDG-PET uptake in the precuneus as the outcomes.

CSF biomarker variables were log-transformed to follow a normal distribution. We calculated the time point at which the CSF sTREM2 levels were significantly different in MC than in NC cross-sectionally as already described elsewhere¹⁹. Cross-sectional analysis focused on the descriptive characteristics at baseline of the different clinical groups, including demographic variables and biomarker values at baseline were done by chi-square tests for categorical variables, and ANOVA or analysis of covariance (ANCOVA) for continuous variables. Age and sex were included as covariates in the ANCOVA studying the differences between biomarkers at baseline across the different groups.

Presymptomatic MC were defined as MC with a CDR score at baseline equal to zero. Symptomatic MC were those MC with a CDR score at baseline higher than zero.

We calculated the raw rate of biomarker change as the individual slope using linear regression for each participant. We used this raw rate of change for illustrational purposes in figures 1, 4 and 5. In order to reassure the validity of our results we defined participants with extreme rate of CSF sTREM2 change as those with a raw rate of CSF sTREM2 change higher or lower than the mean $\pm$ three SD, respectively (7 symptomatic MC and 2 NC). The participants with extreme rate of CSF sTREM2 change are described in the appendix 2 in the supplementary material. We performed the entire analysis by both, excluding and including them (see **appendix 2**), and both sets of results were highly consistent. We show in the main text the results excluding these participants and in the appendix, including them.

We based our longitudinal analysis in Linear Mixed Effects (LME) models. Univariate LME models were used to assess the influence of baseline biomarkers (predictor) on the longitudinal change of the outcome biomarker. The fixed effects in the models included baseline EYO, baseline predictor-biomarker, time from baseline (time), interactions EYO*time and predictor-biomarker*time effects. The random effects included random intercept for each family cluster, individual intercept and slope. The interaction term predictor-biomarker*time was interpreted as the effect of the baseline predictor-biomarker on the subsequent rate of outcome-biomarker change. The models were also evaluated by adjusting for baseline CSF A β ₄₂ and its interaction with time (when analysing the relationship between CSF sTREM2 and tau-related markers), baseline CSF p-tau and its interaction with time (when analysing the relationship between CSF sTREM2 and A β accumulation related markers) or both and their interaction with time (in the case of neuroimaging).

The correlations between the annual rate of change of CSF sTREM2 and that of other outcomes were evaluated using bivariate LME models that simultaneously model the longitudinal courses of both CSF sTREM2 and another biomarker/cognitive outcome^{28,29}. The bivariate LME models included the covariates of baseline parental EYO, baseline CSF p-tau (this is not included if p-tau or t-tau is the outcome), baseline CSF A β ₄₂ (this is not included if A β ₄₂ is the outcome) and their interaction with

time. The random effects included the random intercept for family cluster and random slope for each participant. Unstructured covariance matrix was used for the random effects. Changes from baseline were used as the outcomes instead of the values at each visit that are usually used in LME models. This was done to reduce the dimension of the covariance matrix from 4D (two random intercepts and two random slopes) to 2D (only two random slopes) so that it is easier to converge.

The modification effect of the rate of CSF sTREM2 on the association between the rates of change of A β -deposition and tau-related pathology markers were explored using linear or quadratic regressions. The raw rate of change calculated for each participant based on linear regression were used for this analysis. For each pair of biomarkers, for example CSF A β ₄₂ and PiB PET cortical mean, the rate of change of CSF A β ₄₂ was used as the outcome. Baseline CSF A β ₄₂, baseline PiB PET cortical mean, rate of change of PiB PET cortical mean (and its quadratic term if the model fits better based on AIC), rate of change of CSF sTREM2 group and its interaction with the rate of change of PiB PET cortical mean (and its interaction with the quadratic rate of change of PiB PET cortical mean if the model fits better based on AIC) were used as predictors. The correlations between each pair of the three biomarkers stratified by the CSF sTREM2 rate of change group were also explored using similar bivariate LME models as described above.

Statistical analyses were conducted using SAS 9.4 (SAS Institute Inc., Cary, NC) and R version 3.6.1 (2019-07-05). All p values were based on two-sided tests and values < 0.05 were considered statistically significant.

Data availability

All the data used in this study is available upon request from DIAN at <https://dian.wustl.edu/our-research/observational-study/dianobservationalstudy-investigator-resources/>.

Code availability

The codes used for data analysing in our study can be requested from the corresponding author.

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Author's contributions

E.M.R. and B.N. performed the sTREM2 measurements. E.M.R. and Y.L. performed the statistical analyses. M.S.C., E.M.R. and C.H. designed the study. E.M.R. and C.H. wrote the manuscript. R.F. generated neopeptide specific monoclonal antibodies. E.M.R., G.K. and K.S. developed the new MSD-immunoassay. K.B., B.N. and H.Z. provided CSF samples to validate the new MSD-immunoassay. All other co-authors contributed CSF samples and/or clinical data from DIAN participants. All co-authors contributed to the interpretation of the results and critically reviewed the manuscript.

Conflicts of interest

H.Z. has served at scientific advisory boards for Alector, Eisai, Denali, Roche Diagnostics, Wave, Samumed, Siemens Healthineers, Pinteon Therapeutics, Nervgen, AZTherapies and CogRx and has given lectures in symposia sponsored by Cellectricon, Fujirebio, Alzecure and Biogen. M.S.C has served as a consultant and at

- 1 advisory boards for Roche Diagnostics International Ltd and has given lectures in symposia sponsored by Roche
- 2 Diagnostics, S.L.U and Roche Farma, S.A. C.H. collaborates with Denali Therapeutics, participated on one
- 3 advisory board meeting of Biogen, and received a speaker honorarium from Novartis and Roche. C.H. is chief
- 4 advisor of ISAR Bioscience.

