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Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Morenas-Rodríguez E, Li Y, Nuscher B, et al. Soluble TREM2 in CSF and its association with other biomarkers and cognition in autosomal-dominant Alzheimer´s disease: a longitudinal observational study. *Lancet Neurol* 2022; **21:** 329–41.

Supplementary Material

Supplementary methods

Study design, participants and procedures

Although the procedures and methods used in the Dominantly Inherited Alzheimer Network (DIAN) cohort are well described elsewhere,[1-3] we summarize here the main procedures, relevant for the present study. The DIAN longitudinal observational study comprises 17 sites distributed in USA, Argentina, UK, Germany, Spain, and Australia. Participants or their caregivers provided written informed consent in accordance with their local institutional review board. Asymptomatic individuals were followed with a 2 year-interval until 3 years after their parental age at onset, when the follow-up become annual. Symptomatic participants were followed annually. Non-carriers (NC) participants were the non-affected family members (not carrying the causative familiar mutation) that volunteer to participate in the study. The EYO is calculated for these individuals in the same way as for the mutation carriers (MC) participant's current age relative to parental age at first progressive cognitive decline for each visit.[1]

Participants in the study underwent a comprehensive clinical and neuropsychological evaluation already described.[1] Dementia status was determined by the Clinical Dementia Rating (CDR).[4] The genetic characterization of the autosomal dominant Alzheimer's disease mutations and the Apolipoprotein E (*APOE*) genotyping was performed according to the methods already described.[1] 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 elsewhere.[5] Amyloid β-peptide$_{1-42}$ (Aβ42), Amyloid β-peptide$_{1-40}$ (Aβ40), 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) ≥25% were excluded.

MRI was performed using the Alzheimer's disease Neuroimaging Initiative (ADNI) protocol,[6] 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 elsewhere.[3] In our study, we analysed the averaged measurements of the longitudinal rate of change of cortical thickness in the precuneus, and hippocampal volume. Hippocampal volume was corrected for intracranial volume as already described.[3] Cortical thickness and hippocampal volume measurements were averaged across hemispheres. Amyloid imaging was done using the 11C-Pittsburgh Compound B (11C-PiB) as already described.[3] For the longitudinal analysis, we used the total cortical Aβ uptake in the PIB-PET corrected by a Regional Spread Function (RSF) as it demonstrated a better sensitivity to longitudinal changes.[7]

Novel MSD assay specifically detecting sTREM2 derived from the cleavage of the full length-protein.

Our novel sTREM2 immunoassay is based on the sTREM2 immunoassay already described elsewhere.[8,9] We used a novel detection antibody (1H3) specifically directed against the neo-epitope in the sTREM2 peptide originated after the cleavage of the full-length TREM2 protein. That allowed us to better determine sTREM2 related to the TREM2 signalling in microglia leading to microglial activation. Antibody 1H3 does not detect other soluble forms generated by alternative splicing[10]) as potentially does the sc-373828 (B-3) antibody (Santa Cruz Biotechnology) which was used in previous sTREM2 MSD-immunoassay studies (supplementary figure 2). Monoclonal antibody clone TREMS 1H3 (rat IgG2a/k) was generated by immunization with peptides comprising $_{151}$EDAHVEH-*COOH*$_{157}$ of human TREM2 (PSL GmbH, Heidelberg, Germany). Lou/c rats were immunized subcutaneously and intraperitoneally with a mixture of 40 µg ovalbumin-coupled peptides, 5 nmol CPG oligonucleotide (Tib Molbiol, Berlin), 500 µl PBS and 500 µl incomplete Freund's adjuvant. A boost without adjuvant was given eight weeks after the primary injection. Hybridoma fusion was performed using standard procedures. Supernatants were tested by ELISA for specific binding to biotinylated peptides comprising the neo-epitope and to c-terminally extended peptides as negative controls. Positive supernatants were further validated by Western blotting for binding to soluble full-length ectodomain and ADAM10/17-cleaved TREM2 protein. Hybridoma cells from supernatants that reacted with cleaved protein only were cloned by limiting dilution.

As a brief description of our novel MSD-immunoassay, we used streptavidin-coated 96-well plates (MSD Streptavidin Gold Plates, cat. no. L15SA); a biotinylated polyclonal goat IgG anti-human TREM2 antibody (R&D Systems, cat. no. BAF1828; 0.25 µg/m) as capture antibody; a monoclonal rat IgG anti-human cleaved sTREM2 antibody (clone TREMS 1H3, 2 µg/mL) as a detection antibody; and a SULFO-TAG-labeled goat polyclonal anti-rat IgG secondary antibody (MSD, cat. no. R32AH; 0.5 µg/mL). Recombinant TREM2 protein corresponding to the soluble cleaved fragment of human TREM2 (amino acids 19-157) was purified from the supernatants of HEK293T cells stably overexpressing the protein and was used as standard. All antibodies were diluted in the assay buffer (1% BSA and 0.05% Tween 20 in PBS, pH=7.4). The standard, the blanks, and the CSF samples (duplicates; dilution, 1:6) were diluted in the same assay buffer supplemented with protease inhibitors (Sigma; Cat. # P8340). The plates were blocked overnight at 4°C in blocking buffer [3% bovine serum albumin (BSA) and 0.05% Tween 20 in PBS (pH 7.4); 200 µL/well] and next incubated with the capture antibody (25 µL/well) for 90 minutes at RT. After four washing steps with wash buffer (300 µL/well; 0.05% Tween 20 in PBS), the standard, the blanks, and the CSF samples (50 µL/well) were incubated for 2 hours at RT. Plates were again washed six times followed by an incubation for 1 hour at RT with the detection antibody (50 µL/well). After six additional washing steps, plates were incubated with SULFO-tag conjugated secondary antibody (25 µL/well) for 1 hour in the dark at RT. Thereafter, plates were washed six times with wash buffer followed by two washing steps in PBS (300 µL/well). The light emission after adding 150 µL/well MSD Read buffer T (Cat. # R-92TC) was measured using the MESO QuickPlex SQ 120. Raw values are provided as ng/mL. Duplicate measures had a CV < 15%. All the samples coming from the same participant were measured in the same plate. 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 cohort[9].

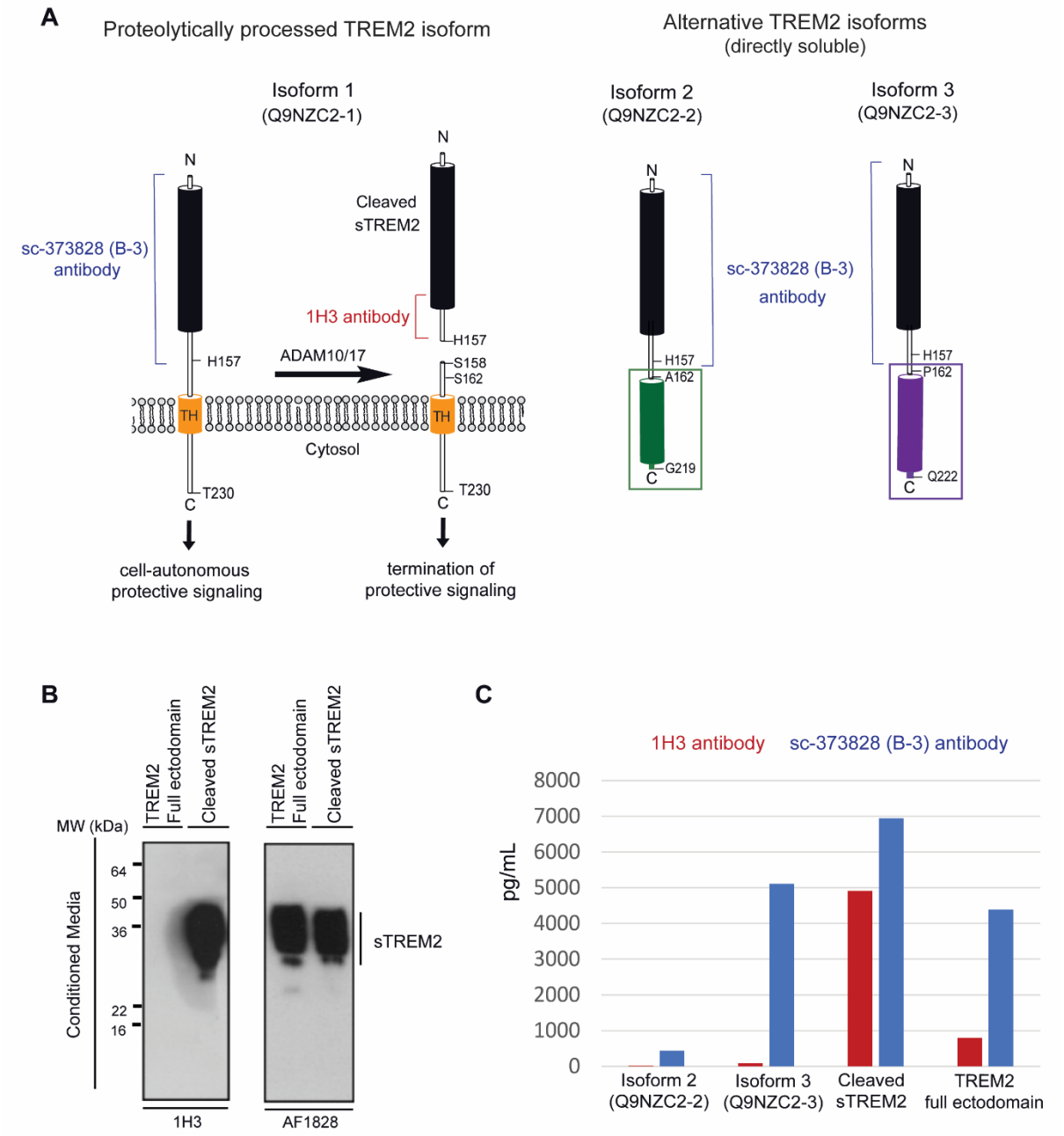
The specificity of the new 1H3 antibody, detecting specifically the sTREM2 coming from cleavage of the full-length protein is shown in supplementary figure 2. The mean recovery percentage in the spike-recovery test was 109.3% (supplementary table 1). The linearity percentage for a 1:6 dilution factor was 94.5%. The lower limit of quantification (LLOQ) was 121 pg/mL. Supplementary figure 3 shows the stability across thaw-freeze cycles and the correlation with the previous sTREM2 immunoassay. CSF samples used for the validation assays consisted of leftovers from diagnostic clinical routine from the Ludwig-Maximilians-Universität München (LMU) department of Neurology (Munich, Germany).

