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Effects of CSF proteins on brain atrophy rates in cognitively healthy older adults

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Abstract

Biomarkers associated with Alzheimer's disease (AD)-like brain atrophy in healthy people may identify mechanisms involved in early stage AD. Aside from cerebrospinal fluid (CSF) β -amyloid₄₂ (A β ₄₂) and tau, no studies have tested associations between CSF proteins and AD-like brain atrophy. We studied 90 healthy elders, who underwent lumbar puncture at baseline, and

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Conflicts of interest

NM, PI, RN, and DT report no conflicts of interest. LMS previously was consultant for Innogenetics and collaborates on quality assessment activities as part of the Alzheimer's Disease Neuroimaging Initiative; serves as a consultant to Janssen AI R & D on biomarker studies. JQT may accrue revenue in the future on patents submitted by the University of Pennsylvania wherein he is co-inventor and he received revenue from the sale of Avid to Eli Lilly as co-inventor on imaging related patents submitted by the University of Pennsylvania; and is the William Maul Measey-Truman G. Schnabel, Jr., M.D. Professor of Geriatric Medicine and Gerontology. MW has been on scientific advisory boards for Pfizer and BOLT Inter-national; has been a consultant for Pfizer Inc., Janssen, KLJ Associates, Easton Associates, Harvard University, inThought, INC Research, Inc., University of California, Los Angeles, Alzheimer's Drug Discovery Foundation and Sanofi-Aventis Groupe; has received funding for travel from Pfizer, AD PD meeting, Paul Sabatier University, Novartis, Tohoku University, MCI Group, France, Travel eDreams, Inc., Neuroscience School of Advanced Studies (NSAS), Danone Trading, BV, CTAD ANT Congres; serves as an associate editor of Alzheimer's & Dementia; has received honoraria from Pfizer, Tohoku University, and Danone Trading, BV; has research support from Merck, Avid, DOD and VA; and has stock options in Synarc and Elan. CRJ provides consulting services for Siemens Healthcare and Janssen Research and Development L.L.C. He receives research funding from the National Institutes of Health (R01-AG011378, R01-AG041851, R01-AG037551, U01-HL096917, U01-AG032438, U01-AG024904), and the Alexander Family Alzheimer's Disease Research Professorship of the Mayo Foundation.

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serial magnetic resonance imaging scans for up to 4 years. We tested statistical effects of baseline CSF proteins (N=70 proteins related to A β 42-metabolism, microglial activity and synaptic/neuronal function) on atrophy rates in 7 AD-related regions. Besides effects of A β 42 and phosphorylated tau (P-tau) that were seen in several regions, novel CSF proteins were found to have effects in inferior and middle temporal cortex (including Apolipoprotein CIII, Apolipoprotein D and Apolipoprotein H). Several proteins (including S100 β and Matrix Metalloproteinase-3) had effects that depended on the presence of brain A β pathology, as measured by CSF A β 42. Other proteins (including P-tau and Apolipoprotein D) had effects even after adjusting for CSF A β 42. The statistical effects in this exploratory study were mild and not significant after correction for multiple comparisons, but some of the identified proteins may be associated with brain atrophy in healthy people. Proteins interacting with CSF A β 42 may be related to A β brain pathology, while proteins associated with atrophy even after adjusting for CSF A β 42 may be related to A β -independent mechanisms.

Keywords

cerebrospinal fluid; biomarkers; atrophy; longitudinal; Alzheimer's disease

1. Introduction

Alzheimer's disease (AD) leads to severe atrophy of cortical brain tissue and ultimately results in dementia. The disease is believed to have a long pre-symptomatic phase with brain pathology accumulating prior to cognitive symptoms. The major disease hallmarks are plaques composed of β -amyloid (A β) peptides and neurofibrillary tangles composed of phosphorylated tau proteins (Braak and Braak, 1991). The view that AD starts prior to any symptoms implies that its earliest changes can only be studied in people who are cognitively normal. However, longitudinal studies on development of cognitive impairment in healthy people are limited by the long follow-up time and large study populations needed to detect significant cognitive changes. An alternative is to study longitudinal atrophy of AD-related brain regions, which are predictive of future cognitive decline (Rusinek et al., 2003). Biomarkers predicting such atrophy in cognitively healthy people may be useful to identify persons at risk for AD, and could inform on biological mechanisms during early disease stages.

Biomarker measurements of A β pathology, using positron emission tomography (PET) or cerebrospinal fluid (CSF), likely become abnormal early in AD, even prior to cognitive symptoms (Bateman et al., 2012; Jack et al., 2010). A few studies have tested if A β pathology predicts longitudinal brain atrophy in cognitively healthy people (Ch  telat et al., 2012; Fjell et al.; Henneman et al., 2009; Schott et al., 2010; Sluimer et al., 2010; Tosun et al., 2011). Some (Ch  telat et al., 2012; Fjell et al., 2010; Schott et al., 2010), but not all (Henneman et al., 2009; Sluimer et al., 2010; Tosun et al., 2011) of these studies found that an AD-like CSF or PET A β biomarker pattern predicted longitudinal atrophy. Studies showing that A β pathology predicts longitudinal AD-like atrophy in cognitively healthy people are consistent with the "dynamic biomarker model" presented by Jack et al. (Jack et al., 2010), but it should be noted that some studies found A β related atrophy also in areas not typically associated with early AD changes (Fjell et al., 2010; Schott et al., 2010).