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22

Table 1

	Mutation non-carriers (n=91)	Mutation carriers (n=148)		p-value
		Presymptomatic MC (n=100)	Symptomatic MC (n=48)	
Age, mean (SD), years	36.29 (11.42)	34.15 (9.49)	46.8 (9.73)	< 0.001*
Sex, % female (n)	59.3 (54)	55 (55)	52.1 (25)	0.686
APOE status, %ε4 carriers (n)	34.01 (31)	33 (33)	35.4 (17)	0.9577
EYO, mean (SD), years	-10.01 (12.05)	-13.78 (9.8)	1.92 (6.55)	< 0.001**
Mean follow up (SD), years	3.37 (1.79)	3.37 (1.8)	2.69 (1.67)	0.062
MMSE, mean (SD)	28.91 (1.37)	29.09 (1.22)	24.42 (4.39)	< 0.001*
Cognitive composite, mean z-score (SD)	-0.14 (0.87)	-0.16 (0.08)	-2.86 (1.73)	< 0.001*
Years of education, mean (SD), years	14.97 (2.32)	14.99 (2.79)	14 (3.8)	0.11
CSF Aβ ₄₂ , mean (SD), pg/mL	749.61 (263.12)	741.12 (417.01)	381.26 (162.49)	< 0.001*
CSF Aβ ₄₀ , mean (SD), pg/mL	8484.8 (2792.57)	9028.6 (3305.92)	8636.21 (2711.02)	0.556
CSF Aβ ₄₂ / Aβ ₄₀ , mean (SD)	0.09 (0.01)	0.08 (0.03)	0.04 (0.01)	< 0.001*
CSF t-tau, mean (SD), pg/mL	267.89 (117.22)	406.48 (289.82)	752.28 (373.21)	< 0.001***
CSF p-tau, mean (SD), pg/mL	29.76 (16.75)	53.02 (48.99)	126.98 (69.53)	< 0.001***
CSF sTREM2, mean (SD), ng/mL	2.71 (1.23)	3.4 (1.61)	4.13 (1.34)	< 0.001****
Total cortical PIB-PET uptake, mean (SD), SURV	1.07 (0.21)	1.61 (0.79)	2.98 (1.07)	< 0.001***
Precuneus cortical thickness, mean (SD), mm	2.38 (0.13)	2.36 (0.16)	2.13 (2.17)	< 0.001*
Hippocampal volume, mean (SD), mm ³	8815.85 (631.48)	8871.4 (752.35)	7497.57 (1246.4)	< 0.001*

2

3 **Demographic data and biomarker levels at baseline of MC and NC.** All p-values regarding demographics
4 are based on ANOVA analysis of the raw data. P-values regarding cognitive data (MMSE and cognitive
5 composite) are based in an ANCOVA on the raw data, adjusted for age and education. The p-values regarding
6 biochemical markers are based on ANCOVA analysis considering the log-transformed variables and in the raw
7 data the rest of variables and neuroimaging markers, adjusted by age and sex. Hippocampal volume was
8 corrected per each participant by the total brain volume. Bonferroni correction for multiple comparisons applied
9 in post-hoc analysis. *Symptomatic MC vs. NC and presymptomatic MC p< 0.001. ** Symptomatic MC vs. NC
10 and presymptomatic MC p < 0.001, presymptomatic MC vs NC, p =0.03. ***Symptomatic MC vs. NC and
11 presymptomatic MC p< 0.001, presymptomatic MC vs. NC, p < 0.001. ****NC vs presymptomatic carriers, p =
12 0.002; NC vs symptomatic MC, p < 0.001; presymptomatic MC vs. symptomatic MC, p = 0.005. One
13 participant was excluded because of incomplete data. SD= Standard Deviation. SUVR= standardized uptake
14 value ratio. CSF=Cerebrospinal Fluid. MC=Mutation Carriers. NC=Mutation Non-Carriers.
15 MMSE=Minimental State Examination. EYO= Expected Year of symptom Onset according to the parental
16 onset. t-tau=total tau. p-tau=phosphorylated tau on threonine 181. PIB-PET=Pittsburgh compound B Positron
17 Emission Tomography.