Supplementary table 1

	sTREM2 concentration (pg/mL)						
	Neat sample	**Spiked (SP 1) sample**	**Recovery (%, SP 1)**	**Spiked (SP 2) sample**	**Recovery (%, SP 2)**	**Spiked (SP 3) sample**	**Recovery (%, SP 3)**
Neat calculated concentration		1932		946.3		222.8	
CSF 1	1569.1	3753.3	113.1	2656.3	114.9	1813.1	109.5
CSF 2	662.7	2696.4	105.3	1761.7	116.1	866.4	91.4
CSF 3	557.5	2635.9	107.6	1600.6	110.2	825.6	120.3
CSF 4	526.3	2490.4	101.7				
Recovery (%)			106.9		113.8		107.1

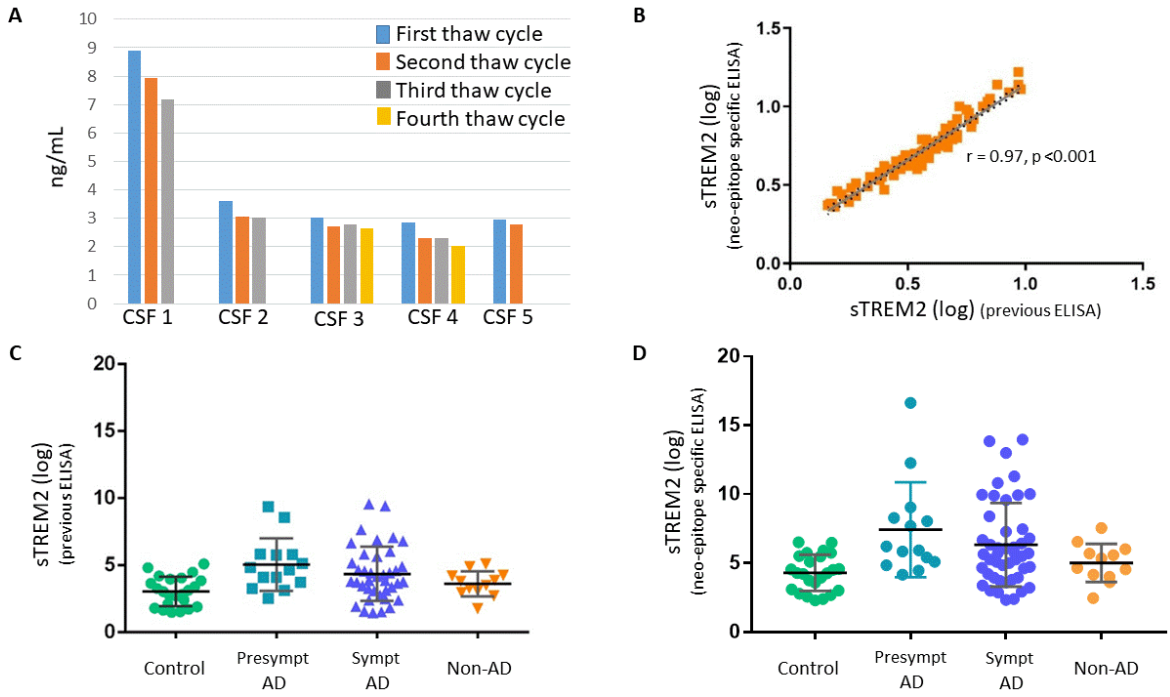
Spike recovery test for CSF cleaved sTREM2 immunoassay. Four CSF samples (dilution factor, 1:6) were spiked with three different concentrations of the recombinant cleaved sTREM2 standard (high concentration -2000 pg/mL, SP 1-, SP 3-, intermediate -1000 pg/mL, SP 2-, and low -250pg/mL-) for assessing the spike-recovery percentage. Neat samples and spiked samples were measured in the same plate. Spike recovery was calculated as the percentage recovery of the signal levels in spiked samples above the signal levels in non-spiked samples. The mean recovery was 109.3%. CSF=Cerebrospinal Fluid. SP=Spike

Supplementary figure 1



Specificity of the novel sTREM2 immunoassay detecting only ADAM10/17 generated sTREM2. (A) Schematic representation of the different TREM2 isoforms and the location of the neo-epitope generated by cleavage of full-length TREM2 and selectivelly recognized by the monoclonal antibody 1H3. Antibody sc-373828 (B-3), previously used as detection antibody in our in-house immunoassay[8,9], binds residues 1-160 and, therefore, potentially detects all TREM2 isoforms. Green and purple squares highlight domains of the isoforms 2 and 3, starting at aminoacid 162. (B) Western blot showing the specificity of antibody 1H3 recognizing selectivelly recombinant $TREM2_{19\text{-}157}$ (corresponding to ADAM10/17 cleaved sTREM2) and not the recombinant full-length TREM2 ectodomain ($sTREM2_{19\text{-}174}$). AF1828 antibody (R&D Systems) served as a control, recognizing all forms of TREM2. Equal amounts of conditioned media (10 µL) of HEK293T cells overexpressing both proteins were loaded. (C) Specificity of 1H3 as a detection antibody in the immunoassay, selectively detecting recombinant $TREM2_{19\text{-}157}$ (cleaved sTREM2). Antibody sc-373828 (B-3) also detects all other TREM2 isoforms. We measured 5000pg/mL of recombinant isoform 2 (Q9NZC2-2), isoform 3 (Q9NZC2-3), $TREM2_{19\text{-}157}$ (cleaved sTREM2) and $sTREM2_{19\text{-}174}$ (the entire TREM2 ectodomain) using our previous immunoassay[8,9], as well as the novel selective assay. Isoform 2 (Q9NZC2-2), isoform 3 (Q9NZC2-3), $TREM2_{19\text{-}157}$ (cleaved sTREM2) and $sTREM2_{19\text{-}174}$ (the entire TREM2 ectodomain) were obtained from supernatants of HEK293T cell lines stably overexpressing each of these proteins. TH=Transmembrane Helix. MW=Molecular Weight.

Supplementary figure 2



Characterization of the novel immunoassay specifically detecting cleaved sTREM2. (A) Stability of the sTREM2 measurements of the novel immunoassay across four thaw-freeze cycles. (B) Correlation of measurements using the novel immunoassay as compared to the previous used one in the Gothenburg AD cohort, previously studied[11]. (C) CSF sTREM2 levels (mean and SD) in the Gothenburg AD cohort using our previously described immunoassay (results already published in Suarez-Calvet *et al*. EMBO Mol Med 2016)[11]. (D) sTREM2 measurements (mean and SD) in the same cohort using our novel immunoassay specifically detecting cleaved sTREM2. CSF=Cerebrospinal Fluid. AD=Alzheimer Disease. Presympt=Presymptomatic. Sympt=Symptomatic. SD=Standard Deviation

Outcomes

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 aim, we analysed the relationship between longitudinal CSF sTREM2 as outcome and baseline markers for Aβ accumulation (CSF $A\beta_{42}$, ratio CSF $A\beta_{42}/A\beta_{40}$ 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 –outcome: longitudinal CSF sTREM2-. The second aim was assessed by analysing the correlation between the longitudinal change of CSF sTREM2 and the longitudinal change of CSF $A\beta_{42}$, total cortical PIB-PET uptake, CSF t-tau and p-tau, cortical thickness in the precuneus, hippocampal volume, and cognition as measured by a cognitive composite already describe elsewhere[12]. For this aim, the outcomes were: longitudinal CSF sTREM2, longitudinal CSF $A\beta_{42}$, t-tau and p-tau, longitudinal total cortical PIB-PET uptake, longitudinal cortical thickness in the precuneus, longitudinal hippocampal volume, and longitudinal cognitive composite. Note, that these correlations were assessed by bivariate LME models, modelling simultaneously two longitudinal outcomes.

Statistical methods

CSF biomarker variables were log-transformed to follow a normal distribution. 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.

For the calculation of the cut-off EYO point where sTREM2 levels and its longitudinal change started to be significantly different in MC versus NC we used the following calculation: The best linear unbiased estimators of the individual's rate of change over the follow-up for log-transformed sTREM2 was estimated using a general linear mixed effects (LME) model and then were plotted against baseline EYO using local regression (LOESS). Based on the LOESS curves, we considered three models: (A) linear mixed effects model (LME), (B) linear spline mixed effects model with one change point and (C) quadratic mixed effects model. Details for the three models are presented below. We then compared the goodness-of-fit of those models using Akaike information criterion (AIC) and selected the model with the best fit (see supplementary table 2 for the AIC). Based on the LME model, we evaluated (1) the baseline EYO point where the rate of change became significantly different between MC and NC, and (2) the baseline EYO point where the significant difference occurred cross-sectionally between MC and NC.

Model A: Linear mixed effects model

$$y_{ijk} = \beta_0 + \beta_1 Mutation + \beta_2 Mutation * t_{ij} + \beta_3 Baseline\ EYO * Mutation + \beta_4 Baseline\ EYO * Mutation * t_{ij} + b_{0i} + b_{1i} * t_{ij} + f_k + \epsilon_{ij}$$

y_{ijk} denote the longitudinal assessments for subject i at time j for group k, $i = 1, 2, \dots, n$, $j = 0, 1, \dots, m_i$,with $j = 0$ representing the baseline visit, and $k = 1, 2$ representing mutation carriers group and non-carriers group. b_{0i} and b_{1i} are the individual random intercept and random slope, and $\begin{pmatrix} b_{0i} \\ b_{1i} \end{pmatrix} \sim N\left(\mathbf{0}, \begin{pmatrix} \sigma^2_{b_0} & \sigma_{b_0}\sigma_{b_1} \\ \sigma_{b_0}\sigma_{b_1} & \sigma^2_{b_1} \end{pmatrix}\right)$. f_k is the random intercept for each family cluster, and $f_k \sim N(0, \sigma^2_k)$. ϵ_{ij} is the within-subject residual and conditioning on the random effect, $\epsilon_{ij} \sim N(0, \sigma^2_\epsilon)$. $\begin{pmatrix} b_{0i} \\ b_{1i} \end{pmatrix}$, f_k, and ϵ_{ij} are assumed to be independent for each participant.

