CSF neurofilament light levels predict hippocampal atrophy in cognitively healthy older adults

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Character count title: 95

Word count Abstract: 228

Word count text: 2999

Number of references: 40

Number of tables: 2

Number of figures: 3

Supplemental data: Supplemental File - tables, figures, and e-references, electronic file name: Idland_Supplemental data, Supplemental File - Contributors, electronic file name: Idland_Contributors, Supplemental File – STROBE checklist, electronic file name: Idland_STROBE checklist

Nonstandard characters list: β = &[beta]

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Study funding: The study was mainly funded by the Medical Student Research Program at the University of Oslo and The National Association for Public Health's dementia research program,

Norway. Further, we have received funding from Innlandet Hospital Trust, the Knut and Alice Wallenberg Foundation, the Swedish Research Council, and the Torsten Söderberg Foundation at the Royal Swedish Academy of Sciences.

Search terms: All Cognitive Disorders/Dementia (25), Alzheimer's disease (26), Cognitive aging (36), MRI (120), Cerebrospinal Fluid (319).

Authors' Contributions:

Mrs. Idland contributed to study concept and design, data acquisition, data analysis and interpretation, study coordination, and drafting and revision of the manuscript.

Dr. Sala-Llonch contributed to data acquisition, data analysis and interpretation, and drafting and revision of the manuscript.

Dr. Borza contributed to data acquisition, data interpretation, study coordination, and revision of the manuscript.

Dr. Watne contributed to study concept and design, data interpretation, and revision of the manuscript.

Dr. Bruun Wyller contributed to study concept and design, data acquisition, data interpretation, study coordination, and revision of the manuscript.

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Dr. Zetterberg contributed to data acquisition, data interpretation, and revision of the manuscript.

Dr. Blennow contributed to study concept and design, data acquisition, data interpretation, and revision of the manuscript.

Dr. Walhovd contributed to study concept and design, data interpretation, and revision of the manuscript.

Dr. Fjell contributed to study concept and design, data acquisition, data analysis and interpretation, and drafting and revision of the manuscript.

Study funding: The study was mainly funded by the Medical Student Research Program at the University of Oslo and The National Association for Public Health's dementia research program, Norway. Further, we have received funding from Innlandet Hospital Trust, the Knut and Alice Wallenberg Foundation, the Swedish Research Council, and the Torsten Söderberg Foundation at the Royal Swedish Academy of Sciences.

Disclosures:

Mrs. Idland reports no disclosures.

Dr. Sala-Llonch reports no disclosures.

Dr. Borza reports no disclosures

Dr. Watne has given a lecture on delirium for Lilly.

Dr. Bruun Wyller has given lectures on delirium for Pfizer, Roche, AstraZeneca and Nycomed.

Dr. Brækhus reports no disclosures.

Dr. Zetterberg reports no disclosures.

Dr. Blennow has served on Advisory Boards for IBL International and Roche Diagnostics.

Dr. Walhovd has given a lecture on lifespan changes in brain and cognition for Shire International Gmbh (2015) and has served in an expert group for ILSI Europe, for both of which honoraria were paid.

Dr. Fjell reports no disclosures.

ABSTRACT

Objective: To test whether cerebrospinal fluid (CSF) neurofilament light (NFL) levels predict hippocampal atrophy rate in cognitively healthy older adults independently of the established CSF Alzheimer's disease (AD) biomarkers.

Methods: Older adults undergoing elective surgery in spinal anesthesia were included in a prospective cohort study. At baseline, participants underwent comprehensive cognitive testing and brain magnetic resonance imaging (MRI), in addition to CSF sampling in conjunction with anesthesia. At two-year follow-up, the cognitive testing and MRI were repeated. 144 cognitively healthy participants with CSF biomarker samples and/or brain MRI were included. Of these, 88 had MRI at both time points and CSF NFL measures.