Early pathological events leading to atrophy in AD may be reflected by other CSF changes, besides altered concentrations of the established biomarkers A β 42 and tau, but to our knowledge, no previous study has examined if other CSF proteins are associated with longitudinal AD-like atrophy. Here we used CSF proteomics data from a multiplex panel, together with longitudinal structural magnetic resonance imaging (MRI) data to identify

novel biochemical predictors of AD-related brain atrophy in cognitively healthy elderly participants in the Alzheimer's Disease Neuroimaging Initiative (ADNI). We studied brain regions typically shown to be vulnerable in AD (although it should be noted that wide spread atrophy may also be seen in cognitively well-screened and longitudinally stable adults with no signs of A β pathology (Oh et al., 2013)). The tested proteins were selected for biological functions possibly altered in early stage AD (A β metabolism, microglia activity, and synaptic/neuronal function). Our primary hypothesis was that baseline protein levels were associated with atrophy rates. Although the dominant view in AD research is that A β pathology is the initiator in the neuropathological cascade, A β -independent processes have also been proposed (Chételat, 2013; Pimplikar et al., 2010). Based on this, we investigated if A β influenced the relationship between proteins and atrophy rates. We tested the specific hypotheses that some proteins interact with A β pathology to effect atrophy rates (suggesting atrophy mechanism linked to A β pathology), and that some proteins effect atrophy rates even when adjusting for the presence of A β pathology (suggesting atrophy mechanism that do not depend on A β pathology).

2. Methods

2.1 Study design

We examined changes in gray matter volume in a priori specified regions of interest (ROI) in cognitively normal people. Structural magnetic resonance imaging brain scans at multiple time points (up to 6 time points: ADNI screening, and 6, 12, 24, 36 and 48 months, median 5 scans per participant [range 2–6]) were acquired at multiple sites using 1.5 Tesla MRI scanners. Using FreeSurfer longitudinal processing framework, regional gray matter volumes were measured at each time point. Linear mixed effects models were performed to test if baseline CSF protein concentrations (N=70 different proteins tested separately) were associated with rates of brain atrophy (i.e., the rate of change in volume).

2.2 Participants

Data used in the preparation of this article were obtained from the ADNI database (adni.loni.ucla.edu). The ADNI was launched in 2003 by the National Institute on Aging (NIA), the National Institute of Biomedical Imaging and Bioengineering (NIBIB), the Food and Drug Administration (FDA), private pharmaceutical companies and non-profit organizations, as a \$60 million, 5-year public-private partnership. The primary goal of ADNI has been to test whether serial MRI, PET, other biological markers, and clinical and neuropsychological assessment can be combined to measure the progression of mild cognitive impairment (MCI) and early AD. Determination of sensitive and specific markers of very early AD progression is intended to aid researchers and clinicians to develop new treatments and monitor their effectiveness, as well as lessen the time and cost of clinical trials. The Principal Investigator of this initiative is Michael W. Weiner, MD, VA Medical Center and University of California – San Francisco. ADNI is the result of efforts of many co-investigators from a broad range of academic institutions and private corporations, and participants have been recruited from over 50 sites across the U.S. and Canada. The initial goal of ADNI was to recruit 800 participants but ADNI has been followed by ADNI-GO and ADNI-2. To date these three protocols have recruited over 1500 adults, ages 55 to 90, to participate in the research, consisting of cognitively normal older individuals, people with early or late MCI, and people with early AD. For up-to-date information, see www.adni-info.org. The population in this study included ADNI-1 participants with valid results for a CSF multiplex protein panel (described below) and successful longitudinal FreeSurfer processing of MR images from at least two time points.

2.3 Structural MRI acquisition

The participants underwent a standardized 1.5 Tesla MRI protocol, which included T1-weighted MRI scans using a sagittal volumetric magnetization prepared rapid gradient echo (MP-RAGE) sequence, as previously described (Jack et al., 2008).

2.4 FreeSurfer longitudinal MR image processing

Automated cortical volume measures and hippocampal segmentation were performed with FreeSurfer software package, version 4.4 (<http://surfer.nmr.mgh.harvard.edu/fswiki>), as previously described (Fischl et al., 2004, 2002). To reduce the confounding effect of intra-participant morphological variability, each participant's longitudinal data series was processed by FreeSurfer longitudinal workflow (<http://surfer.nmr.mgh.harvard.edu/fswiki/LongitudinalProcessing>). A previous test-retest study validated that the longitudinal processing provides consistent ROI segmentation (Reuter et al., 2012). All images underwent standardized quality control procedures (<http://www.loni.ucla.edu/twiki/pub/ADNI/ADNIPostProc/UCS-FFreeSurferMethodsSummary.pdf>). Participants with complete segmentation failure or gross errors throughout all brain regions were rated as complete failure. Participants with gross errors in one or more specific brain regions (i.e., temporal lobe regions, superior regions, occipital regions, and insula) were given partial pass rating. Participants with partial pass rating were included in analyses of appropriate brain regions only. For this study, we used volumetric measurements of entorhinal cortex, hippocampus, inferior parietal cortex, inferior temporal cortex, middle temporal cortex, posterior cingulate cortex, and precuneus (combining left and right hemisphere data).