Model B: linear spline mixed effects model

$$y_{ijk} = \beta_0 + \beta_1 Mutation + \beta_2 Mutation * t_{ij} + \beta_3 Baseline\ EYO * Mutation + \beta_4 Baseline\ EYO * Mutation * t_{ij} + \beta_5 CP\ EYO * Mutation + \beta_6 CP\ EYO * Mutation * t_{ij} + b_{0i} + b_{1i} * t_{ij} + f_k + \epsilon_{ij}.$$

Where, $CP\ EYO = \max\big((Baseline\ EYO - EYO\ change\ point), 0\big)$. A range of EYO change point were considered, from -25 to 10 by 1.

Model C: quadratic mixed effects model

$$y_{ijk} = \beta_0 + \beta_1 Mutation + \beta_2 Mutation * t_{ij} + \beta_3 Baseline\ EYO * Mutation + \beta_4 Baseline\ EYO * Mutation * t_{ij} + \beta_5 Baseline\ EYO^2 * Mutation + \beta_6 Baseline\ EYO^2 * Mutation * t_{ij} + b_{0i} + b_{1i} * t_{ij} + f_k + \epsilon_{ij}$$

Supplementary table 2

Model	AIC
Linear model (A)	395
Linear spline with one change point (B)	≥434 for all change point we considered (baseline EYO -25 to 10 by 1)
Quadratic model (C)	457

AIC from models performed to assess the cut-off EYO point for significantly different sTREM2 in MC from NC. AIC=Akaike information criterion. EYO=Estimated Years to/from symptom Onset

The raw rate of change for each biomarker or cognitive outcome was calculated as the individual slope per participant in a linear regression biomarker/cognitive outcome by time.

Univariate LME models were used to assess the influence of baseline biomarkers (predictor) on the longitudinal change of the outcome biomarker and were performed separately and independently for each predictor. We explain the individual univariate LME models with an example pseudocode for R (version 3.6.1 (2019-07-05), lme4 package) as follows. 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 family cluster is the family from which each participant originates, considering they were recruited from families carrying APP, PS1 and PS2 mutations as described in the Method section. The interaction term predictor-biomarker*time was interpreted as the effect of the baseline predictor-biomarker on the subsequent rate of outcome-biomarker change. This is the target effect we aimed to study according to our first objective, stated above, that we extracted from the models and summarized in the results. The models were also evaluated by adjusting for baseline CSF Aβ42 and its interaction with time (when analysing the relationship between sTREM2 and tau-related markers), baseline CSF p-tau and its interaction with time (when analysing the relationship between sTREM2 and Aβ accumulation related markers) or both and their interaction with time, in the case of neuroimaging markers (neuronal damage).

Univariate LME models studying the influence of baseline demographics (predictor/independent variable) on the subsequent rate of sTREM2 change (outcome/dependent variable) were performed following the next example and individually assessing EYO (example), age at baseline, APOE-ε4 (binary variable carriers vs. NC), sex and years of education (one individual model per predictor variable, and separately performed in MC and NC):

```
lmer(Longitudinal_LgsTREM2 ~ time_from_baseline_yrs  + EYO_baseline +
                             time_from_baseline_yrs*EYO_baseline  +
                             (1 + time_from_baseline_yrs|Individual_ID) + (1|Family), DIAN_dataset)
```

The inclusion or exclusion of family cluster as a random factor arose similar results per all univariate LME models on demographics.

In the case of Mutation status (MC vs. NC) and Mutation type (APP vs. PS1 vs. PS2 / Dutch Mutation Carriers vs. rest of MC) the models were adjusted by EYO at baseline as follows:

```
lmer(Longitudinal_LgsTREM2 ~ time_from_baseline_yrs  + EYO_baseline + Mutation_status
                             time_from_baseline_yrs*EYO_baseline  +
                             time_from_baseline_yrs* Mutation_status +
                             (1 + time_from_baseline_yrs|Individual_ID) + (1|Family), DIAN_dataset)
```

We extracted the effect of each predictor on the rate of sTREM2 from the model according to the effect of the interaction term predictor (at baseline)*Time(from baseline)

Univariate LME models studying the influence of baseline biomarkers (predictor/independent variable) on the subsequent rate of sTREM2 change (outcome/dependent variable) were performed individually per each predictor and separately in MC and NC as follows:

Aβ-deposition related markers (CSF Aβ42, ratio Aβ42/Aβ40 and PiB-PET)

```
lmer(Longitudinal_lgsTREM2 ~ time_from_baseline_yrs + EYO_baseline  + Amyloid_marker_baseline +
                             CSF_ptau_baseline + time_from_baseline_yrs* EYO_baseline  +
                             time_from_baseline_yrs*Amyloid_marker_baseline +
                             time_from_baseline_yrs*CSF_ptau_baseline +
                             (1 + time_from_baseline_yrs|Individual_ID) + (1|Family), DIAN_dataset)
```

Models adjusted or not by baseline CSF p-tau (by introducing CSF p-tau at baseline and its interaction with time from baseline as fixed factors in the model) or family cluster had similar results. The individual model assessing the ratio Aβ42/Aβ40 failed to converge without adjustments, thus, to enable a better comparison between a Aβ-deposition related markers, we show results coming from the adjusted models in the three cases.

Tau-pathology related markers (CSF t-tau and p-tau)

lmer(Longitudinal_lgsTREM2 ~ time_from_baseline_yrs + EYO_baseline + Tau_marker_baseline +
time_from_baseline_yrs* EYO_baseline +
time_from_baseline_yrs* Tau_marker_baseline +
(1 + time_from_baseline_yrs|Individual_ID) + (1|Family), DIAN_dataset)

The results were similar when introducing or not family as a random factor and also when controlling or not by baseline CSF Aβ42 (by introducing CSF Aβ42 at baseline and its interaction with time from baseline as fixed factors in the model). We show results adjusted just by family cluster to simplify as much as possible the model, considering the relationship between CSF tau markers and Aβ-markers.

Neuroimaging variables (cortical thickness in the precuneus and hipposcampal volume)

lmer(Longitudinal_lgsTREM2 ~ time_from_baseline_yrs + EYO_baseline + Neuroimaging_baseline +
CSF_Ab42_baseline + CSF_ptau_baseline +
time_from_baseline_yrs* EYO_baseline +
time_from_baseline_yrs*Neuroimaging_baseline +
time_from_baseline_yrs*CSF_Ab42_baseline +
time_from_baseline_yrs*CSF_ptau_baseline +
(1 + time_from_baseline_yrs|Individual_ID) + (1|Family), DIAN_dataset)

In the case of the neuroimaging variables, the individual univariate LME models were adjusted by baseline CSF p-tau, baseline CSF Aβ42 and their interactions with time to isolate the effect of each neuroimaging marker at baseline from the effect of CSF p-tau and CSF Aβ42 on the subsequent longitudinal sTREM2 change.

For all the models, we extracted the effect of each predictor on the rate of sTREM2 from the model according to the effect of the interaction term predictor (at baseline)*Time(from baseline)

Univariate LME models studying the influence of baseline sTREM2 levels (predictor/independent variable) on the subsequent rate of change of other biomarkers (outcome/dependent variable) –longitudinal CSF Aβ42, t-tau and p-tau, PiB-PET, cortical thickness in the precuneus and hippocampal volume- were performed individually per each dependent variable in MC as follows:

lmer(Longitudinal_biomarker ~ time_from_baseline_yrs + EYO_baseline + sTREM2_baseline +
time_from_baseline_yrs* EYO_baseline +
time_from_baseline_yrs*sTREM2_baseline +
(1 + time_from_baseline_yrs|Individual_ID) + (1|Family), DIAN_data set)

In the case of Aβ-deposition related markers (longitudinal CSF Aβ42 and PiB-PET), the adjustment for CSF p-tau or t-tau (by introducing baseline CSF p-tau or t-tau and its interaction with time from baseline as fixed factor in the model) did not originate different results. In the case of tau-pathology related markers (longitudinal CSF t-tau and p-tau), the adjustment for CSF Aβ42 (by introducing baseline CSF Aβ42 and its interaction with time from baseline as fixed factor in the model) did not affect the results.

In the case of the neuroimaging variables (cortical thickness in the precuneus and hippocampal volume), the individual univariate LME models were adjusted by baseline CSF p-tau and its interaction with time to isolate the effect of sTREM2 at baseline from the effect of tau pathology at baseline.

lmer(Longitudinal_biomarker ~ time_from_baseline_yrs + EYO_baseline + sTREM2_baseline +
CSF_ptau_baseline +
time_from_baseline_yrs* EYO_baseline +
time_from_baseline_yrs* sTREM2_baseline +
time_from_baseline_yrs*CSF_ptau_baseline +
(1 + time_from_baseline_yrs|Individual_ID) + (1|Family), DIAN_dataset)

The relationship between the rates of change of two biomarkers or biomarker/cognitive composite was studied using separated bivariate LME models per each pair of variables. In each separate bivariate LME model both variables of study were the outcome. Changes from baseline were used as the outcomes instead of the values at each visit that are usually used in LME models. This is 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 bivariate LME models included the covariates of baseline parental EYO, baseline outcomes of interest, baseline CSF p-tau (this is not included if p-tau or t-tau is the outcome), baseline CSF Aβ42 (this is not included if Aβ42 is the outcome) and their interaction with time and group (sTREM2 and another outcome). 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. Statistical model details and related SAS code can be found in Luo *et al*.[13] We also report the associations between each pair of biomarkers without adjusting for covariates in the supplementary table 14.