Results: CSF NFL levels predicted hippocampal atrophy rate independently of age, CSF β-amyloid 1-42 and phosphorylated tau (P-tau) levels. Including NFL, P-tau and age in the same linear regression model, higher NFL levels and lower P-tau levels predicted significantly higher hippocampal atrophy rate (full model adjusted R^2=.20, NFL β=-.34, P-tau β=.27). The results were upheld in a subgroup of participants with very low AD risk based on genetic, cognitive and biomarker information.

Conclusion: CSF NFL predicts neurodegeneration in older adults with very low probability of AD. The present results suggest that factors previously shown to be important for brain degeneration in mild cognitive impairment may also impact brain changes in normal aging, demonstrating that NFL is likely to be a marker of AD-independent, age-expected neurodegeneration.

INTRODUCTION

Hippocampal atrophy rates are higher in patients with Alzheimer's disease (AD) than in cognitively healthy older adults.[1] However, hippocampal atrophy is known to accelerate from midlife onwards also in persons with low AD-risk,[2] and hippocampus is one of the brain areas with highest atrophy rate in aging,[2] reported to be around 1% annually.[2, 3] Thus, identification of biomarkers predicting hippocampal atrophy is critical for understanding brain changes both in normal aging and early AD. Interestingly, a recent study showed that cerebrospinal fluid (CSF) neurofilament light subunit (NFL) levels predicted hippocampal atrophy in mild cognitive impairment (MCI) patients,[4] indicating that CSF NFL could be a progression marker in AD.

Neurofilaments are important cytoskeletal components of neuronal axons, and CSF NFL levels are believed to reflect axonal degeneration.[5, 6] CSF NFL levels are associated with age,[7-9] white matter lesions,[10] AD,[4, 9, 11] and other neurodegenerative diseases.[9, 11-14] Previous studies have indicated a relationship between high CSF NFL and lower brain volume in frontotemporal lobe dementia,[15] and non-demented older adults,[8, 16] but studies are mostly cross-sectional and results have not been consistent.[17, 18] The relationship between CSF NFL levels and hippocampal atrophy in cognitively healthy older adults has never been tested, but is critical for understanding whether NFL is a general or disease-specific atrophy marker. Thus, the objective of this study was to test whether CSF NFL levels predict hippocampal atrophy rate in cognitively healthy older adults independently of the established CSF AD biomarkers β-amyloid 1-42 (Aβ42) and phosphorylated tau (P-tau).[19]

METHODS

Participants

We recruited patients scheduled for elective surgery in spinal anesthesia turning 65 years or older the year of inclusion. Dementia, previous stroke with sequela, Parkinson's disease and other neurodegenerative diseases likely to affect cognition were exclusion criteria. The participants were assessed with a multi-domain battery of cognitive tests before surgery, comprising the Mini Mental Status Examination (MMSE),[20] Clock Drawing Test,[21] Word List Memory Task,[22] Trail Making Test A and B,[23] Kendrick Object Learning Test,[24] and verbal fluency (FAS test and Animal Naming),[25] giving 11 test scores. Blood and CSF samples were collected by the anesthesiologist in conjunction with spinal anesthesia, and the participants underwent magnetic resonance imaging (MRI) after surgery. The mean time between CSF sampling and MRI at baseline was 8 weeks (standard deviation [SD] [range]: 6 [-20 to 24]). Participants underwent a second MRI and were tested with the same battery of cognitive tests at two-year follow-up (mean time between MRIs 2.2 years, SD [range]: 0.3, [1.6 to 2.9]) (see table 1).

[Insert table 1 around here]

We selected participants as shown in figure e-1. Only participants with CSF biomarker analyses and/or brain MRI(s) were included. We selected only cognitively healthy participants based on the following procedure: First, participants offered referral to cognitive assessment were excluded. Next, we included all participants with MMSE score ≥ 27. Last, for participants with

MMSE score < 27, only those with none or one other abnormal test score(s) when last tested were included. Abnormal score was defined as more than 1.5 SD below the mean normal value for age, sex, and educational level. 4 participants with CSF NFL levels > 4000 pg/mL were excluded (CSF NFL levels were more than ± 3 SD from the mean value). From this remaining sample, we selected two separate groups for subgroup analyses: 1) we excluded participants with additional conditions that could possibly affect hippocampal atrophy (details table e-1) 2) we selected participants with very low risk of AD fulfilling all the three following criteria: 1) Aβ42 > 550 pg/mL[26] 2) no apolipoprotein E (APOE) 4 alleles 3) stable or improved delayed recall score on Word List Memory Task at two-year follow-up compared to baseline.