2.5 CSF biomarker concentrations

A CSF sample was collected at study baseline by lumbar puncture, and shipped to the ADNI Biomarker Core laboratory at the University of Pennsylvania Medical Center for long term storage in -80° C. The established CSF AD biomarkers A β 42, T-tau and P-tau were measured using the multiplex xMAP Luminex platform (Luminex Corp, Austin, TX, USA) with the INNOBIA AlzBio3 kit (Innogenetics, Ghent, Belgium) (Olsson et al., 2005; Shaw et al., 2009). CSF multiplex proteomics was done using an xMAP multiplex panel developed by Rules Based Medicine (MyriadRBM). Previous versions of the MyriadRBM panel had been used to explore CSF or plasma proteins in AD (Craig-Schapiro et al., 2011; Hu et al., 2012, 2010). Details regarding the assay technology, validation and quality control can be found online in a data primer for these CSF analyses (<http://adni.loni.ucla.edu/>). In brief, the panel included 159 biomarkers. Seventy-six analytes were adequately quantifiable in the CSF samples in this study (the other analytes were mostly below the assay detection limit or had other assay limitations). For these, we conducted a systematic literature review to find previous evidence of associations with A β metabolism, microglial activity, synaptic/neuronal function, which identified 67 analytes of interest (Supplementary Table 1). In all, this resulted in 70 proteins (including A β 42, T-tau and P-tau) available for this particular study. Some analytes were used after log transformation as described in the assay documentation (<http://adni.loni.ucla.edu/>).

2.6 Statistical analyses

Potentially confounding effects by age, sex, education and *APOE* genotype on explanatory proteins were evaluated by non-parametric tests (Mann-Whitney test and Spearman Correlation tests, as appropriate). We used generalized linear mixed models to test if proteins were associated with a binary missing indicator for each visit. Effects of explanatory proteins on regional rates of atrophy in brain regions associated with AD were tested by linear mixed-effects models. Regional brain volumes were used as response variables and the time (years) from the first image measurement, protein, *protein:time*

interaction, age, sex and intracranial volume (ICV) were used as fixed effects. Each brain region was tested separately with each explanatory protein. All proteins (including A β 42) were used as continuous measurements in this analysis. All models included a random intercept and a random slope.

To specifically test if the effects of proteins were influenced by the presence of brain A β pathology, all models were run with and without adjusting for A β 42 as a fixed effect. For this we used A β 42 dichotomized by the cut-off 192 ng/L, since this cut-off has been found to be highly discriminate of brain A β pathology, as evidenced by PET imaging (Weigand et al., 2011) or autopsy (Shaw et al., 2009). For each protein and region, we also tested the *protein:A β 42:time* interaction, again using A β 42 dichotomized by the cut-off 192 ng/L, and including all sub-effects.

After inspection of the correlation within participants, a compound symmetry structure was assumed. We assessed the applicability of the models by evaluating linearity of regional volumes over time within participants, and the normality of the model residuals. Two participants were outliers (in standardized residuals) for models of entorhinal and hippocampal atrophy rates, respectively. The overall results did not change after removing these observations, and we kept all observations in the final models.

Due to the large number of tested proteins and brain regions, we corrected for multiple comparisons using a false discovery rate correction. Details on how the false discovery rate correction was applied are presented in the table legends. Volumes and proteins were standardized (centered and divided by the standard deviation). All tests were two-sided. All statistics were done using R (v. 2.15.2, The R Foundation for Statistical Computing).

3. Results

The study included 90 participants (44 females, 22 *APOE* ϵ 4 carriers, mean age 76 years [range 62–90]). At the 48 month follow-up visit, 12 participants had converted from NL to MCI and 1 to AD dementia. We used survival analysis to test if any protein was related to conversion. The only proteins significantly associated with conversion were MMP2 (Log MMP2, β =0.76, P =0.048) and PAI1 (Log PAI1, β =-1.27, P =0.049). There was a tendency to significant association for A β 42 (β =0.0022, P =0.067). No protein was significant after correcting for multiple comparisons (mean levels and ranges for converters and non-converters are available in Supplementary Table 2).

3.1 Potential confounding factors

Age, sex, education and *APOE* genotype were potential confounders of the relationship between the investigated proteins and regional brain volumes. We tested for imbalance between proteins and these demographic factors, using non-parametric tests (Supplementary Table 3). Age, sex and education were associated with several different proteins. *APOE* genotype was only associated with A β 42 and P-tau. Based on these results, we included age, sex and education as covariates in all analyses.

3.2 Missing data

Several participants lacked MRI data for one or more visit (data were missing for 2 participants at the ADNI screening visit, 2 for the month 6 visit, 5 for the month 12 visit, 18 for the month 24 visit, 30 for the month 36 visit, and 73 for the month 48 visit). Using generalized linear mixed models, the *protein:time* effect was significantly associated with missing visits for 2 proteins (p -values 0.03–0.05). This number of significant associations is expected given the number of tests (N =70), under the null hypothesis.