Bivariate LME models

Outcome	Fixed effects
sTREM2 and one of the other outcomes (cognitive composite, hippocampal volume, precuneus thickness, FDG precuneus)	baseline outcome of interest, baseline EYO, baseline CSF pTau, baseline CSF Aβ42 and their interaction with time and group
sTREM2 and one of the other outcomes (CSF Aβ42, PiB PET cortical mean)	baseline outcome of interest, baseline EYO, baseline CSF pTau, and their interaction with time and group
sTREM2 and one of the other outcomes (CSF tau, pTau)	baseline outcome of interest, baseline EYO, baseline CSF Aβ42 and their interaction with time and group

The modification effect of the rate of CSF sTREM2 (1) on the association between the longitudinal changes of CSF Aβ42 and PiB-PET signal and (2) on the association between the longitudinal changes of CSF p-tau and PiB-PET signal were explored using linear or quadratic regression models. The raw rate of change of each biomarker, calculated as explained above, was used for this analysis. We compared linear and quadratic regression models based on the AIC and Bayesian Information criterion (BIC) (see the supplementary table 3 below). CSF sTREM2 increase rate was considered as high when the raw rate of sTREM2 change was higher that the median and low when it was equal or lower than the median. The binary variable high vs. low raw rate of sTREM2 change was used in the regression models and for graphical representations and was determined separately in presymptomatic MC and symptomatic MC

For (1), the linear model consisted on the raw rate of CSF $A\beta_{42}$ change as dependent variable (outcome) and the raw rate of PiB-PET total cortical uptake, baseline PiB-PET total cortical uptake, baseline CSF $A\beta_{42}$ raw rate of sTREM2 change -binary- and the interaction term raw 'rate of PiB-PET total cortical uptake'*'raw rate of sTREM2 change -binary-' as the independent variables:

lm(raw_Ab42_change_rate ~ CSF_Ab42_baseline + PiB_PET_baseline +
raw_PIB_change_rate + raw_sTREM2_change_binary
raw_sTREM2_change_binary*raw_PIB_change_rate, DIAN_dataset)

In the quadratic model we additionally introduced (raw rate of PiB-PET total cortical uptake)^2 and the '(rate of PiB-PET total cortical uptake)^2'*'raw rate of sTREM2 change -binary- as independent variables:

lm(raw_Ab42_change_rate ~ CSF_Ab42_baseline + PiB_PET_baseline +
raw_PIB_change_rate + raw_PIB_change_rate^2 + raw_sTREM2_change_binary
raw_sTREM2_change_binary*raw_PIB_change_rate +
raw_sTREM2_change_binary*raw_PIB_change_rate^2, DIAN_dataset)

For (2) the linear and quadratic regression models were analogous to the ones previously described but accounting for the raw rate of CSF p-tau change as the dependent variable (outcome) and the baseline CSF p-tau as independent variable instead of baseline CSF $A\beta_{42}$.

These regression models were run separately in presymptomatic MC and symptomatic MC.

The relationship between the longitudinal changes of amyloid markers and between longitudinal changes of CSF p-tau and PiB-PET total cortical uptake stratified by the CSF sTREM2 rate of change group were also explored using similar bivariate LME models as described above.

Supplementary table 3

	Longitudinal CSF p-tau and PiB-PET		**Longitudinal CSF $A\beta_{42}$ and PiB-PET**	
	Presymptomatic MC	Symptomatic MC	Presymptomatic MC	Symptomatic MC
AIC quadratic model	-163.13	-31.26	-143.61	-26.98
AIC linear model	-161.86	-34.07	-121.58	-22.75
BIC quadratic model	-144.28	-23.76	-124.77	-19.48
BIC linear model	-147.2	-28.24	-106.92	-16.92

AIC and BIC of the quadratic and regression models performed to study the interaction of the longitudinal sTREM2 change on the relationship between the longitudinal changes of Aβ markers and between the longitudinal changes of CSF p-tau and PiB-PET. AIC= Akaike information criterion. BIC= Bayesian Information Criterion. CSF=Cerebrospinal Fluid. p-tau= phosphorylated tau on threonine 181. PIB-PET= Pittsburgh compound B Positron Emission Tomography.

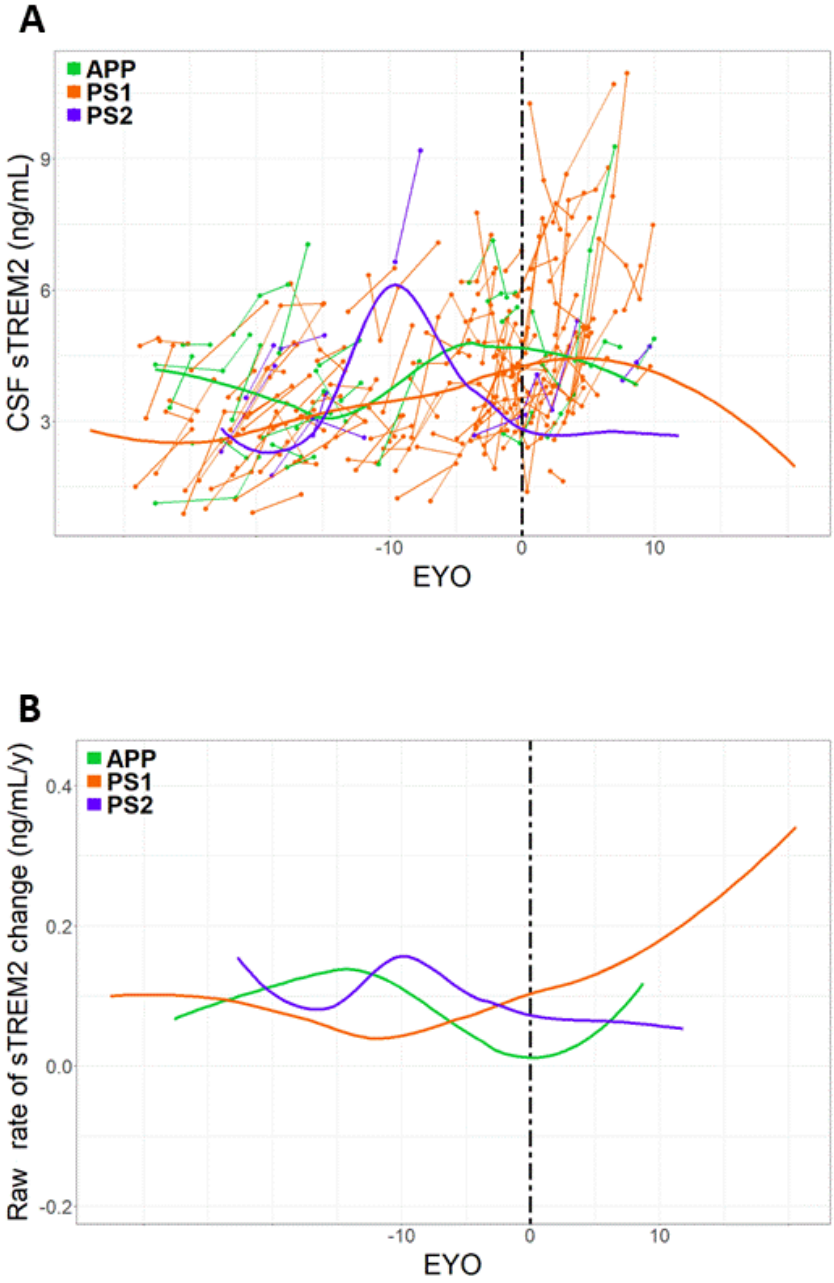
Supplementary results

Supplementary table 4

	All participants (n = 239)			MC (n = 148)			NC (n = 91)		
Predictor	**β-coefficient**	**SE**	**p-value**	**β-coefficient**	**SE**	**p-value**	**β-coefficient**	**SE**	**p-value**
Time*EYO at baseline	$1.05x10^{-4}$	$4.8x10^{-4}$	0.828	$-3.56x10^{-4}$	$5.8x10^{-4}$	0.542	$1.08x10^{-3}$	$8.39x10^{-4}$	0.206
Time*Age at baseline	$3.35x10^{-5}$	$4.79x10^{-4}$	0.944	$8.56x10^{-5}$	$5.93x10^{-4}$	0.886	$9.49x10^{-5}$	$8.18x10^{-4}$	0.908
Time*Sex (female)	$-7.77x10^{-3}$	0.011	0.471	$-5.87x10^{-4}$	0.014	0.965	-0.022	0.017	0.219
Time*Educational level	$-1.27x10^{-3}$	$2.07x10^{-3}$	0.540	$-1.46x10^{-3}$	$2.41x10^{-3}$	0.574	$2.31x10^{-3}$	$4.06x10^{-3}$	0.573
Time*APOE-ε4 –NC-	$1.13x10^{-2}$	0.012	0.341	$7.69x10^{-3}$	0.015	0.614	$1.87x10^{-2}$	0.019	0.323
Time*Mutation (NC vs. MC)*	0.0178	0.011	0.107	-	-	-	-	-	-
Time*Mutation type (PS1 vs. APP)*	-	-	-	0.0298	0.02	0.141	-	-	-
Time*Mutation type (PS2 vs. APP)*	-	-	-	0.0145	0.029	0.619	-	-	-
Time*Dutch mutation (MC vs other)**	-	-	-	0.0199	0.024	0.418	-	-	-

Influence of demographics, mutation status and mutation type on the subsequent longitudinal change of CSF sTREM2. We summarize only the β-coefficient and p-values for the interaction term Time(from baseline)*Demographics at baseline/Mutation status/ Mutation Type. Each interaction term comes from separate models and it represents in each separate model the effect of the predictor at baseline on the subsequent rate of sTREM2 change. These separate univariate LME models included longitudinal CSF sTREM2 (dependent variable) by time-from-baseline, predictor biomarker at baseline and interaction between time-from-baseline*predictor-biomarker as fixed effects, and individual slope and intercept as random factors (see model details in the supplementary material pages 8-9). The results were similar when also introducing family as a random factor. *In the case of Mutation status and Mutation type, we also introduced in the models EYO at baseline and the interaction term Time*EYO-at-baseline as fixed factor. **Dutch mutation carriers (n=6) vs rest of MC (n=148). CSF=Cerebrospinal Fluid. LME=Linear Mixed Effects. MC=Mutation Carriers. NC=mutation Non-Carriers. SE=Standard Error. PS1=Presenilin 1. PS2=Presenilin 2. APP=Amyloid Precursor Protein. EYO=Estimated Years to/from symptom Onset.

Supplementary figure 3



Longitudinal sTREM2 levels in CSF and raw rate of sTREM2 change along EYO in MC according the mutation type (APP, PS1 or PS2). (A) Spaghetti plot showing the longitudinal levels of CSF sTREM2 from MC as a function of EYO, divided into three groups according to their mutation type: APP (light green, n = 26), PS1 (orange, n = 112), PS2 (purple, n = 10). The continuous lines overlapping the spaghetti plot are the LOESS curves fitting the baseline sTREM2 values for each participant representing the cross-sectional differences across groups. The bold dot line at EYO = zero is pointing the expected onset per each participant according their parental onset. The representation of the individual participants is limited to EYO from -30 to 10 and excluding the extremes to maintain the participants' confidentiality. (B) Estimated raw rate of sTREM2 change (slope in the linear regression longitudinal sTREM2 by time) plotted against EYO at baseline for each mutation-type group. Individual values are not shown to preserve the participants' confidentiality. This graph has only illustrational purposes and no statistical meaning. Our results regarding the influence of the mutation type on the rate of sTREM2 change are based in the LME models showed in table 2. CSF=Cerebrospinal fluid. EYO=Estimated Years to/from symptom Onset. APP= Amyloid Precursor Protein. PS1= Presenilin 1. PS2= Presenilin 2. MC=Mutation carriers. LOESS=Locally Weighted Scatterplot Smoothing. LME=Linear Mixed Effects.