Standard protocol approvals, registrations, and patient consents

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

Magnetic Resonance Imaging acquisition and processing

T1-weighted MPRAGE 3D images were acquired with a 1.5 T Siemens Avanto scanner using a 12-channel head coil (TR=2400 ms, TE=3.79ms, Field of View=240mm, slice thickness=1.20mm, pixel size=1.25x1.25mm).

The scans were processed with FreeSurfer (version 5.3) and its specific longitudinal stream (https://surfer.nmr.mgh.harvard.edu). For each MRI, the standard Freesurfer pipeline performs a set of automated procedures for the cortical reconstruction and volumetric segmentation of the individual scans, documented elsewhere.[27, 28] We used hippocampi volume measures and white matter hypointensities (WM-hypointensities) estimations obtained from a segmentation based on both a subject-independent probabilistic atlas and subject-specific intensity measures. In addition, it is important to note that the Freesurfer longitudinal stream includes a set of methods designed to minimize the bias to any time point in a participant and which are shown to lead to increased statistical power, better separation of groups based on atrophy, and higher reproducibility. These methods include the generation of a subject-specific intermediate template followed by a projection of each time point to this template.[29, 30]

APOE genotyping

Blood samples were genotyped for *APOE* (gene map locus 19q13.2) using TaqMan Allelic Discrimination technology (Applied Biosystems, Carlsbad, CA, USA). Genotypes were obtained for the two SNPs that are used to unambiguously define the ε2, ε3, and ε4 alleles (rs7412 and rs429358).

CSF collection and analyses

CSF was collected in polypropylene tubes, centrifuged at room temperature for 10 minutes, the supernatant aliquoted into polypropylene tubes, and frozen at -80 °C pending analyses. Mean

time from CSF sampling to freezing was 85 minutes (SD, [range]: 22, [16 to 127]). Samples were sent on dry ice to the Clinical Neurochemistry Laboratory at Sahlgrenska University Hospital, Mölndal, Sweden, for analyses. CSF Aβ42, total tau (T-tau) and P-tau concentrations were determined using INNOTEST enzyme-linked immunosorbent assays (Fujirebio, Ghent, Belgium) and CSF NFL concentrations using a commercial ELISA (UmanDiagnostics, Umeå, Sweden). Analyses were performed by board-certified laboratory technicians masked to clinical data. Intra-assay coefficients of variation were 9-13% and the lower limit of detection for NFL was 50 pg/mL. We found a strong correlation between CSF T-tau and P-tau levels ($r=.96$, $p<.001$), thus we only used CSF P-tau in statistical analyses.

Statistical analysis

We calculated hippocampal atrophy rate as the annual percent change in hippocampal volume (average of both hemispheres), normalized by the average volume across time points and divided by years between scans. We also calculated the average WM-hypointensities volume across time points for use as a control variable.

We tested associations between CSF biomarkers, age and hippocampal atrophy rate using SPSS (version 22). Generalized Additive Mixed Models (GAMM) implemented in R (www.r-project.org) using the package "mgcv"[31] was used to derive the age-function for hippocampal atrophy and for the relationship between hippocampal change and CSF NFL levels, taking advantage of all longitudinal and cross-sectional observations, run through the PING data portal (http://pingstudy.ucsd.edu/welcome.html).[32] Akaike Information Criterion (AIC) [33] was used to

guide model selection and help guard against over-fitting. For analyses including CSF biomarkers and not MRI measures, we used age at the day of CSF sampling. For analyses including MRI measures, we used the age at the day of baseline MRI.