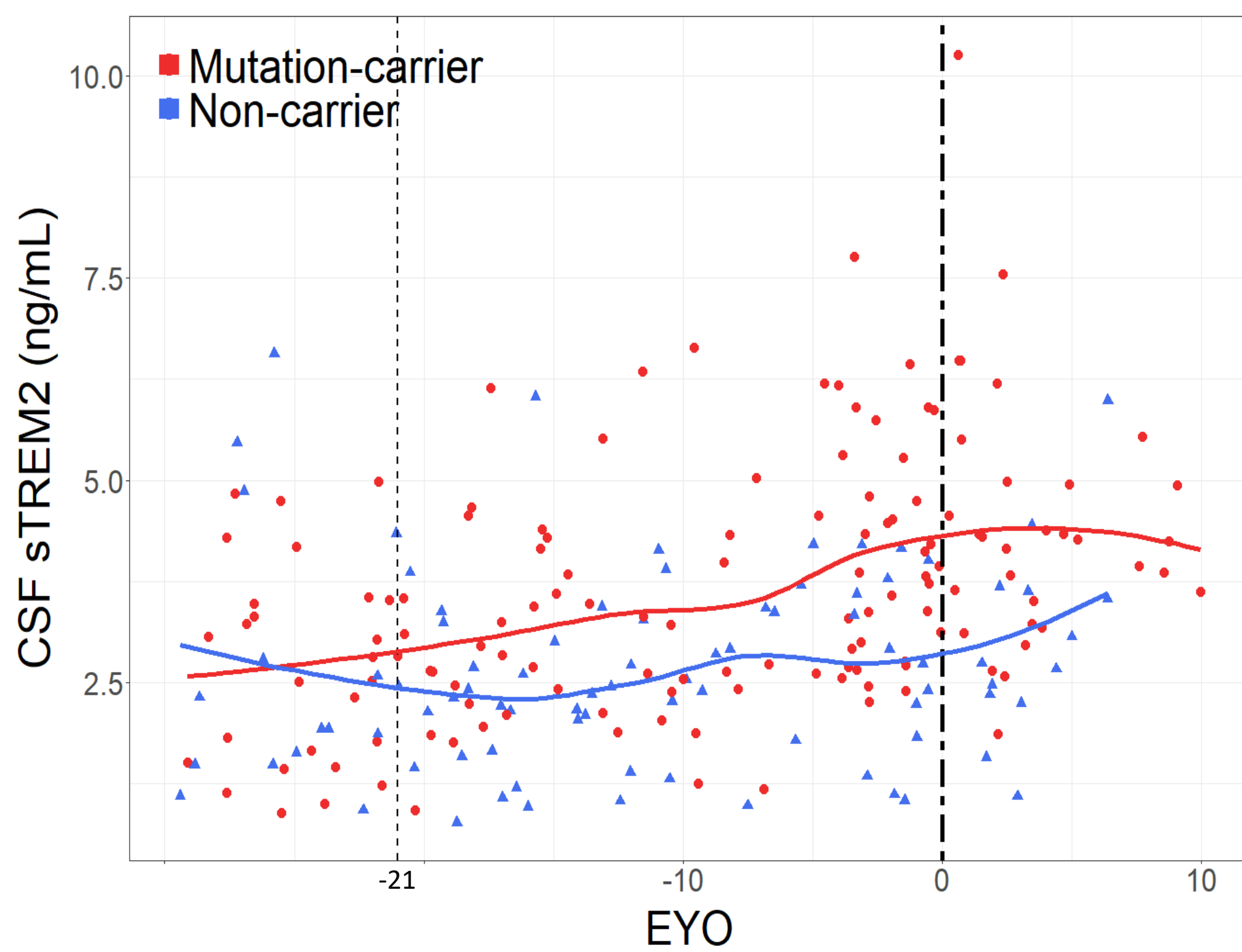
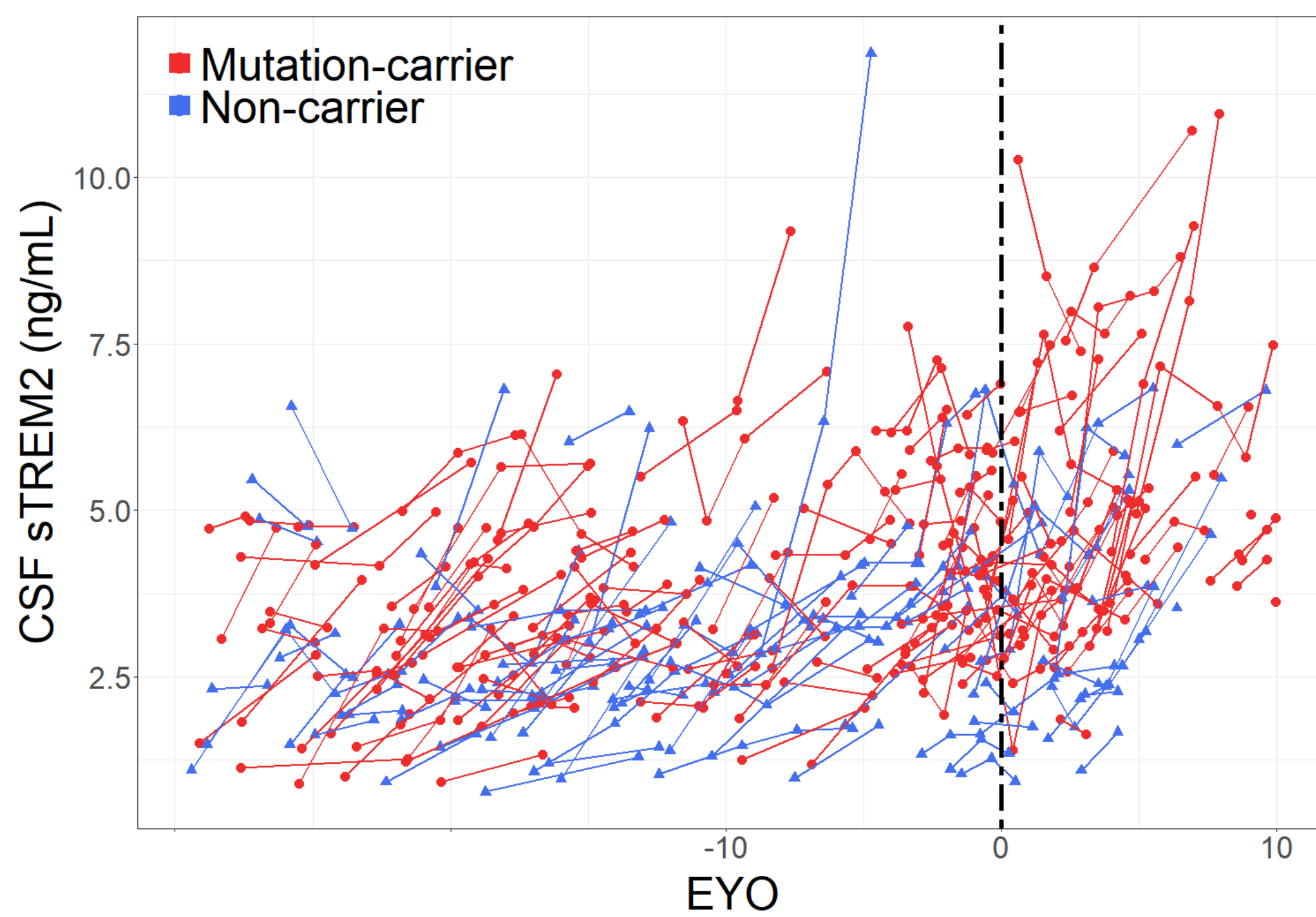
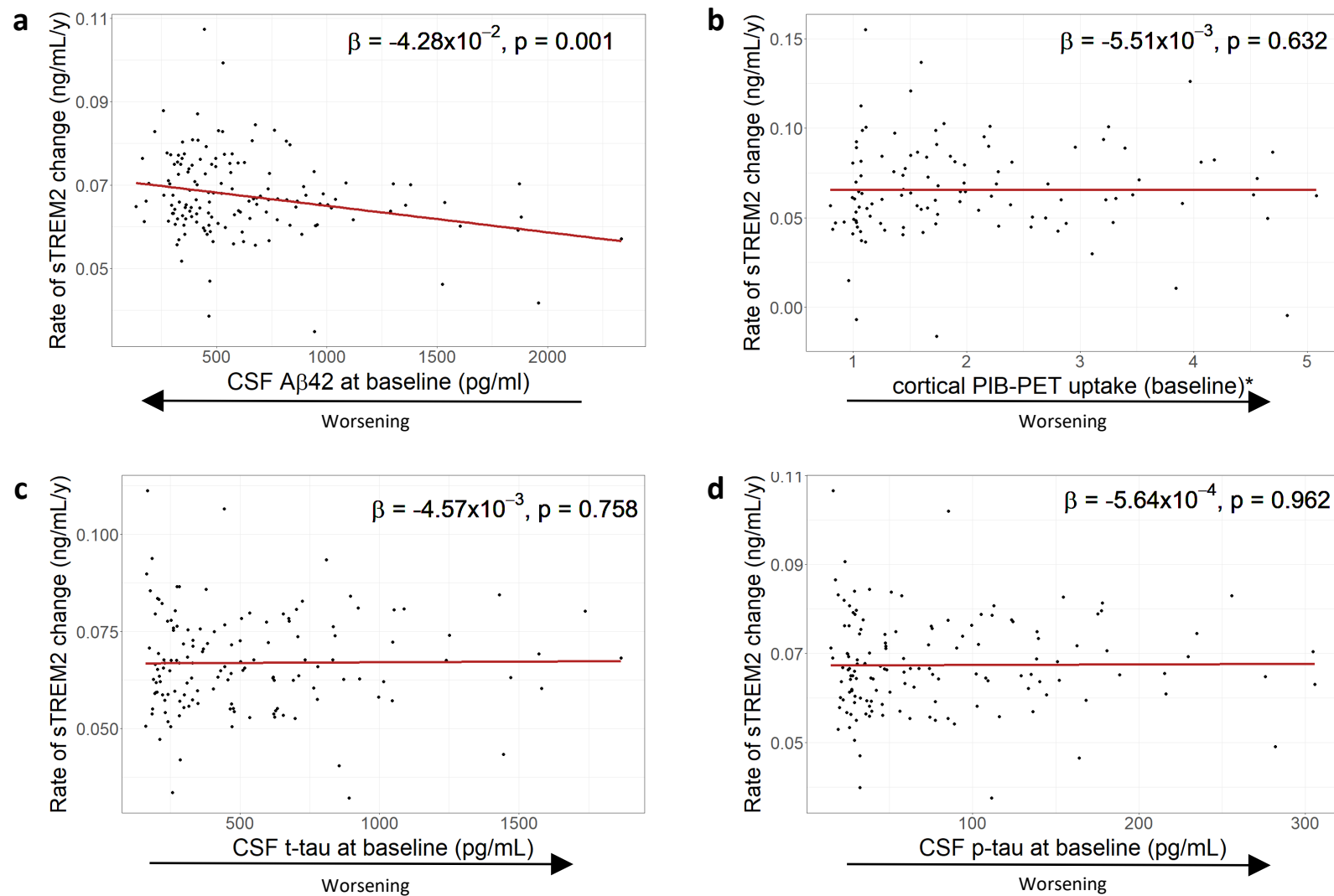
a**b****Fig. 1**