Supplementary table 5

	MC (n = 148)			NC (n = 91)		
Predictor	**β-coefficient**	**SE**	**p-value**	**β-coefficient**	**SE**	**p-value**
Time*CSF Aβ42*	-0.04283	0.013	0.001	0.0336	0.026	0.2
Time*CSF Aβ42/Aß40*	-0.6565	0.249	0.011	0.6054	1.144	0.598
Time*PIB-PET uptake*	-0.00551	0.011	0.632	0.0386	0.098	0.693
Time*CSF p-tau	$-5.64x10^{-4}$	0.012	0.962	0.0139	0.028	0.618
Time*CSF t-tau	-0.00457	0.015	0.758	0.024	0.031	0.436
Time*Cortical thickness (precuneus)**	-0.0437	0.051	0.396	-0.0118	0.076	0.877
Time*Hippocampal Volume**	$-5.27x10^{-6}$	$8.7x10^{-6}$	0.546	$2.58x10^{-6}$	$1.57x10^{-6}$	0.107

Influence of AD-related biomarkers at baseline on the subsequent longitudinal change of CSF sTREM2. We summarize only the β-coefficient and p-values for the interaction term Time(from baseline)*predictor biomarker at baseline, which represents in each separate univariate LME model the effect of the predictor at baseline on the subsequent rate of sTREM2 change. These separate LME models consisted on longitudinal CSF sTREM2 (dependent variable) by time–from-baseline, EYO at baseline, predictor biomarker at baseline and interactions Time*EYO at baseline and Time*Predictor at baseline as fixed factors and individual slope, intercept and family as random factors. The models adjusted or no adjusted by CSF p-tau, $A\beta_{42}$ or both had similar results. *Models adjusted by CSF p-tau and its interaction by time. Albeit results adjusted or unadjusted were similar, we show the adjusted results in the three models to allow comparison of amyloid deposition markers, we show the adjusted results in the three models (see supplementary methods, page 9, for details). **Models adjusted by CSF p-tau and $A\beta_{42}$ at baseline and their interaction with time. MC=Mutation Carriers. NC= Mutation Non-Carriers. CSF=Cerebrospinal Fluid. t-tau= total tau. p-tau= phosphorylated tau on threonine 181. PIB-PET= Pittsburgh compound B Positron Emission Tomography. SE= Standard error. EYO=Estimated Years to/from symptom Onset. LME=Linear Mixed Effects

Supplementary table 6

	Presymptomatic MC (n = 62)			Symptomatic MC (n = 24)		
Term	β-coefficient	SE	p-value	β-coefficient	SE	p-value
Intercept	-0.082	0.047	0.09	0.0001	0.029	0.996
Baseline CSF p-tau	0.02	0.015	0.189	-	-	-
Baseline PiB-PET cortical mean	0.006	0.012	0.632	-	-	-
Rate of PiB-PET change	0.272	0.114	0.02	0.062	0.253	0.244
Rate of sTREM2 change (binary - High)	0.024	0.018	0.188	0.048	0.037	0.219
PiB-PET change * sTREM2 Change (High)	-0.394	0.137	0.006	-0.271	0.288	0.362
Model R-squared	0.23			0.18		
Model p-value	0.01			0.38		

Modification effect of the rate of sTREM2 on the association between CSF p-tau and PiB-PET rates of change. Regression models were fitted separately in presymptomatic and symptomatic MC with CSF p-tau as the outcome. In symptomatic MC the model is unadjusted by baseline biomarkers or EYO as including them resulted in overfitting. MC= Mutation Carriers. CSF=Cerebrospinal Fluid. p-tau=phosphorylated tau on threonine 181. PiB-PET=Pittsburgh compound B Positron Emission Tomography. EYO=Estimated Years to/from symptom Onset

Supplementary table 7

	Presymptomatic MC (n = 62)			Symptomatic MC (n = 24)		
Term	β-coefficient	SE	p-value	β-coefficient	SE	p-value
Intercept	-0.089	0.161	0.584	1.622	0.462	0.007
Baseline CSF Aβ42	0.0005	0.022	0.984	-0.274	0.078	0.006
Baseline PiB-PET cortical mean	0.007	0.014	0.637	-0.017	0.019	0.408
Rate of PiB-PET change	-0.7	0.302	0.024	0.205	0.469	0.671
Rate of PiB-PET change^2	5.015	1.091	<0.001	2.802	3.911	0.492
Rate of sTREM2 change (binary - High)	0.029	0.024	0.238	0.016	0.048	0.752
PiB-PET change * sTREM2 Change (High)	0.974	0.318	0.003	-2.105	3.948	0.607
PiB-PET change^2 * sTREM2 Change (High)	-6.24	1.135	<0.001	-0.632	0.542	0.274
Model R-squared	0.51			0.66		
Model p-value	<0.001			0.097		

Modification effect of the rate of sTREM2 on the association between CSF Aβ42 and PiB-PET rates of change. Regression models were fitted separately in presymptomatic and symptomatic MC with CSF $A\beta_{42}$ as the outcome. MC=Mutation Carriers. CSF=Cerebrospinal Fluid. PiB-PET=Pittsburgh compound B Positron Emission Tomography.

Supplementary table 8

	Presymptomatic MC (n = 100)			Symptomatic MC (n = 48)		
Term	β-coefficient	SE	p-value	β-coefficient	SE	p-value
Intercept	0.15	0.184	0.42	1.305	0.444	0.012
Baseline CSF Aβ42	-0.033	0.026	0.212	-0.224	0.075	0.012
Baseline PiB-PET cortical mean	-0.0004	0.017	0.979	0.001	0.018	0.936
Rate of PiB-PET change	0.297	0.121	0.017	-0.015	0.271	0.956
Rate of PiB-PET change^2	-0.439	0.475	0.360	-0.188	0.768	0.811
Model R-squared	0.149			0.47		
Model p-value	0.06			0.084		

Regression model assessing the relationship between CSF Aβ42 and PiB-PET rates of change without accounting for the interaction with the rate of sTREM2 change. Regression models were fitted separately in presymptomatic and symptomatic MC with CSF $A\beta_{42}$ as the outcome. MC=Mutation Carriers. CSF= Cerebrospinal Fluid. PiB-PET=Pittsburgh compound B Positron Emission Tomography.

Supplementary table 9

		Low rate of sTREM2 change (n = 50)			High rate of sTREM2 change (n = 48)		
Longitudinal variable pairs -bivariate LME models-	**Model**	**r-value**	**SE**	**p-value**	**r-value**	**SE**	**p-value**
PiB-PET / CSF Aβ42	Adjusted by baseline EYO	0.40	0.25	0.11	-0.31	0.40	0.44
	Unadjusted	0.26	0.28	0.36	-0.37	0.53	0.49
PiB-PET / p-tau	Adjusted by baseline EYO	0.45	0.21	0.032	0.22	0.74	0.76
	Unadjusted	0.51	0.19	0.009	0.25	0.83	0.76

Correlation values for each studied variable pair based on bivariate LME models. Bivariate LME models were performed in asymptomatic MC to assess the relationship between the rates of change of each pair of studied variables stratifying by the raw rate of sTREM2 change (below or above the median). We found a significant relationship between a higher increase rate of PiB-PET change and a higher increase rate in CSF p-tau in the group with low raw rate of sTREM2 change, while in the other the relationship was not significant. In the case of the relationship between PiB-PET and CSF $A\beta_{42}$, and CSF $A\beta_{42}$ and p-tau the models do not show significant associations, but the directions of the associations are opposite in MC with low or high rate of CSF sTREM2 change, supporting the modification effect of the rate of sTREM2 change on both relationships. Bivariate LME models were not performed in symptomatic MC stratified by their raw rate of sTREM2 change due to the small n-number in each subgroup that would compromise the validity of these models. MC= Mutation Carriers. SE=Standard Error. CSF= Cerebrospinal Fluid. PiB-PET=Pittsburgh compound B Positron Emission Tomography. LME=Linear Mixed Effects. p-tau=phosphorylated tau on threonine 181. EYO=Estimated Years to/from symptom Onset

Supplementary table 10

	MC (n = 148) – sTREM2 at baseline*Time		
Outcome	**β-coefficient**	**SE**	**p-value**
CSF Aβ42	$1x10^{-3}$	0.013	0.939
PIB-PET uptake	$7.82x10^{-3}$	0.022	0.718
CSF p-tau	0.017	0.017	0.308
CSF t-tau	$-9.51x10^{-3}$	0.015	0.534
Cortical Thickness (Precuneus)*	$5.91x10^{-3}$	$6.15x10^{-3}$	0.339
Hippocampal Volume*	66.06	28.94	0.02

Effect of CSF sTREM2 at baseline on the subsequent change of different AD biomarkers. We summarize the β-coefficient and p-values of the interaction term sTREM2-baseline*Time-from_baseline, representing the effect of baseline sTREM2 (predictor) on the subsequent longitudinal change of CSF $A\beta_{42}$, PIB-PET uptake, CSF p-tau, t-tau (dependent variable) from separate univariate LME models per each dependent variable. These models consisted on the dependent longitudinal variable (outcome) by sTREM2 and EYO at baseline and their interactions with time-from-baseline as fixed factors and family, individual slope and intercept as random factors. In the case of β-amyloid markers, the introduction of p-tau or t-tau and their interactions with time-from baseline as fixed factors did not have a significant effect on the results. In the case of tau pathology-related markers, the introduction of CSF $A\beta_{42}$ and its interactions with time-from-baseline as fixed factors did not alter the results. *Results based on the univariate LME model: longitudinal cortical thickness (precuneus) or hippocampal volume by sTREM2, EYO and p-tau at baseline and their interactions with time-from-baseline as fixed factors and family, individual slope and intercept as random factors. MC=Mutation Carriers. SE = Standard Error. CSF=Cerebrospinal Fluid. EYO=Estimated Years to/from symptom Onset. t-tau= total tau. p-tau= phosphorylated tau on threonine 181. PIB-PET= Pittsburgh compound B Positron Emission Tomography. LME=Linear Mixed Effects

Appendix 1. Results including participants with extreme rate of sTREM2 change.