We tested correlations between CSF biomarkers, age and hippocampal atrophy rate using Pearson correlations, and hippocampal volume change using paired samples T-test. We performed multiple linear regression analyses to test associations between age, CSF biomarkers and hippocampal atrophy rate. The regressions were performed in several steps. The first model included NFL and age as predictors of hippocampal atrophy rate. Next, we tested the predictive power of Aβ42 and P-tau levels separately in conjunction with NFL in the model. The resulting regression model was tested for stability by including sex and WM-hypointensities separately. We also tested the final regression model within two low risk subgroups: 1) the group without additional conditions and 2) the very low AD risk subgroup. Sensitivity analyses were performed with and without outliers (defined as studentized residuals > ± 2) for all regression models. Finally, we ran mediation analyses using the SPSS macro INDIRECT.[34] Mediation is present if the relationship between the predictor variable and the dependent variable (c) attenuates when accounting for a third variable (the mediator) (c'). The % reduction was calculated as (c-c')/c. The significance of the indirect effect (a*b) was tested using bootstrapped confidence intervals (CI). Standardized coefficients were obtained using z-scores.

RESULTS

CSF biomarkers, hippocampal volume and demographic factors

Demographics, and CSF biomarker and MRI characteristics are shown in table 1. NFL levels correlated positively with age (r=.41, p<.001). P-tau levels also correlated positively with age (r=.21 p=.02), whereas Aβ42 levels did not correlate with age (r=-.002, p=.98). High NFL levels correlated with high P-tau levels (r=.28, p=.001), but not with Aβ42 levels (r=.02, p=.85).

CSF NFL levels and hippocampal atrophy rate

Hippocampal atrophy was significant (mean [SD], range: -45.38 mm^3 [75.35], -30.43 to -60.34, t=6.02, p<.001), and the mean annual atrophy rate was -0.61% (n=100). Age correlated with higher atrophy rate (r=-.29, p=.003), indicating accelerated atrophy with increasing age. This relationship was confirmed with GAMM for the full sample, as illustrated in figure 1. We ran a multiple regression analysis using NFL and age as predictors of hippocampal atrophy rate. Higher NFL levels predicted higher hippocampal atrophy rate (p = .02) (see table 2). Age was not a significant predictor in this model. In the next step, Aβ42 level was also introduced as a possible predictor, and did not predict hippocampal atrophy rate independently of NFL, while NFL was still significant (table 2). The last step included P-tau as a predictor together with age and NFL, and we obtained a model with higher NFL levels and lower P-tau levels predicting higher hippocampal atrophy rate (table 2) independently of age. Regression analyses results were unchanged when excluding 5-6 outliers per analysis.

The relationship between NFL and hippocampal volume was also tested with GAMM to take advantage of all data points, obtaining an optimal fit based on both cross-sectional and longitudinal information. The sample was divided into NFL+ and NFL- by a median split, and the relationship between hippocampal volume and age was plotted in each group, with sex as a covariate. AIC for the model was 2587 and NFL status yielded a highly significant contribution ($t = -2.96$, $p < .005$). Removing NFL increased AIC to 2595, indicating a worse fit. Adding P-tau as a covariate did not improve the model fit (AIC = 2588), and P-tau did not contribute significantly ($t = -0.18$, $p = 0.85$) while NFL still did ($t = -2.66$, $p < .01$). Thus, the initial model was preferred and plotted in figure 2.

[Insert table 2, figure 1 and figure 2 around here]

Adjusting for effect of white matter hypointensities and sex on hippocampal atrophy rate

Since vascular brain pathology may affect the relationship between NFL and hippocampal atrophy rates,[10] we entered WM-hypointensities into the regression model including age, NFL and P-tau levels as predictors of hippocampal atrophy rate. NFL and P-tau levels were still significant predictors of hippocampal atrophy rate, whereas WM-hypointensities did not predict hippocampal atrophy rate (data not shown). Results were unchanged after exclusion of 6 outliers. We adjusted for sex in the same way as for WM-hypointensities. Also in this model, NFL and P-tau levels were the only significant predictors of hippocampal atrophy rate (data not shown). Sex was not a significant predictor, however after exclusion of 6 outliers, sex was also a significant predictor (higher atrophy rates in males).