3.3 CSF proteins associated with longitudinal brain atrophy

During the period of longitudinal observation, there was significant atrophy in all tested regions (Figure 1). Our main objective was to test if baseline measurements of proteins were associated with atrophy rates. We tested this by linear mixed effects models for each protein and brain region. The results of the regression are presented in figure 2 (as T-values for the *protein:time* interaction). Some proteins were associated with atrophy rates at the significance threshold $p < 0.01$, including P-tau, where high concentrations were associated with atrophy rates in hippocampus and inferior temporal cortex; apolipoprotein D (ApoD), where high concentrations were associated with atrophy rates in middle temporal cortex; and ApoCIII, where high concentrations were associated with atrophy rates in inferior temporal cortex.

Due to the exploratory nature of the study, we here also present proteins that were significant at a more permissive threshold, $p < 0.05$ (only presenting proteins significant in at least 2 regions, because of suspected spurious findings for proteins that were only significant in one region). At this threshold, several proteins were associated with atrophy rates in hippocampus (low A β 42), inferior temporal cortex (high ApoD, α -1-Microglobulin [A1M], ApoH and interleukin 16 [IL-16]), middle temporal cortex (low A β 42, and high A1M, ApoCIII, ApoH and IL-16), and posterior cingulate (low A β 42).

Results for all proteins significant at $p < 0.05$ are presented in Table 1. Note that the associations were mild and that no protein had a significant effect after correcting for multiple comparisons using false discovery correction.

3.4 CSF proteins associated with longitudinal brain atrophy: effects of A β 42-interaction

As seen in Table 1, A β 42 was associated with atrophy rates in hippocampus, middle temporal cortex and posterior cingulate (using continuous A β 42 data). For the remaining part of the study we examined how A β 42 influenced associations between other proteins and atrophy rates. We tested if associations between proteins and atrophy rates differed by brain A β status, by examining the *protein:A β 42:time* interaction (using β 42 dichotomized by the cutoff 192 ng/L, as explained in the methods section). For several proteins, we found interactions in at least one brain region (Table 2A and Figure 2). As seen in Figure 3, most of these proteins were associated with atrophy rates mainly in participants with signs of brain A β pathology, where high levels some proteins (ferritin [FRTN], S100 β , ApoCIII, ApoH, and Hepatocyte Growth Factor [HGF]) were associated with increased atrophy rates. Again, these associations were mild and were not significant after correcting for multiple comparisons.

3.5 CSF proteins associated with longitudinal brain atrophy: A β 42-independent effects

Finally, we tested if proteins were associated with atrophy rates independent of A β 42. We did this by comparing estimates for the *protein:time* interaction with and without adjusting for A β 42 as a covariate (again using A β 42 dichotomized by the cutoff 192 ng/L, as explained in the methods section). Note that this was not meaningful to do for proteins and regions where the *protein:Ab42:time* interaction was significant, since we had already concluded that A β 42 influenced effects of proteins in those regions (Table 2A). For all other proteins and regions, we tested the *protein:time* interaction with and without adjusting for A β 42. Several proteins were associated with atrophy rates when the models were not adjusted for A β 42 (Table 2B). For all these, the effects were unchanged when adjusting the models for A β 42 (β -coefficients changed only minimally, $< 6\%$). Our interpretation of this is that the presence of brain A β pathology at baseline did not mediate the detected effects on regional atrophy rates for the proteins listed in Table 2B.

4. Discussion

The key findings of this study were that (1) several CSF proteins obtained at baseline were associated with atrophy rates in AD-related brain regions over a follow-up period of 4 years, suggesting that these proteins may be novel prognostic biomarkers of AD-related atrophy in cognitively normal people; (2) several CSF proteins had interactions with CSF A β 42, indicating that the presence of A β pathology may influence their effects on longitudinal AD-like atrophy; (3) some CSF proteins were associated with atrophy rates even when the models were adjusted for CSF A β 42, suggesting that these proteins may be associated with atrophy partly independent of A β pathology. Several previous studies have been performed on CSF proteomics in AD (Craig-Schapiro et al., 2011; Hu et al., 2010), but to our knowledge this is the first study linking large scale CSF proteomics to imaging measurements of atrophy in healthy older adults. Previous studies integrating CSF proteins and imaging measurements in this field have mainly examined CSF A β 42, P-tau and T-tau (Fjell et al.; Schott et al., 2010; Tosun et al., 2011). Our results are in agreement with a few previous studies showing that reduced CSF A β 42 predicts longitudinal brain atrophy in cognitively healthy persons (Fjell et al.; Schott et al., 2010). One earlier study from our group failed to show this association (Tosun et al., 2011), which may be due to shorter follow-up time and/or use of other imaging analysis methods. The present study gives novel information on links between a large number of CSF proteins and longitudinal AD-like atrophy.