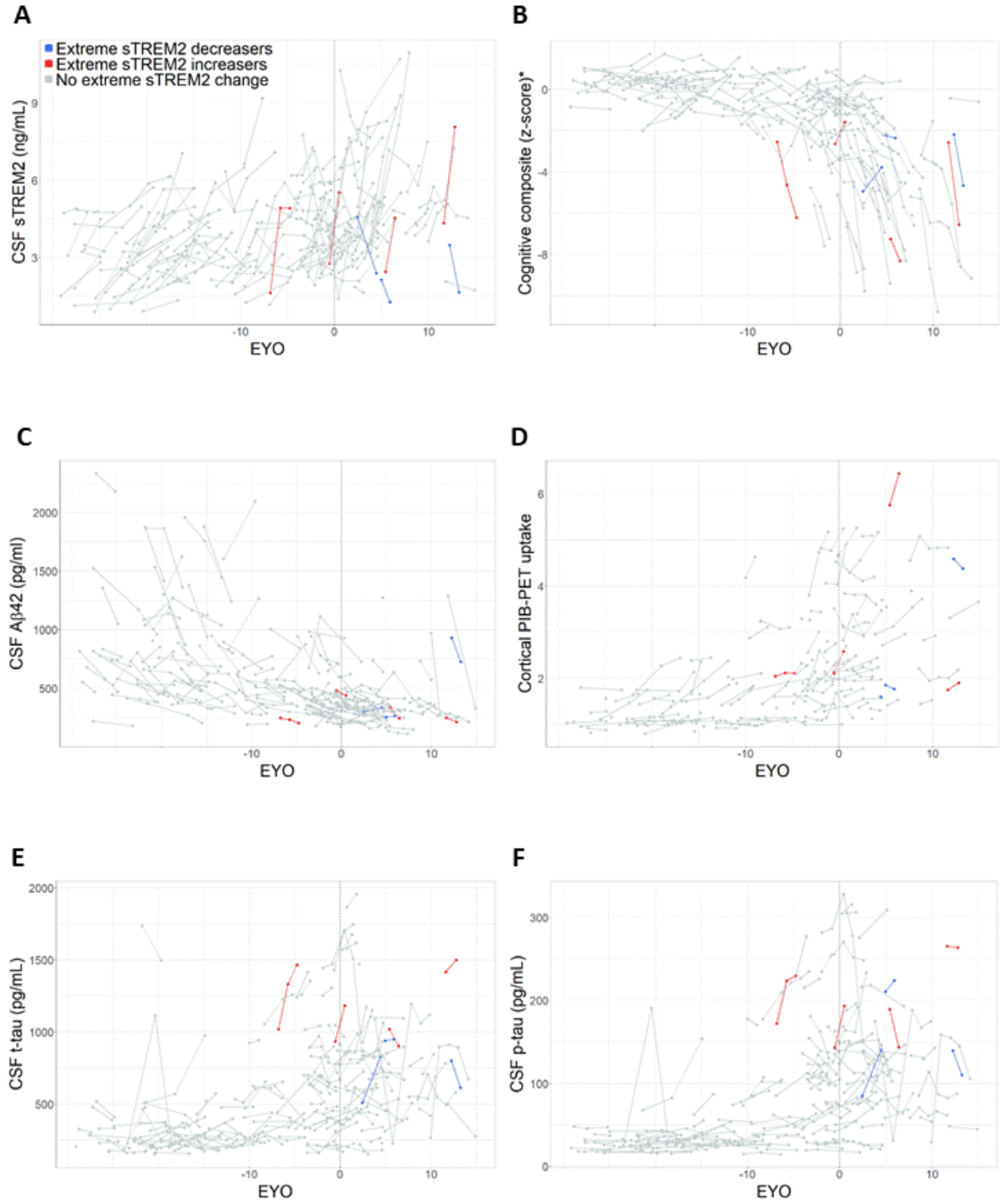
In this Appendix, we show the main results including those participants with an extreme rate of sTREM2 change, defined as those with a raw rate of sTREM2 change higher or lower than the mean plus or minus 3SD, respectively. We found two NC and seven MC with extreme rate of sTREM2 change. Their characteristics at baseline and their biomarker trajectories are described below in supplementary table 9 and supplementary figure 4. All MC with extreme sTREM2 change were symptomatic, therefore, the exclusion or inclusion of these participants do not affect the results in the presymptomatic MC group where we found our main results. We run the same models including and excluding these participants, with the only difference of not including family as a random factor in the univariate models per MC including participants with extreme rate of sTREM2 change as the models including family in this group did not converge (supplementary tables 12 and 13). Regarding the bivariate models, only the one assessing the relationship between the rate of sTREM2 change and the hippocampal shrinkage in symptomatic MC did not converge when including the subjects with extreme rate of sTREM2 change (supplementary table 12). Results arisen from the analysis including or excluding the participants with extreme rate of sTREM2 change were highly consistent.

Supplementary table 11

	MC with extreme sTREM2 increase rate (n=4)	**MC with extreme sTREM2 decrease rate (n=3)**
Age, mean (SD), years	40.17 (10.7)	46.2 (5.1)
Sex, % female (n)	75 (3)	33.33 (1)
APOE status, %ε4 carriers (n)	0 (0)	66.67 (2)
EYO, mean (SD), years	2.42 (7.9)	6.54 (5.1)
Mean follow up (SD), years	1.31 (0.5)	1.32 (0.6)
MMSE, mean (SD)	20.67 (7.6)	25 (4.4)
Cognitive composite, mean z-score (SD)	-3.74 (2.3)	-3.12 (1.6)
CSF Aβ42, mean (SD), pg/mL	328.5 (109.4)	496.33 (378.1)
CSF Aβ40, mean (SD), pg/mL	7969 (1077.8)	7894 (2100.1)
CSF t-tau, mean (SD), pg/mL	749.33 (220.4)	1098 (217.15)
CSF p-tau, mean (SD), pg/mL	192.4 (52.4)	145.07 (63.1)
CSF sTREM2, mean (SD), ng/mL	2.79 (1.1)	3.39 (1.2)

Demographics and biomarker levels at baseline of participants with extreme rate of sTREM2 change. Data from the NC with extreme rate of sTREM2 change are not shown, as there was only one participant with extreme sTREM2 increase rate and other one participant with extreme sTREM2 decrease rate. SD= Standard Deviation. CSF=Cerebrospinal Fluid. MC=Mutation Carriers. NC=Mutation Non-Carriers. APOE=Apolipoprotein E. MMSE=Minimental State Examination. EYO=Estimated Years to/from symptom Onset. t-tau= total tau. p-tau= phosphorylated tau on threonine 181.

Supplementary figure 4



Biomarker trajectories for MC with extreme rate of sTREM2 change. (A), (B), (C), (D), (E) and (F) show the spaghetti plots for different studied biomarkers (sTREM2, cognitive composite, CSF $A\beta_{42}$, total cortical uptake in the PIB-PET, CSF t-tau and CSF p-tau respectively) in MC, highlighting in red those with an extreme sTREM2 increase rate (n = 4), and, in blue, those with an extreme sTREM2 decrease rate (n=3). The rest of MC are represented in grey (n=148). *The cognitive composite was assessed as already published (Bateman *et al.* 2017) as a composite measurement comprising the z-scores per each participant of the following tests: DIAN Word List Test, Logical Memory delayed recall, Digit Symbol Coding test (total score), and MMSE. CSF= Cerebrospinal Fluid. MC= Mutation Carriers. EYO=Estimated Years to/from symptom Onset. t-tau= total tau. p-tau=phosphorylated tau on threonine 181. MMSE=Minimental State Examination. PIB-PET= Pittsburgh compound B Positron Emission Tomography.

Supplementary table 12

	All participants (n = 248)			MC (n = 155)			NC (n = 93)		
Predictor	**β-coefficient**	**SE**	**p-value**	**β-coefficient**	**SE**	**p-value**	**β-coefficient**	**SE**	**p-value**
Time*EYO at baseline	$-5.16x10^{-5}$	$4.75x10^{-4}$	0.914	$-3.89x10^{-4}$	$5.77x10^{-4}$	0.503	$8.02x10^{-4}$	$8.38x10^{-4}$	0.345
Time*Age at baseline	$-8.21x10^{-5}$	$4.75x10^{-4}$	0.863	$1.438x10^{-5}$	$5.93x10^{-4}$	0.981	$-8.48x10^{-5}$	$4.75x10^{-4}$	0.918
Time*Sex (female)	$-6.53x10^{-3}$	0.011	0.543	$7.11 x10^{-4}$	0.014	0.958	-0.0204	0.017	0.236
Time*Educational level*	$-1.28x10^{-3}$	$2.08x10^{-3}$	0.539	$-1.12x10^{-3}$	$2.46x10^{-3}$	0.649	$-3.08x10^{-3}$	$4.04x10^{-3}$	0.452
Time*Mutation (NC vs. MC)*	0.0167	0.012	0.128	-	-	-	-	-	-
Time*Mutation type (PS1 vs. APP)*	-	-	-	0.0367	0.029	0.072	-	-	-
Time*Mutation type (PS2 vs. APP)*	-	-	-	0.0169	0.0289	0.563	-	-	-
Time*Dutch mutation (Dutch mutation NC vs MC)*	-	-	-	0.0209	0.0239	0.386	-	-	-

Influence of demographics, mutation status and mutation type on the subsequent longitudinal change of CSF sTREM2 - not excluding individuals with extreme rate of sTREM2 change. We summarize only the β-coefficient and p-values for the interaction term Time(from baseline)*Demographics at baseline/Mutation status/ Mutation Type, which represents in each separate model the effect of the predictor at baseline on the subsequent rate of sTREM2 change. These separate univariate LME models consisted of longitudinal CSF sTREM2 (dependent variable) by time-from-baseline, predictor biomarker at baseline and interaction between time-from-baseline*predictor-biomarker as fixed factors, and individual slope and intercept as random factors. *Models studying the influence of mutation status and type and educational level on the rate of sTREM2 change also added EYO and its interaction with time-from-baseline as fixed factors. MC=Mutation Carriers. NC=Mutation Non-Carriers. SE=Standard Error. PS1=Presenilin 1. PS2=Presenilin 2. APP=Amyloid Precursor Protein. EYO=Estimated Years to/from symptom Onset. LME=Linear Mixed Effects