CSF NFL levels and hippocampal atrophy in low risk subgroups

We further applied our final regression model including age, NFL, and P-tau levels as predictors of hippocampal atrophy rate in two low risk subgroups: 1) in the subgroup without additional conditions, NFL was the only significant predictor (table e-2). However, when excluding 5 outliers, P-tau was also a significant predictor as in the full sample. 2) in the subgroup of Aβ42 negative participants without APOE4 alleles, and stable memory function between baseline and follow up, higher NFL levels and lower P-tau levels predicted higher hippocampal atrophy rate independent of age as in the full sample (table e-3). The results were unchanged when increasing the Aβ42 cutoff from 550 to 650 pg/mL, and also when excluding 2-3 outliers per analysis.

Mediation analyses

We tested the mediating (indirect) effect of NFL on the relationship between age and hippocampal atrophy rate (figure 3). NFL was a significant mediator, with bootstrapped 95 % confidence interval of -.24 to -.01, and accounted for 36 % of the age effect on hippocampal atrophy rate. In our model, the total effect of age on hippocampal atrophy was β=- .23 equal to the sum of the direct effect of age (β=#.15) and the indirect effect through the relationship with NFL (β=-.08).

[Insert figure 3 around here]

DISCUSSION

High CSF NFL levels predicted higher hippocampal atrophy rate in cognitively healthy older adults. While previous studies have demonstrated this in samples of high-risk participants, i.e. MCI patients,[4] here we show that the relationship was replicated in a sample with very low AD-risk, and that NFL predicted hippocampal atrophy independently of the established AD CSF biomarkers Aβ42 and P-tau. This suggests that CSF NFL may be an important marker of neurodegeneration both in normal aging and in age-related neurodegenerative diseases.

The only previous study assessing CSF NFL in relation to longitudinal volume change in older adults found that higher NFL levels were associated with deterioration in whole-brain, ventricular and hippocampal volume in MCI patients.[4] However, cross-sectional studies in non-demented adults have been more inconsistent. One study found that high CSF NFL correlated with ventricular size, but not with sulcal atrophy,[16] a second study found a correlation between brain parenchymal fraction and CSF NFL that did not survive adjustment for age,[8] while a third study found no relationship between baseline CSF NFL levels and gray matter volumes 3.5 years later.[18] In frontotemporal dementia, higher CSF NFL is associated with lower gray and white matter volumes, including in the temporal lobe,[15] while findings in multiple sclerosis (MS) and related disorders are less straightforward.[17, 35] Thus, previous literature on the association between CSF NFL and brain volumes is scarce and inconsistent, but the only longitudinal study in older adults is in line with our findings,[4] showing that high CSF NFL predicts more hippocampal atrophy in MCI patients. The present study takes these results further by showing

that the NFL-atrophy association is likely not caused by AD-specific mechanisms, but is important also in AD-independent, age-expected hippocampal decline.

Neurofilaments are abundant in neuronal axons where they are essential for axon radial growth,[5] but are also found in soma and dendrites of neurons.[36] NFL is expressed in neurons in both the central and the peripheral nervous system,[36] including the hippocampus.[37] Thus, when a neuron is damaged, NFL is believed be released into the extracellular compartment resulting in increased CSF NFL levels.[5] Age is associated with increasing CSF NFL levels in several studies,[7-9, 12, 17] suggesting that CSF NFL levels increase with normal aging. Interestingly, we found that NFL levels could explain more than one third of the age-related increase in hippocampal atrophy rates. As accelerated decline of the hippocampus also in normal aging is observed independently of AD-related pathology,[2] this is an important finding. Thus, our results indicate that CSF NFL levels reflect processes characterizing normal aging.