Fig. 1. Longitudinal and cross-sectional sTREM2 levels in CSF along EYO in MC and NC. **a**, sTREM2 baseline levels plotted against EYO at baseline for MC (red, n = 148) and NC (blue, n = 91). The dotted line at EYO = -21 indicates the time point at which cross-sectional sTREM2 levels start to be statistically higher in MC than in NC, according the method described by McDade *et al.* 2018. The red (MC) and blue (NC) lines implicates the LOESS best-fitting curves. **b**, spaghetti plot showing the longitudinal levels of CSF sTREM2 from MC (red, n = 148) and NC (blue, n = 91) as a function of EYO. The bold dotted line at EYO = zero is pointing the expected symptom onset. The negative EYO values represent the expected presymptomatic phase and while the positive values indicate the expected symptomatic phase of the disease. Due to the low number of participants located at the extremes of the graph, individual participants are not shown in the timeframe before EYO= -30 and after EYO= 10 to maintain their confidentiality. CSF=Cerebrospinal Fluid. EYO=Estimated Years from expected symptom Onset. MC=Mutation Carrier. NC=Mutation Non-Carrier.



1 **Fig. 2. Lower levels of CSF A β ₄₂ at baseline are associated with a higher increase rate of CSF sTREM2 in**
2 **MC. a,** representation of the relationship between the lower CSF A β ₄₂ levels at baseline and the subsequent
3 higher rate of sTREM2 change according to the respective LME model. The β -value and the p-value in the
4 graph indicate the effect and statistical significance of the interaction term time*CSF A β ₄₂. **b, c and d.** we did
5 not find any significant effect of the total cortical PIB-PET uptake, t-tau, or p-tau levels at baseline, respectively,
6 on the subsequent rate of sTREM2 change estimated by the LME models already described in table 3. *PIB-
7 PET uptake is measured by SUVR. CSF=Cerebrospinal Fluid. t-tau=total tau. p-tau=phosphorylated tau on
8 threonine 181. PIB-PET= Pittsburgh compound B Positron Emission Tomography. SUVR=Standardized Uptake
9 Value Ratio.