Supplementary table 13

	MC (n = 155)			NC (n = 93)		
Predictor	**β-coefficient**	**SE**	**p-value**	**β-coefficient**	**SE**	**p-value**
Time*CSF Aβ42*	-0.046	0.013	<0.001	0.0365	0.037	0.33
Time*CSF Aβ42/Aβ40*	-0.59	0.248	0.02	0.452	1.18	0.7
Time*PIB-PET uptake*	$-1.67x10^{-4}$	0.011	0.99	0.04	0.098	0.68
Time*CSF p-tau	0.0066	0.012	0.58	0.0049	0.028	0.86
Time*CSF t-tau	0.0038	0.015	0.8	0.0182	0.031	0.56
Time*Cortical thickness**	-0.061	0.049	0.22	0.0102	0.085	0.91
Time*Hippocampal Volume**	$-3.34x10^{-6}$	$8.54x10^{-6}$	0.7	$8.68x10^{-6}$	$1.87x10^{-5}$	0.65

Influence of AD-related biomarkers at baseline on the subsequent longitudinal change of CSF sTREM2 – not excluding participants with an extreme sTREM2 change. We summarize only the β-coefficient and p-values for the interaction term Time(from baseline)*predictor biomarker at baseline, which represents in each separate univariate LME model the effect of the predictor at baseline on the subsequent rate of sTREM2 change. These separate LME models consisted on longitudinal CSF sTREM2 (dependent variable) by time–from-baseline, EYO at baseline, predictor biomarker at baseline and interactions Time*EYO at baseline and Time*Predictor at baseline as fixed factors and individual slope, intercept and family as random factors. The models adjusted or no adjusted by CSF p-tau, $A\beta_{42}$ or both had similar results in all the models, so we are showing unadjusted results if nothing else is specified. *Models adjusted by CSF p-tau (introducing CSF p-tau log-transformed values at baseline and its interaction with time-from-baseline as fixed factors in the model). Albeit results adjusted or unadjusted were similar, we show the adjusted results in the three models to allow comparison of amyloid deposition markers, we show the adjusted results in the three models (see supplementary methods, page 9, for details)**We are showing the results adjusted by CSF p-tau and $A\beta_{42}$ at baseline (introducing the baseline biomarker values and their interaction with time-from-baseline as fixed factors in the model) in the case of the structural neuroimaging variables. Adjusted and unadjusted results were similar, but the unadjusted model in NC in the case of cortical thickness in the precuneus did not converge. To make them comparable, we are showing the adjusted results for all the structural neuroimaging biomarkers. MC=Mutation Carriers. NC= Mutation Non-Carriers. SE = Standard Error. CSF=Cerebrospinal Fluid. EYO=Estimated Years to/from symptom Onset. t-tau= total tau. p-tau= phosphorylated tau on threonine 181. PIB-PET= Pittsburgh compound B Positron Emission Tomography. LME=Linear Mixed Effects

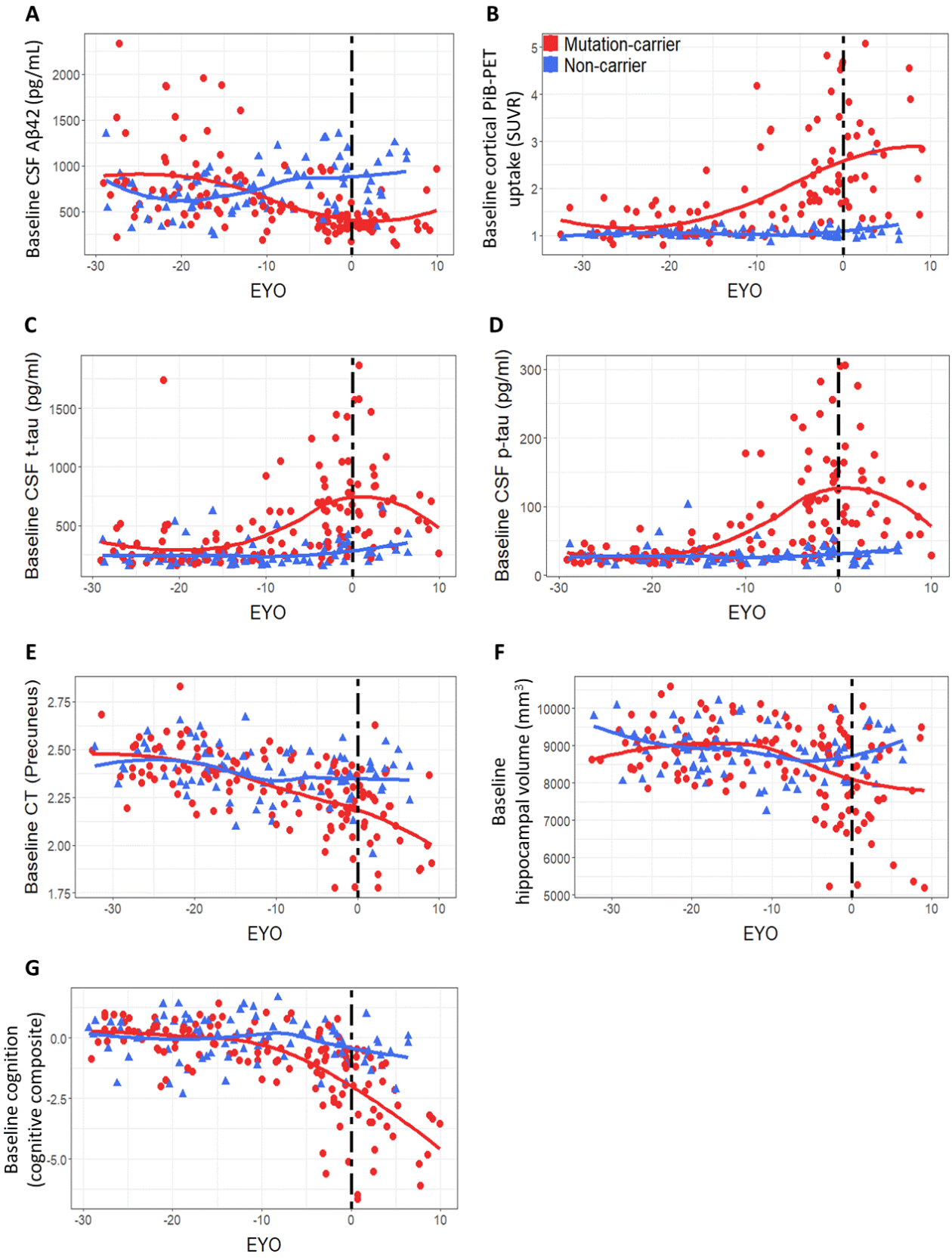
Supplementary table 14

		Controlling for covariates				Not controlling for covariates			
		All MC		Excluding participants with extreme sTREM2 change		All MC		Excluding participants with extreme sTREM2 change	
Longitudinal variable pairs -bivariate LME models-	**Group**	**r-value (SE)**	**p-value**	**r-value (SE)**	**p-value**	**r-value (SE)**	**p-value**	**r-value (SE)**	**p-value**
sTREM2 / Aβ42	All MC	0.19 (0.19)	0.31	0.25 (0.17)	0.14	0.26 (0.15)	0.08	0.29 (0.14)	0.04
	AS MC	0.56 (0.22)	0.011	0.56 (0.22)	0.011	0.44 (0.18)	0.01	0.44 (0.18)	0.01
	Symp MC	-0.02 (0.29)	0.94	0.37 (0.26)	0.16	0.14 (0.23)	0.56	0.28 (0.21)	0.18
sTREM2 / t-tau	All MC	0.39 (0.21)	0.06	0.34 (0.19)	0.08	0.35 (0.18)	0.05	0.32 (0.17)	0.06
	AS MC	0.35 (0.28)	0.22	0.35 (0.28)	0.22	0.37 (0.25)	0.14	0.37 (0.25)	0.14
	Symp MC	0.58 (0.44)	0.19	0.16 (0.85)	0.85	0.38 (0.25)	0.14	0.32 (0.25)	0.19
sTREM2 / p-tau	All MC	0.21 (0.21)	0.32	0.18 (0.19)	0.35	0.15 (0.18)	0.4	0.14 (0.17)	0.41
	AS MC	-0.04 (0.36)	0.92	-0.04 (0.36)	0.92	0.2 (0.28)	0.48	0.2 (0.28)	0.48
	Symp MC	0.33 (0.45)	0.46	-0.02 (0.58)	0.98	0.23 (0.27)	0.38	0.22 (0.25)	0.38
sTREM2 / Hippocampal volume	All MC	0.09 (0.22)	0.68	0.06 (0.2)	0.74	-0.006 (0.16)	0.97	0.03 (0.15)	0.82
	AS MC	-0.01 (0.28)	0.97	-0.01 (0.28)	0.97	0.13 (0.18)	0.48	0.13 (0.18)	0.48
	Symp MC	0.08 (0.3)	0.79	0.16 (0.31)	0.60	0.14 (0.25)	0.57	0.29 (0.24)	0.23
sTREM2 / CT –precuneus-	All MC	0.19 (0.21)	0.36	0.25 (0.19)	0.18	0.02 (0.16)	0.88	0.12 (0.15)	0.4
	AS MC	0.46 (0.22)	0.04	0.46 (0.22)	0.04	0.32 (0.17)	0.07	0.32 (0.17)	0.07
	Symp MC	0.28 (0.32)	0.39	0.56 (0.29)	0.06	0.07 (0.26)	0.8	0.45 (0.35)	0.2
sTREM2 / PIB-PET uptake	All MC	-0.38 (0.21)	0.07	-0.46 (0.17)	0.007	-0.36 (0.18)	0.05	-0.42 (0.15)	0.006
	AS MC	-0.19 (0.25)	0.45	-0.19 (0.25)	0.45	-0.31 (0.19)	0.1	-0.31 (0.19)	0.1
	Symp MC	-0.25 (0.39)	0.51	-0.67 (0.25)	0.006	-0.13 (0.31)	0.67	-0.58 (0.21)	0.006
sTREM2 / cognitive composite	All MC	-0.21 (0.18)	0.25	-0.01 (0.17)	0.95	-0.2 (0.14)	0.16	-0.06 (0.13)	0.66
	AS MC	0.67 (0.22)	0.002	0.67 (0.22)	0.002	0.34 (0.16)	0.03	0.34 (0.16)	0.03
	Symp MC	-0.46 (0.24)	0.05	-0.07 (0.28)	0.79	-0.33 (0.23)	0.16	0.03 (0.25)	0.92

Correlations between sTREM2 rate of change and the rate of change of the other studied biomarkers based on bivariate LME models excluding and not excluding participants with an extreme rate of sTREM2 change, and controlling and not controlling for confounders. The overall results do not change significantly when including or excluding MC with extreme sTREM2 change. The models were controlled by p-tau at baseline in the case of sTREM2 vs. $A\beta_{42}$, and total cortical PIB-PET uptake; by $A\beta_{42}$ in the case of t-tau and p-tau, and by $A\beta_{42}$ and p-tau in the case of CT in the precuneus, hippocampal volume and cognitive composite[12]. When not controlling for covariates, we obtained similar results, but with a lower statistical significance. MC=Mutation Carriers. SE=Standard Error. LME=Linear Mixed Effects. t-tau=total tau. p-tau=phosphorylated tau on threonine 181. PiB-PET=Pittsburgh compound B Positron Emission Tomography. CT=Cortical Thickness. Symp=Symptomatic. AS=Asymptomatic.