There has recently been increasing focus on amyloid-independent neurodegeneration in aging, often referred to as suspected non-Alzheimer pathology (SNAP), making it important to map out correlates of atrophy also in AD-typical areas in Aβ42 negative older adults.[38] Thus, we created a subgroup with very low AD risk (only Aβ42 negative participants), in which our finding was upheld. This bolsters that AD brain pathology is not a confounder of the relationship between CSF NFL and hippocampal atrophy rate, and suggests that CSF NFL most likely reflects neurodegeneration processes in normal aging. Further, in another subgroup analysis, CSF NFL predicted hippocampal atrophy rate after exclusion of participants with additional risk conditions,

supporting that CSF NFL likely reflects normal aging processes. Previous studies suggest that CSF NFL may reflect the rate of ongoing neurodegeneration. High CSF NFL levels are seen days after a bout in amateur boxing,[6] with subsequent decrease during the next months, CSF NFL levels are highest in MS patients with an ongoing relapse,[39] and high CSF NFL levels are associated with progression of neurodegenerative diseases.[4, 9, 12, 13] Accordingly, CSF NFL levels are higher in the rapidly progressing neurodegenerative disease amyotrophic lateral sclerosis than in AD which progresses more slowly,[12] and MCI patients have CSF NFL levels that are intermediate between those of AD patients and controls.[4] Thus, CSF NFL may reflect that similar neurodegenerative processes are ongoing in both normal aging and diseases, and the CSF NFL levels may reflect the progression rate of the processes.

Unexpectedly,[40] in the final model, higher P-tau levels predicted lower hippocampal atrophy rates. One explanation for this finding may be that our study could have excluded individuals with high CSF P-tau levels and high hippocampal atrophy rates, as they are more likely to have dementia or cognitive impairment. Therefore, this result should be interpreted with caution. Also, it must be noted that P-tau was not significantly related to atrophy when no covariates were included in the model, nor in GAMM, and we can thus not exclude the possibility that the unexpected relationship with hippocampal atrophy is due to shared variance with the other covariates NFL and age.

The main limitation is that although the likelihood of confounding by presymptomatic AD is low, we cannot rule out the possibility that presymptomatic neurodegenerative pathology of other

etiologies may in part account for some of the relationship between CSF NFL and hippocampal atrophy. Further, although the participants were followed for two years, we cannot be sure that these participants do not develop neurodegenerative diseases later.

CONCLUSION

CSF NFL predicts neurodegeneration in older adults with very low probability of AD. The present results suggest that factors previously shown to be important for brain degeneration in MCI may also impact brain changes in normal aging, demonstrating that NFL is likely to be a marker of AD-independent, age-expected neurodegeneration.

ACKNOWLEDGEMENTS

The authors would like to thank Knut Engedal, MD, PhD (University of Oslo and Vestfold Hospital Trust) and Anette Hylen Ranhoff, MD, PhD (Diakonhjemmet Hospital and Kavli Research Center for Geriatrics and Dementia) for general support, Gry Torsæter Dahl, MD (Diakonhjemmet Hospital), Randi Støen, MD (Oslo University Hospital), Arne Myklebust, MD (Oslo University Hospital) and Dagfinn Tore Kollerøs, MD (Oslo University Hospital) for collection of data. The authors would also like to thank the study participants and the contributions of the Department of Gynecology, the Department of Urology, and the Department of Anesthesiology at Oslo University Hospital, the Department of Orthopedic Surgery and the Department of Anesthesiology at Diakonhjemmet Hospital in Oslo, Norway.

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FIGURE LEGENDS

Figure 1. Relationship between age and hippocampal volume.

Legend: Adjusted for sex. The graph shows mean slope with 95 % confidence interval. Data points from participants with MRI are displayed, including within-person changes for those with MRI at both time points. MRI = magnetic resonance imaging.

Figure 2. Relationship between age and hippocampal volume in NFL+ and NFL- participants.