Our first major finding was that several CSF proteins obtained at baseline were associated with longitudinal atrophy of hippocampus, inferior temporal cortex, or middle temporal cortex in cognitively healthy people. Elevated P-tau and reduced A β 42 were associated with atrophy rates in hippocampus, elevated ApoCIII, P-tau, ApoD, A1M, ApoH and IL-16 with atrophy rates in inferior temporal cortex, elevated ApoD, ApoCIII, P-tau, A1M, IL-16 and ApoH and reduced A β 42 with atrophy rates in middle temporal cortex, and reduced A β 42 with atrophy rates in posterior cingulate. This is in agreement with previous studies of reduced CSF A β 42 and elevated CSF P-tau (Blennow et al., 2010) and ApoD (Craig-Schapiro et al., 2011; Terrisse et al., 1998) in patients with AD or other causes of cognitive decline, as well as increased plasma A1M in AD subjects with reduced whole brain volume (Thambisetty et al., 2011) and increased plasma IL-16 in AD (Motta et al., 2007). Several different mechanisms may link these proteins to AD-like brain atrophy. Increased P-tau is widely reported to be associated with AD (Blennow et al., 2010), and probably reflects lysis of neurons containing tau pathology (Buerger et al., 2006). ApoD has been suggested to be involved in neuroprotection, since it reduces effects of excitotoxic injury (He et al., 2009), and is absent from injured human neurons (Navarro et al., 2008). Taken together, our findings are consistent with the hypothesis that several different CSF proteins are related to longitudinal atrophy rates in AD-related brain regions. However, the effects were mild and not significant after correction for multiple comparisons. The present study should be regarded as a pilot study and the identified proteins may be explored further in future studies. To reduce the risk of reporting spurious findings, we only report proteins that were associated with atrophy rates in at least two regions. However, it should be noted that the effects of some proteins still differed between the investigated brain regions, and no protein was significantly associated with atrophy rates in all regions. The reasons for such inconsistencies are unclear. We suggest that proteins with significant effects across several adjacent regions, including P-tau and ApoD for inferior temporal and medial temporal cortex, represent the best candidate protein biomarkers for future studies on proteins related to atrophy rates. Future voxel-wise studies may allow determining in more details the exact brain distribution of these relationships.

The second major finding was that there were significant interactions between CSF A β 42 and several proteins, in several cases for proteins previously linked to A β -metabolism (See supplementary Table 1). Since reduced CSF A β 42 is a good estimate of brain A β load (Fagan et al., 2006), the interactions of these proteins with CSF A β 42 suggests that brain A β pathology influenced the identified proteins' effects on atrophy rates. In participants with reduced CSF A β 42, high levels of some proteins (FRTN, S100 β , ApoCIII, ApoH and HGF) were associated with increased atrophy rates. For some of the proteins, our findings are in line with previous reports. For example, patients with AD or other types of cognitive decline have been described to have increased CSF levels of ApoH (Abdi et al., 2006; Ohrfelt et al., 2011), S100 β (Fagan and Perrin, 2012), and increased brain levels of HGF (Fenton et al., 1998). A possible interpretation of these findings is that the identified proteins mediate neurotrophic effects dependent on and downstream from A β pathology. Another possibility is that they reflect biological pathways that are independent from A β pathology, but which only results in neuronal atrophy if they are present together with A β pathology, constituting a "dual hit" mechanism, where A β pathology is a necessary prerequisite for damaging effects of other biological pathways. A third possibility is that they reflect pathways upstream of A β pathology, and which A β pathology to some extent depends on. Studies simultaneously examining protein levels and longitudinal development of A β pathology and atrophy may help to reveal the precise relationships between the identified proteins, atrophy and A β pathology.

The third major finding of this study was that several CSF proteins obtained at baseline were associated with atrophy rates independent of A β 42. We identified such proteins for hippocampus (P-tau), inferior temporal cortex (P-tau, ApoD), and middle temporal cortex (P-tau, ApoCIII, ApoD, TM, A1M, IL-16, AAT and ApoH). The finding that P-tau was associated with longitudinal atrophy of several AD-related regions independently of A β 42 is intriguing. Both A β and tau pathology are common in elderly people without cognitive impairment, indicating that both types of pathology are well established in AD prior to symptom debut (Savva et al., 2009). Tau pathology may also be present in the brain in very young age, although it is not known if such deposits constitute initial stages of AD (Braak et al., 2011). Our results could be taken to support that tau pathology, as reflected by CSF P-tau, modulates AD-like atrophy partly independent of A β pathology. Likewise, ApoCIII, ApoD, A1M, IL-16, AAT, ApoH and TM levels may also represent mechanisms of atrophy that are partly independent of A β pathology. Currently it is not clear to what extent these mechanisms of atrophy are relevant specifically for AD, or whether they rather represent atrophy processes of normal aging or other degenerative processes.

Limitations of the study include the examination of a small number of brain regions, which was done to reduce the problem of multiple comparisons. The regions were chosen a priori to any analysis, and it is possible that examination of other regions would have produced different results. It should also be noted that wide-spread atrophy is seen in normal aging, which makes it difficult to define "preserved regions" for comparison to the regions included in this study (for example, in this cohort, atrophy rates in the lateral occipital lobe was of a similar magnitude as in entorhinal cortex or precuneus). Another limitation of the study is that the results may be biased by the selection of the examined proteins, which were included mainly due to known associations with A β metabolism, microglia activity or synaptic function, limiting our capacity to detect proteins associated with unexpected pathways. Future studies may include larger sets of brain regions (or be done using a voxel-based approach), and/or include larger unbiased proteomics data sets, but such studies will encounter statistical difficulties due to problems with multiple comparisons. Finally, we acknowledge the need for replication of these findings in another cohort, especially since the detected statistical effects were small and not significant when correcting for multiple comparisons.