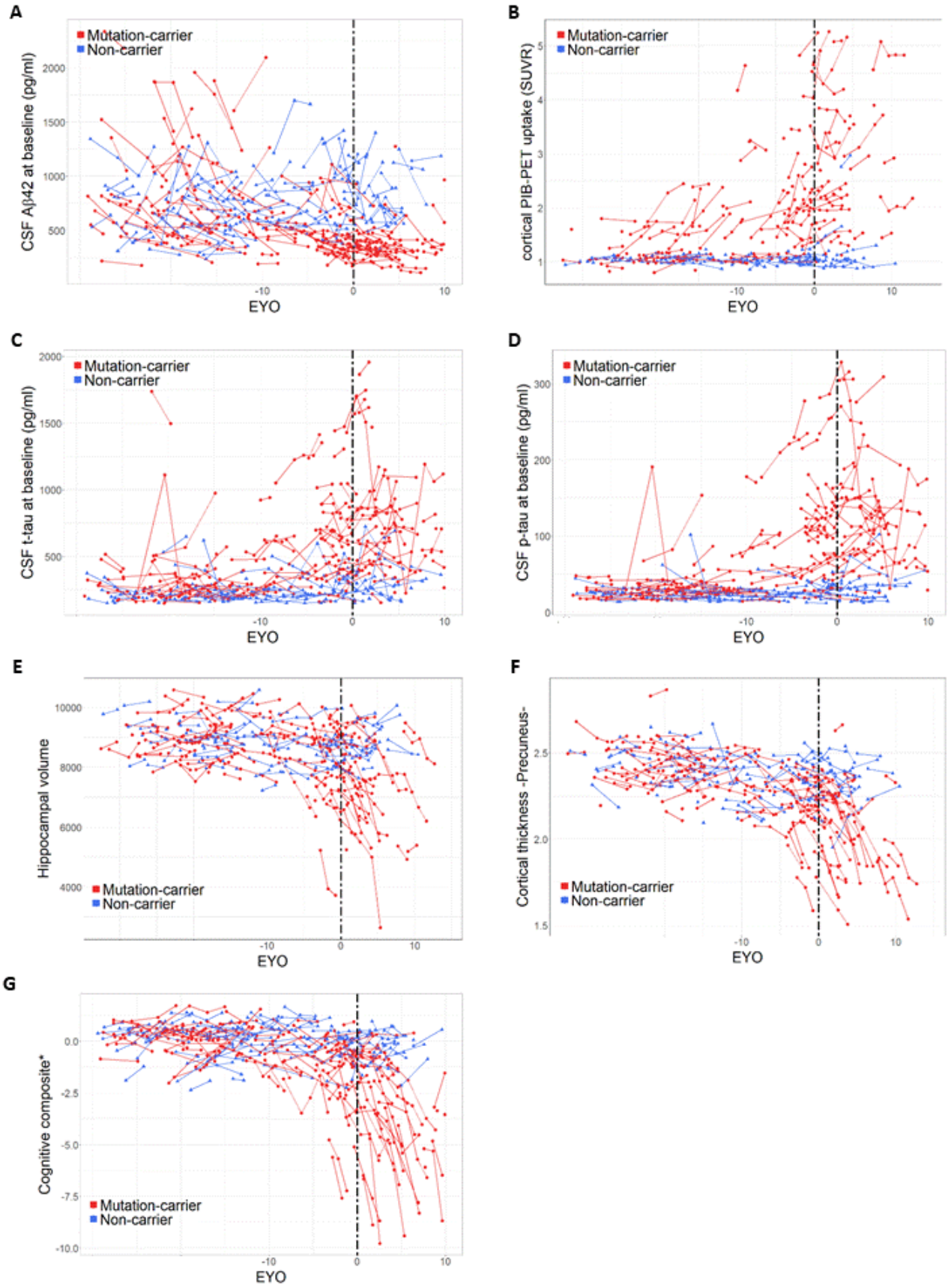
<u>Appendix 2</u>. Baseline and Longitudinal measurements of CSF Aβ42, PiB-PET, CSF t-tau and p-tau, cortical thickness in the precuneus, hippocampal volume and cognitive composite.

Supplementary figure 5



Baseline biomarker values. (A), (B), (C), (D), (E), (F) and (G) show the baseline biomarker values for mutation carriers (red) and non-carriers (blue) along EYO **(**CSF $A\beta_{42}$, total cortical uptake in the PIB-PET, CSF t-tau and CSF p-tau, cortical thickness in the precuneus (mm), hippocampal volume (mm^3), and cognitive composite, respectively). Some of these data were already shown elsewhere[14]. Continuous red (mutation carriers) and blue (non-carriers) lines implicate the LOESS best-fitting curves. Dashed lines pointed to EYO = 0, indicating the expected symptom onset according to the parental onset. CSF= Cerebrospinal Fluid. EYO=Estimated Years to/from symptom Onset. t-tau= total tau. p-tau=phosphorylated tau on threonine 181. PIB-PET= Pittsburgh compound B Positron Emission Tomography. SUVR=Standardized Uptake Value Ratio

Supplementary figure 6



Biomarker trajectories. (A), (B), (C), (D), (E), (F) and (G) show the spaghetti plots for different studied biomarkers **(**CSF $A\beta_{42}$, total cortical uptake in the PIB-PET, CSF t-tau and CSF p-tau, Hippocampal volume (mm^3), cortical thickness in the precuneus (mm), and cognitive composite, respectively) in mutation carriers (red) and non-carriers (blue). Some of these data have already been shown elsewhere.[14] Dashed lines pointed to EYO = 0, indicating the expected symptom onset according to the parental onset. CSF= Cerebrospinal Fluid. EYO=Estimated Years to/from symptom Onset. *We used the cognitive composite previously described.[12] t-tau= total tau. p-tau=phosphorylated tau on threonine 181. PIB-PET= Pittsburgh compound B Positron Emission Tomography.

Appendix 3. Consortia

the Dominantly Inherited Alzheimer Network

Sarah Adams, Ricardo Allegri, Aki Araki, Nicolas Barthelemy, Randy Bateman, Jacob Bechara, Tammie Benzinger, Sarah Berman, Courtney Bodge, Susan Brandon, William (Bill) Brooks, Jared Brosch, Jill Buck, Virginia Buckles, Kathleen Carter, Lisa Cash, Charlie Chen, Jasmeer Chhatwal, Patricio Chrem Mendez, Jasmin Chua, Helena Chui, Carlos Cruchaga, Gregory S Day, Chrismary De La Cruz, Darcy Denner, Anna Diffenbacher, Aylin Dincer, Tamara Donahue, Jane Douglas, Duc, Duong, Noelia Egido, Bianca Espósito, Anne Fagan, Martin Farlow, Becca Feldman, Colleen Fitzpatrick, Shaney Flores, Nick Fox, Erin Franklin, Nelly Friedrichsen, Hisako Fujii, Samantha Gardener, Bernardino Ghetti, Alison Goate, Sarah Goldberg, Jill Goldman, Alyssa González, Brian Gordon, Susanne Gräber-Sultan, Neill Graff-Radford, Morgan Graham, Julia Gray, Emily Gremminger, Miguel Grilo, Alexander Groves, Christian Haass, Lisa Häasler, Jason Hassenstab, Cortaiga Hellm, Elizabeth Herries, Laura Hoechst-Swisher, Anna Hofmann, David Holtzman, Russ Hornbeck, Igor Yakushev, Ihara Ryoko, Takeshi Ikeuchi, Snezana Ikonomovic, Kenji Ishii, Clifford Jack, Gina Jerome, Erik Johnson, Mathias Jucker, Celeste Karch, Stephan Käaser, Kensaku Kasuga, Sarah Keefe, William (Bill) Klunk, Robert Koeppe, Deb Koudelis, Elke Kuder-Buletta, Christoph Laske, Allan Levey, Johannes Levin, Yan Li, Oscar López, Jacob Marsh, Rita Martínez, Ralph Martins, Neal Scott Mason, Colin Masters, Kwasi Mawuenyega, Austin McCullough, Eric McDade, Arlene Mejía, Estrella Morenas-Rodriguez, John Morris, James Mountz, Cath Mummery, Neelesh Nadkarni, Akemi Nagamatsu, Katie Neimeyer, Yoshiki Niimi, James Noble, Joanne Norton, Brigitte Nuscher, Antoinette O'Connor, Ulricke Obermüller, Riddhi Patira, Richard Perrin, Lingyan Ping, Oliver Preische, Alan Renton, John Ringman, Stephen Salloway, Peter Schofield, Michio Senda, Nick Seyfried, Kristine Shady, Hiroyuki Shimada, Wendy Sigurdson, Jennifer Smith, Lori Smith, Beth Snitz, Hamid Sohrabi, Sochenda Stephens, Kevin Taddei, Sarah Thompson, Jonathan Vöglein, Peter Wang, Qing Wang, Elise Weamer, Chengjie Xiong, Jinbin Xu & Xiong Xu

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