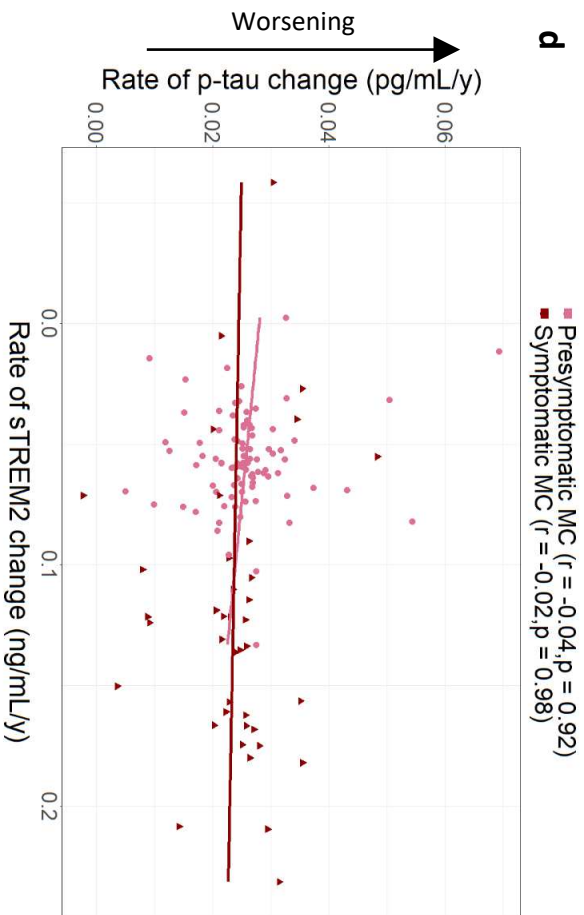
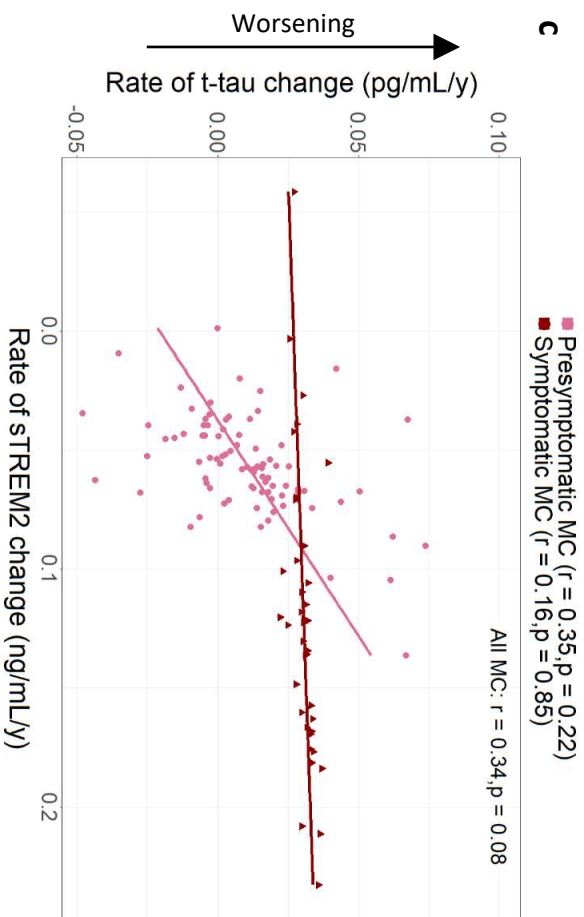
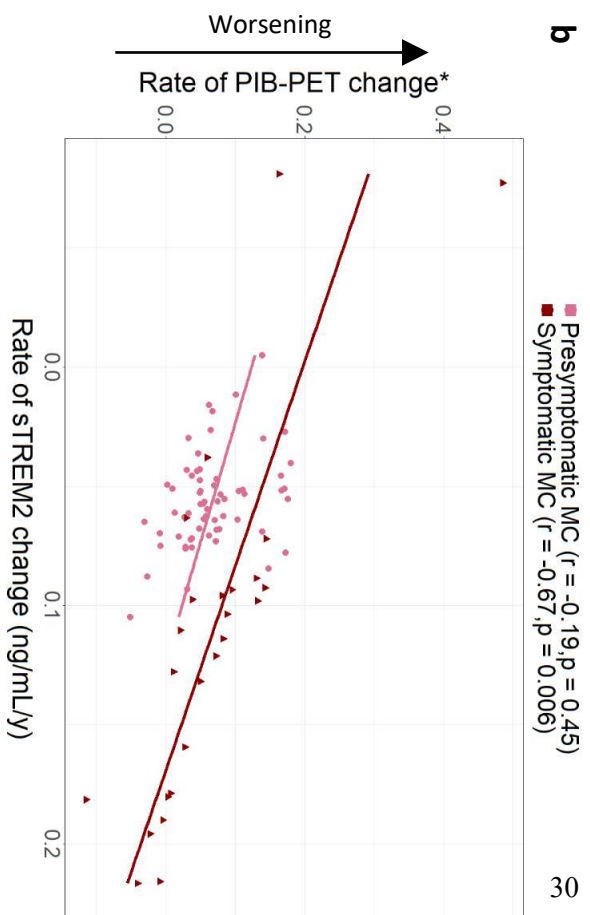
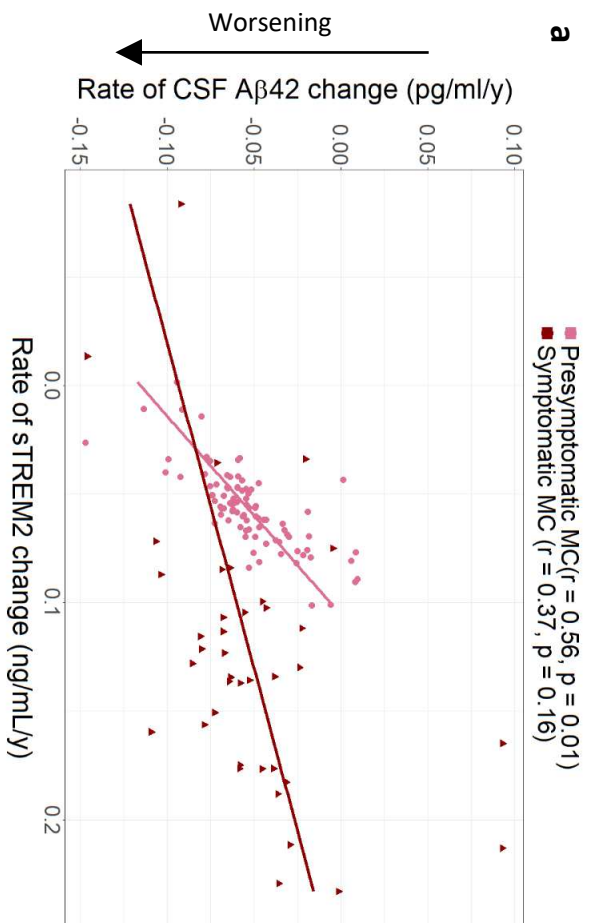