5. Conclusion

We have identified several CSF proteins, primarily linked to microglia activity and/or A β -metabolism, where baseline levels are associated with atrophy rates in AD-related brain regions over a follow-up period of 4 years in cognitively healthy elders. These proteins may be involved in biological pathways involved in early stage AD, and may be explored further for prediction of future AD risk. Several proteins interacted with baseline CSF A β 42, indicating that A β pathology moderated their effects on atrophy rates. These proteins may represent key pathological mechanisms linking A β pathology to neuronal loss. Some proteins, including P-tau, were related to atrophy rates even when the models were adjusted for A β 42, and may thus represent mechanisms of neuronal loss that are at least partly independent of A β pathology. However, the effects were mild and no proteins had significant effects when correcting for multiple comparisons. The findings thus need to be replicated in other cohorts.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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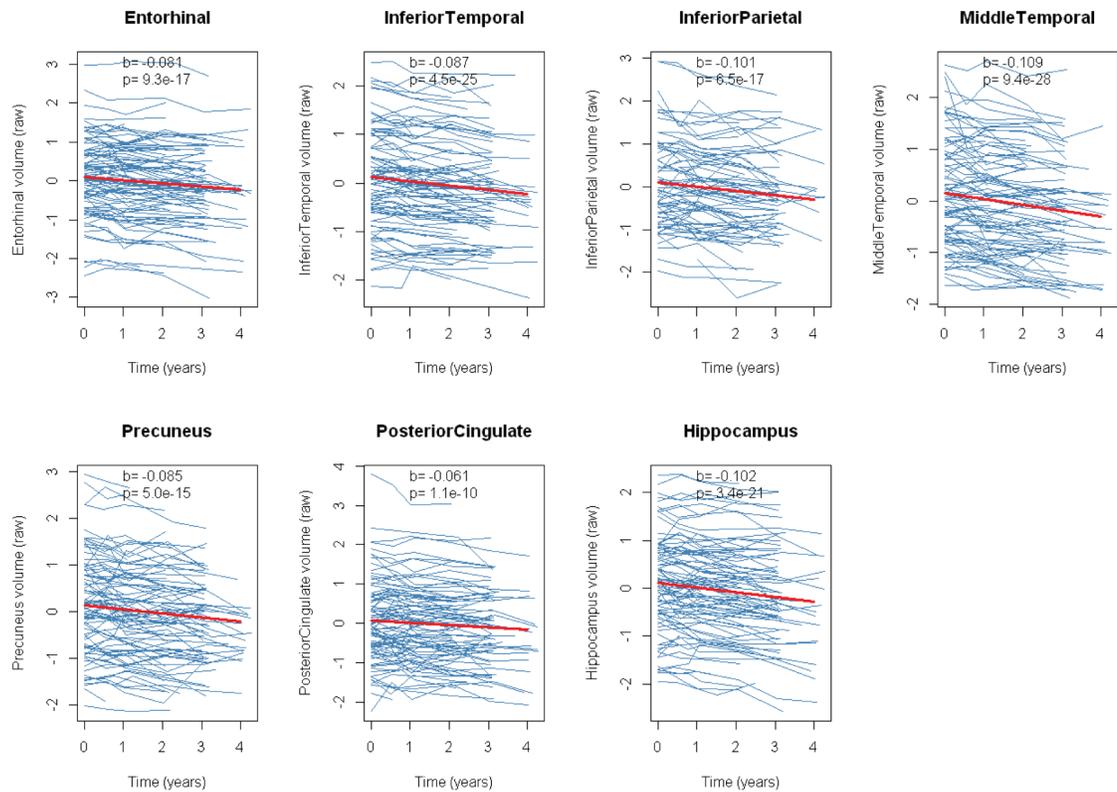


Figure 1. Longitudinal atrophy of regional brain volumes

Thick lines are regression curves calculated by linear mixed effects models, adjusted for age, sex, education and intracranial volume, with random intercepts and slopes. Beta-coefficients and p-values for the fixed time effect are presented in each panel. Volume measurements (y-axes) are standardized. In a few cases, we noted increased volume measurement over time. This likely reflects measurement noise, possibly due to differences in partial volume effects between different scans (these scans were inspected manually to exclude major imaging artifacts).