Legend: Adjusted for sex. NFL+ (> 902 pg/mL) and NFL- (≤ 902pg/mL) participants are defined by a median split. Estimated group slopes with 95 % confidence intervals are displayed. Data from participants with MRI at one or both time points are used. NFL = cerebrospinal fluid neurofilament light. MRI = magnetic resonance imaging.

Figure 3. NFL mediates the effect of age on hippocampal atrophy rate.

Legend: Path analyses showing that NFL mediates the effect of age on hippocampal atrophy rate. Standardized regression coefficients for the paths are presented; A) c = the direct association between age and hippocampal atrophy rate, B) a = the association between age and NFL, b = the association between NFL and hippocampal atrophy rate adjusted for age, and c' the association between age and hippocampal atrophy rate adjusting for NFL. The regression coefficient for the mediation effect (c-c' = a*b) and the % reduction of the effect of age on hippocampal atrophy rate are also presented. The bootstrapped 95 % confidence interval for the mediation effect was -

.24 to -.01, showing that the mediation effect is significant. NFL = cerebrospinal fluid neurofilament light level.

TABLES

Table 1. Demographics, CSF biomarkers and hippocampal measures.

	All participants (n=144)	Participants with MRI at both time points and CSF NFL analyses (n=88)
Age at baseline, years	73 (6), 64 to 91	73 (6), 64 to 89
Sex, male	68 (47)	43 (49)
Education, years	14 (4), 7 to 23	15 (3), 8 to 23
MMSE score, baseline	29 (1.2), 25 to 30	29 (1.3), 25 to 30
MMSE score, 2-year follow-up	29 (1.4), 21 to 30[a]	29 (1.2), 24 to 30
APOE genotype[b]		
E3/E2	12 (9)	4 (5)
E3/E3	68 (53)	44 (53)
E4/E2	1 (1)	1 (1)
E4/E3	44 (34)	31 (37)
E4/E4	4 (3)	3 (4)
CSF Aβ42, pg/mL	718 (208), 275 to 1179[c]	724 (203), 275 to 1175
CSF P-tau, pg/mL	60 (20), 25 to 115[c]	61 (19), 26 to 110
CSF NFL, pg/mL	1163 (507), 487 to 3123[d]	1141 (558), 510 to 3123

Aβ42+ (< 550 pg/mL)	34 (26)[c]	24 (27)
Months between MRIs	-	26 (3), 19 to 35
Hippocampal volume, baseline, mm^3	-	3505 (396), 2337 to 4544
Hippocampal volume, 2-year follow up, mm^3	-	3464 (407), 2425 to 4514
Hippocampal volume, % annual change	-	-.55 (1.08), -4.24 to 2.14

Legend: Values are n (%) and mean (SD), range. [a]n=115, [b]n=129 and n=83, respectively, [c]n=130,[d]n=128. MMSE = Mini Mental Status Examination. APOE = Apolipoprotein E. CSF = cerebrospinal fluid. Aβ42= β-amyloid 1-42. P-tau = phosphorylated tau. NFL = neurofilament light. MRI = magnetic resonance imaging.

Table 2. Multiple linear regression with hippocampal atrophy rate as dependent variable (full sample).

Independent variables	R^2	B	95 % CI	β	P value
Age	.13	-.024	-.064 to .016	-.14	.23
NFL		-.001	-.001 to -.00001	-.28	.02
Age	.16	-.025	-.064 to .015	-.14	.22
NFL		-.001	-.001 to -.0001	-.29	.01

Aβ42		.001	-.0002 to .002	.16	.11
Age	.20	-.023	-.062 to .015	-.13	.23
NFL		-.001	-.001 to -.0002	-.34	.003
P-tau		.016	.004 to .027	.27	.009

Legend: Aβ42= cerebrospinal fluid β-amyloid 1-42. P-tau = cerebrospinal fluid phosphorylated tau. NFL = cerebrospinal fluid neurofilament light.

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