Fig. 3

Fig. 3. Association in MC between sTREM2 increase rate and the rates of change of biomarkers related to amyloid deposition and tau-related pathology. **a**, higher sTREM2 increase rates correlated with a slower CSF A β ₄₂ decrease in presymptomatic MC (pale violet, n = 100). No significant correlation was found in symptomatic MC (dark red, n = 48). **b**, a significant association between a higher sTREM2 increase rate and a slower increase in the cortical PIB-PET uptake is observed in symptomatic MC (dark red, n = 48), while a trend for an association is seen in presymptomatic MC (pale violet, n = 100). **c**, only a trend for an association between a higher sTREM2 and t-tau increase rates was observed upon studying all MC together. **d**, no significant relationship between the rate of sTREM2 change and the rate of p-tau change in MC was observed (neither in the presymptomatic or symptomatic MC, nor in the entire MC group). Presymptomatic and symptomatic MC were defined by a CDR at baseline equal or higher than zero, respectively. The r- and p-values per group are the correlation value for the association between both rates of change and its statistical significance based on the correspondent bivariate LME models. The r- and p-values for the entire MC group only appear in the graphs when its value was statistically significant or showed a trend for a statistical association. The rates of change represented in each graph were extracted from the correspondent bivariate model. *Rate of PIB-PET change measured by SUVR/y. MC=Mutation Carriers. CSF=Cerebrospinal Fluid. t-tau=total tau. p-tau=phosphorylated tau on threonine 181. PIB-PET=Pittsburgh compound B Positron Emission Tomography. CDR=Clinical Dementia Rating. LME=Lineal Mixed Effects. SUVR= Standardized Uptake Value Ratio

Presymptomatic MC

Symptomatic MC

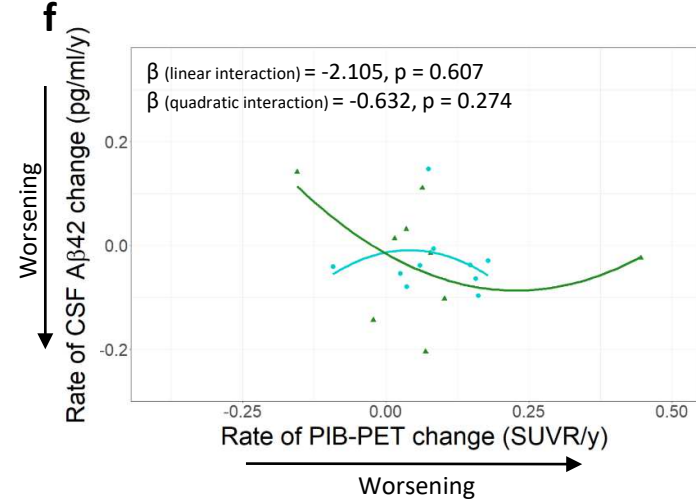
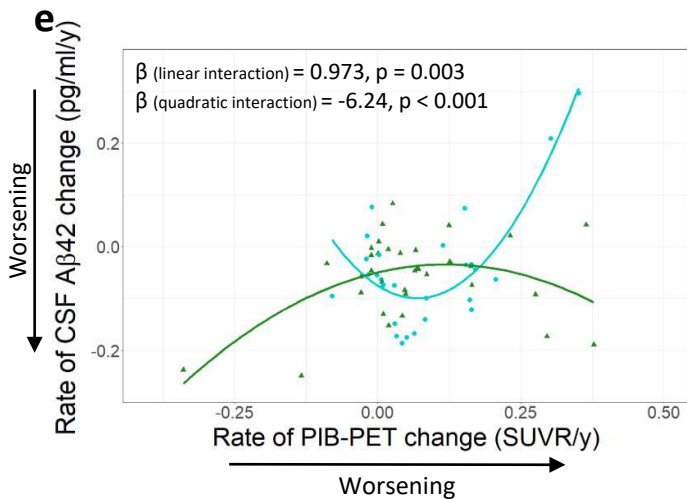
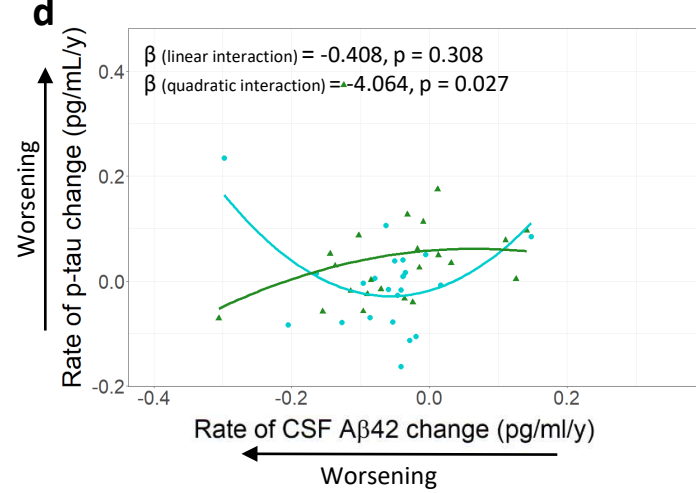
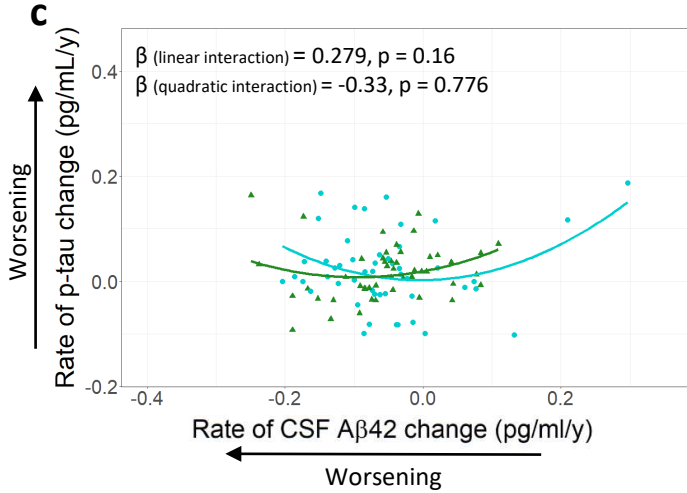
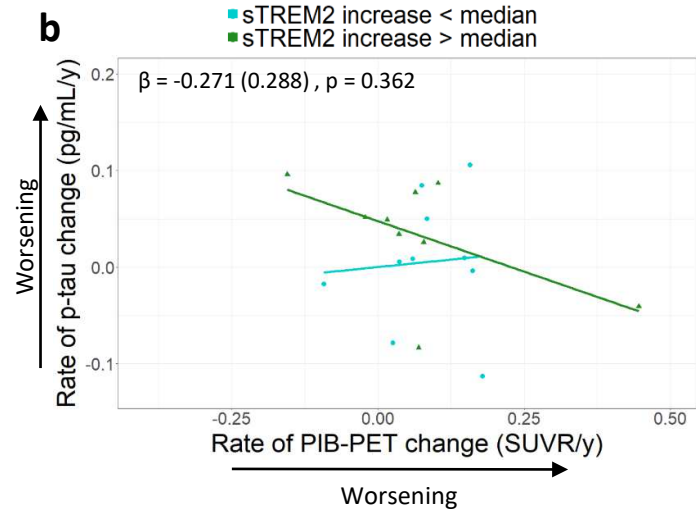
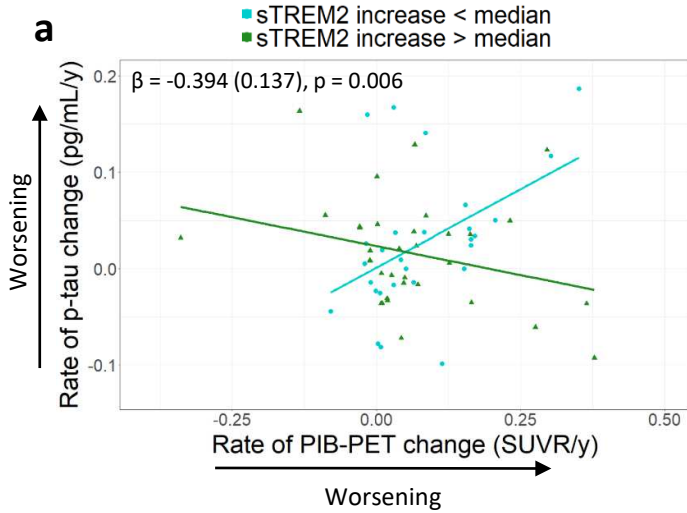