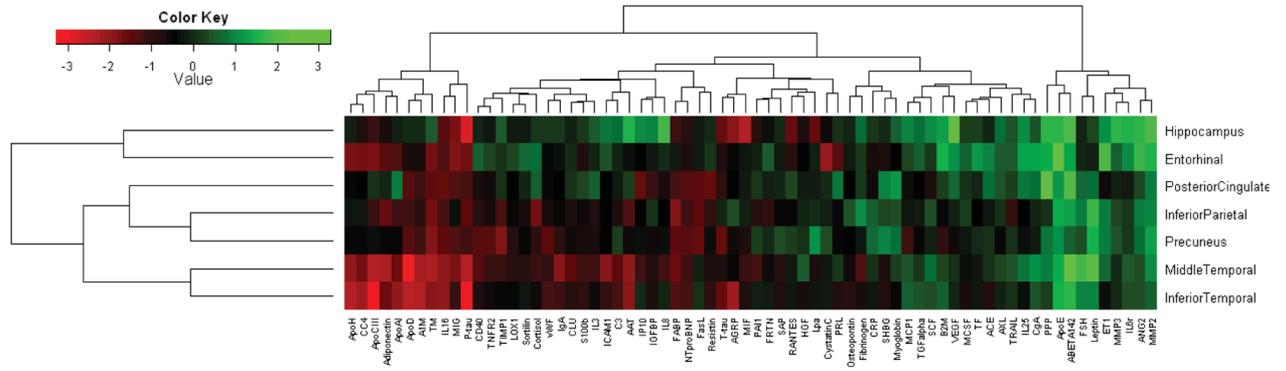


Figure 2. Heat map of baseline CSF proteins and atrophy rates of regional brain volumes T-values (β -coefficients divided by standard error) for the *protein:time* interaction for all CSF proteins and regions from linear mixed effects models, irrespective of p-value. The models were adjusted for age, sex, education and intracranial volume. Red colors indicate low t-values (high proteins levels were associated with high atrophy rates) and green colors indicate high t-values (low protein levels were associated with high atrophy rates).

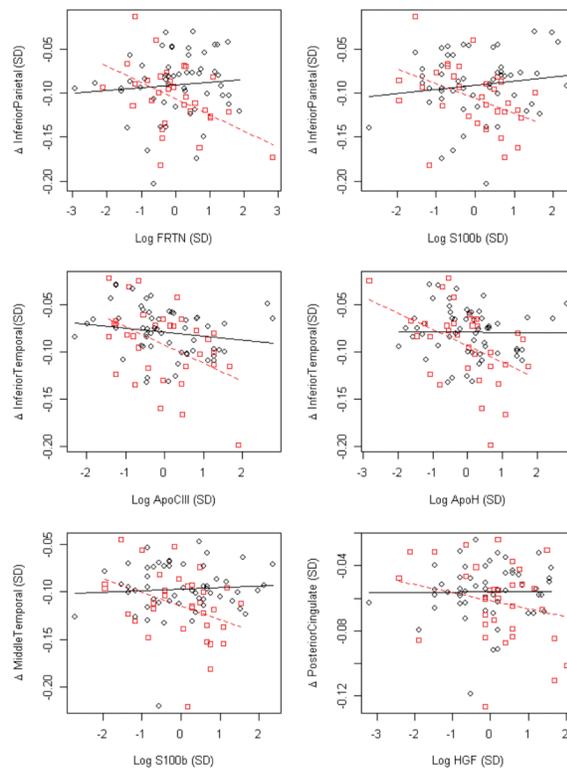


Figure 3. Interactions between baseline CSF proteins and CSF A β 42 on atrophy rates
 All proteins and regions where the *protein:A β 42:time* interaction was significantly associated with regional atrophy rates at $p < 0.01$. Y-axes show yearly atrophy rates (standardized), and x-axes show baseline protein concentrations. CSF A β 42 was dichotomized by the cutoff 192 ng/L. Participants with low CSF A β 42 are shown in red boxes (dashed regression lines) and participants with normal CSF A β 42 in black circles (solid regression lines). Regression lines are adjusted for age, sex, education and intracranial volume.

Table 1

CSF proteins significantly associated with regional atrophy rates

Region	CSF protein	protein:time interaction				
		β	p	N _{par}	N _{obs}	P _{dir}
Hippocampus	A β 42	0.020	0.045	89	406	0.79
	Log P-tau	-0.028	0.005	89	406	0.58
	Log A1M*	-0.017	0.017	90	404	0.70
Inferior Temporal Cortex	Log ApoCIII*	-0.022	0.002	90	404	0.39
	Log ApoD	-0.018	0.015	90	404	0.70
	Log ApoH*	-0.018	0.017	90	404	0.70
	Log IL16	-0.015	0.040	90	404	0.78
	Log P-tau	-0.022	0.002	89	400	0.39
Middle Temporal Cortex	A β 42	0.021	0.019	89	400	0.70
	Log A1M	-0.019	0.026	90	404	0.71
	Log ApoCIII	-0.021	0.016	90	404	0.70
	Log ApoD	-0.027	0.001	90	404	0.39
	Log ApoH	-0.017	0.045	90	404	0.79
Posterior Cingulate	Log IL16	-0.018	0.040	90	404	0.78
	Log P-tau	-0.021	0.016	89	400	0.70
Inferior Parietal Cortex Precuneus	A β 42	0.019	0.040	89	406	0.78
No significant proteins						

Results from linear mixed effects models, showing tests significant before correcting for multiple comparisons, for proteins significant in at least two regions (13 proteins were significant in one region only and were considered likely spurious findings). The rate of change of regional brain volume was used as response variable. The explanatory protein, time from the baseline visit, interaction *protein:time*, age, sex, intracranial volume, and education were used as fixed effects. All models had a random intercept and slope. Each brain region was tested separately with each explanatory protein. Results were similar when *APOE* was included as a fixed effect (data not shown). N_{par}: number of participants; N_{obs}: number of observations; P_{dir}: corrected using a false discovery rate correction applied on 490 tests (70 proteins tested in 7 regions). Biomarkers and brain volumes were centered and standardized (for example, for Log CSF P-tau levels one SD above the mean, the volume of the hippocampus decreased by 0.028 SD per year).

* For these models the *protein:A β 42:time* interaction effect was found to be significant, as explained in the results and presented in Table 2.

Table 2
CSF proteins significantly associated with regional atrophy rates, A β 42-dependent and A β 42-independent effects

Region	CSF protein	A protein:A β 42:time interaction			B protein:time interaction Unadjusted for A β 42		
		β	P	P _{tdr}	β	P	P _{tdr}
Entorhinal	IL25	0.042	0.024	0.40	NA	NA	NA
	Log FRTN	-0.039	0.043	0.51	NA	NA	NA
	Log CC4	-0.043	0.045	0.51	NA	NA	NA
	Log HGF	-0.037	0.047	0.53	NA	NA	NA
	Log Cystatin C				-0.019	0.038	0.87
Hippocampus	Log P-tau				-0.028	0.005	0.69
	Log FRTN	-0.069	0.003	0.37	NA	NA	NA
Inferior Parietal	Log S100b	-0.061	0.009	0.37	NA	NA	NA
	ICAM1	-0.061	0.014	0.37	NA	NA	NA
	Log HGF	-0.052	0.015	0.37	NA	NA	NA
	Log NTproBNP	-0.056	0.019	0.40	NA	NA	NA
	Log Cystatin C	0.058	0.022	0.40	NA	NA	NA
	Log MMP3	-0.052	0.029	0.41	NA	NA	NA
	Log ApoCIII	-0.045	0.002	0.37	NA	NA	NA
Inferior Temporal	Log ApoH	-0.041	0.004	0.37	NA	NA	NA
	Log A1M	-0.040	0.011	0.37	NA	NA	NA
	Log CC4	-0.040	0.015	0.37	NA	NA	NA
	Log IL16	-0.036	0.020	0.40	NA	NA	NA
	Log AAT	-0.037	0.024	0.40	NA	NA	NA
	Log S100b	-0.035	0.027	0.40	NA	NA	NA
	ICAM1	-0.034	0.041	0.50	NA	NA	NA
	Log MMP3	-0.030	0.049	0.53	NA	NA	NA
	Log TM	-0.028	0.050	0.53	NA	NA	NA
	Log P-tau				-0.022	0.002	0.52
Log ApoD				-0.018	0.015	0.87	

Region	CSF protein	A <i>protein:Aβ42:time</i> interaction			B <i>protein:time</i> interaction Unadjusted for Aβ42		
		β	P	P _{fidr}	β	P	P _{fidr}
Middle Temporal	Log S100b	-0.047	0.009	89	NA	NA	NA
	Log FRTN	-0.040	0.025	89	NA	NA	NA
	Log ApoD				-0.027	0.001	0.51
	Log ApoCIII				-0.021	0.016	0.87
	Log P-tau				-0.021	0.016	0.87
	Log TM				-0.020	0.020	0.87
	Log AIM				-0.019	0.026	0.87
	Log IL16				-0.018	0.040	0.87
	Log AAT				-0.018	0.042	0.87
	Log ApoH				-0.017	0.045	0.87
Precuneus	Log PPP	0.052	0.015	0.37	NA	NA	NA
	IL25	-0.051	0.028	0.40	NA	NA	NA
	Log NTproBNP	-0.046	0.033	0.46	NA	NA	NA
Posterior Cingulate	ICAMI	0.052	0.015	0.37	NA	NA	NA
	Log HGF	-0.045	0.005	0.37	NA	NA	NA
	ICAMI	-0.047	0.015	0.37	NA	NA	NA
	Log PPP	0.035	0.027	0.40	NA	NA	NA

Results from linear mixed effects models for proteins significantly associated with atrophy rates in at least two regions (23 proteins were significant in one region only and were considered likely spurious findings). Regional atrophy rates were used as response variables. The time from the baseline visit, explanatory protein, age, sex, intracranial volume, and education were used as fixed effects. Each brain region was tested separately with each explanatory protein. Results are given for models that included the *protein:Aβ42:time* interaction effect (A), *protein:time* or the interaction effect unadjusted for Aβ42 (B). For proteins listed in (B), coefficients and p-values did not change when including Aβ42 as a predictor in the models (not shown). Results were similar when the models were adjusted for APOE genotype (not shown). The numbers of participants were 84–90, and the numbers of observations 348–406. P_{fidr} corrected using a false discovery rate correction (for models of the *protein:Aβ42:time* interaction effect, the correction was applied on 483 tests [69 proteins tested in 7 regions], for models of the *protein:time* interaction, the correction was applied on 438 tests [all proteins and regions except the 45 proteins and regions where *protein:Aβ42:time* was significant]). Biomarkers and brain volumes were centered and standardized.