Fig. 4

Fig. 4. Relationships between PiB-PET cortical uptake, CSF A β ₄₂ and p-tau longitudinal changes are modified by the sTREM2 increase rate. **a**, in presymptomatic MC, the relationship between PiB-PET and CSF p-tau raw rates of change is modified by the raw rate of sTREM2 change with opposite associations in the subgroup with a low rate (below the median, light blue, n = 50) and the subgroup with a high rate (above the median, green, n = 48). **b**, this interaction effect was not significant in symptomatic MC. **c**, the relationship between CSF A β ₄₂ and CSF p-tau raw rates of change in presymptomatic MC was not modified by the raw rate of sTREM2 change in presymptomatic MC. **d**, symptomatic MC showed a significant interaction effect between the raw rate of sTREM2 change and CSF A β ₄₂ in the quadratic regression model predicting p-tau longitudinal change. **e**, presymptomatic MC with a high raw rate of sTREM2 change (above the median, green, n = 50) and those with a low raw rate of sTREM2 change (light blue, n = 48) showed opposite relationships between CSF A β ₄₂ and PiB-PET longitudinal changes. **f**, this interaction effect was not significant in symptomatic MC. The β -values in each figure are the β -coefficient for the linear and quadratic interaction terms in each linear or quadratic regression model assessing the relationships between each pair of biomarkers, summarized in appendix 1. MC=Mutation Carriers. CSF=Cerebrospinal Fluid. p-tau=phosphorylated tau on threonine 181. PiB-PET=Pittsburgh compound B Positron Emission Tomography. CDR=Clinical Dementia Rating. SUVR=Standardized Uptake Value Ratio

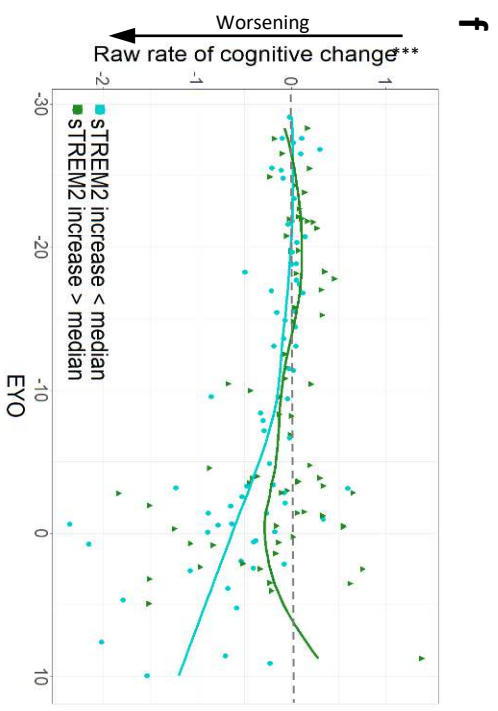
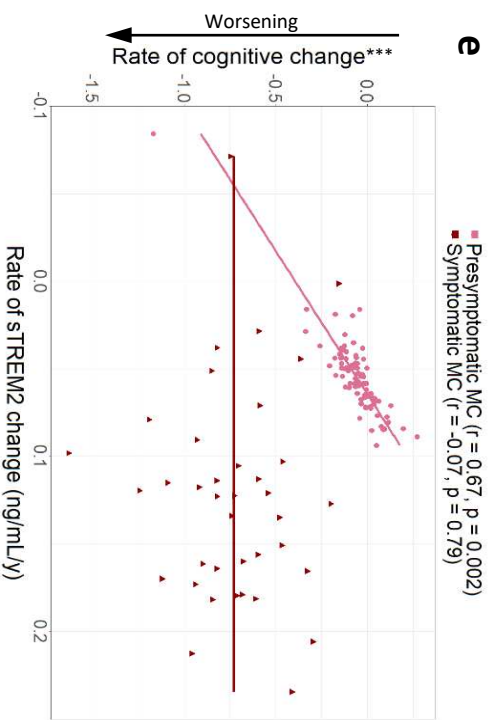
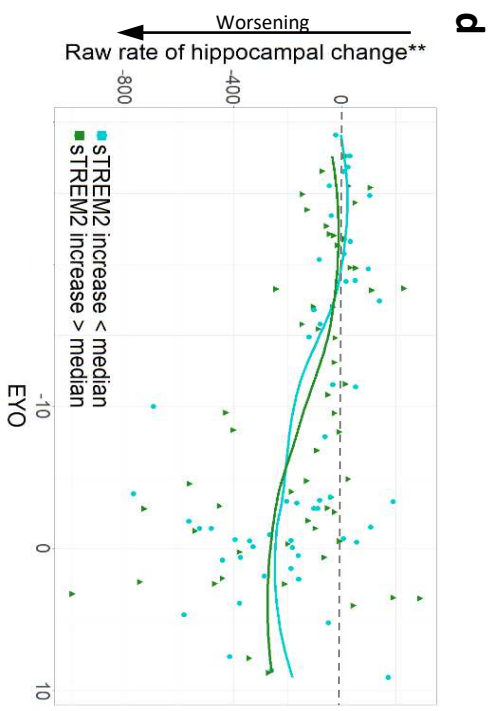
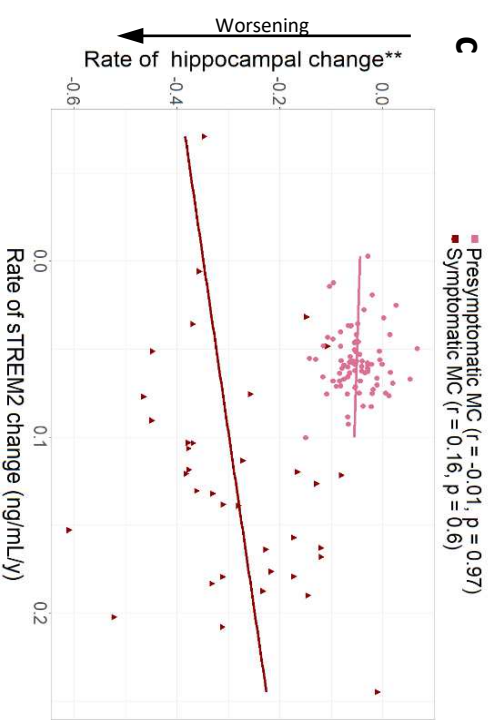
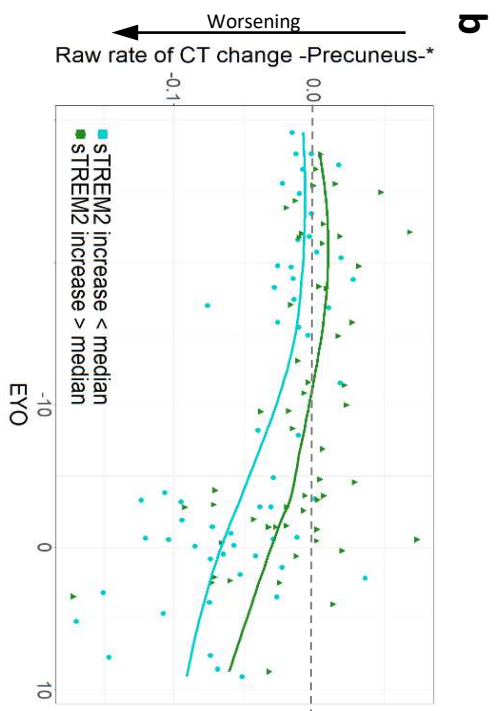
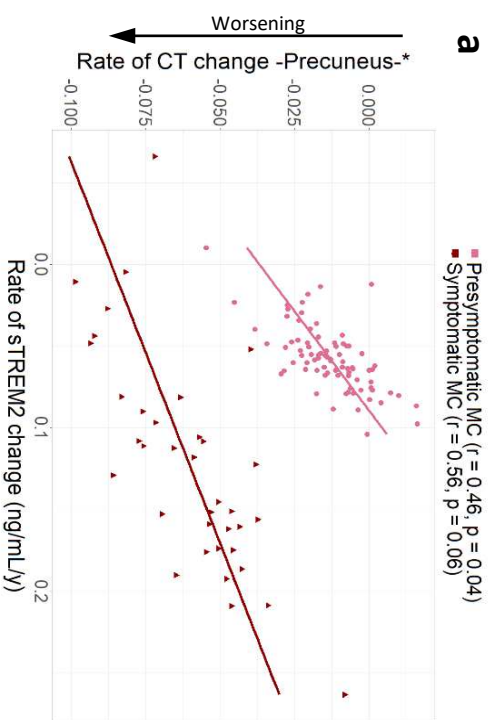


Fig. 5

Fig. 5. Higher sTREM2 increase rate is related to slower cortical shrinkage in the precuneus and slower cognitive decline in presymptomatic MC. **a**, significant association between a higher sTREM2 increase rate and slower cortical shrinkage in the precuneus in the presymptomatic MC (pale violet, n=100) and a trend for a similar association in the symptomatic MC group (dark red, n=48). **b**, shows the raw rate of cortical shrinkage in the precuneus over the EYO in MC. MC were divided in two groups according to their raw rate of sTREM2 (above the median, in green, or below the median, in light blue). **c**, no significant relationship between the rate of sTREM2 change and the hippocampal shrinkage rate in the presymptomatic or symptomatic MC was observed. **d**, raw rate of hippocampal shrinkage over the EYO in MC divided in two groups according to their raw rate of sTREM2 (above the median, in green, or below the median, in light blue). **e**, a strong correlation between higher sTREM2 increase rates and slower cognitive decline in presymptomatic MC (pale violet, n=100), but not in the symptomatic MC (dark red, n=48) was observed. **f**, raw rate of cognitive decline over the EYO in MC divided in two groups according to their raw rate of sTREM2 (above the median, in green, or below the median, in light blue). The r- and p-values per group in **a**, **c**, and **d**, are the correlation value for the relationship between both rates of change and its statistical significance based on the correspondent bivariate LME model. The rates of change represented in **a**, **c**, and **d**, were extracted from the correspondent bivariate LME model. The dashed lines in **b**, **d**, and **f**, point to change = zero, indicating stability. *Rate of cortical thickness change in the precuneus is measured by mm/y **Hippocampal rate of change (volume) is measured by mm³/y. ***The cognitive change was calculated based on the cognitive composite already described (Bateman *et al.* 2017). MC=Mutation Carriers. CT=Cortical Thickness. EYO= Estimated Years from expected symptom Onset. LME=Lineal Mixed Effects. CDR=Clinical Dementia Rating.

Supplementary Files

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