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Cerebrospinal fluid levels of synaptic and neuronal integrity correlate with gray matter volume and amyloid load in the precuneus of cognitively intact older adults

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ABSTRACT

The main pathophysiological alterations of Alzheimer's disease (AD) include loss of neuronal and synaptic integrity, amyloidogenic processing and neuroinflammation. Similar alterations can, however, also be observed in cognitively intact older subjects and may prelude the clinical manifestation of AD. The objectives of this prospective cross-sectional study in a cohort of 38 cognitively intact older adults were twofold: (i) to investigate the latent relationship among cerebrospinal fluid (CSF) biomarkers reflecting the main pathophysiological processes of AD, and (ii) to assess the correlation between these biomarkers and gray matter volume as well as amyloid load. All subjects underwent extensive neuropsychological examinations, CSF sampling, [^{18}F]-flutemetamol amyloid positron emission tomography (PET) and T_1-weighted magnetic resonance imaging (MRI). A factor analysis revealed one factor that explained most of the variance in the CSF biomarker dataset clustering t-tau, α-synuclein, p-tau$_{181}$, neurogranin, BACE1, VILIP-1, YKL-40, Aβ1$_{-40}$ and Aβ$_{1-38}$. Higher scores on this factor correlated with lower gray matter volume and with higher amyloid load in the precuneus. At the level of individual CSF biomarkers, levels of VILIP-1, neurogranin, BACE1, Aβ$_{1-40}$, Aβ$_{1-38}$ and YKL-40 all correlated inversely with gray matter volume of the precuneus. These findings

demonstrate that in cognitively intact older subjects, CSF levels of synaptic and neuronal integrity biomarkers, amyloidogenic processing and measures of innate immunity (YKL-40) display a latent structure of common variance, which is associated with loss of structural integrity of brain regions implicated in the earliest stages of AD.

KEYWORDS

Cerebrospinal fluid biomarkers; neurogranin; VILIP-1; BACE1; precuneus; Alzheimer's disease.

ABBREVIATIONS

Aβ: amyloid-β; AD: Alzheimer's disease; APOE: apolipoprotein; APP: amyloid precursor protein; AVF: animal verbal fluency; AVLT: Rey's auditory verbal learning; A.U.: arbitrary unit; BACE1: β-secretase amyloid precursor protein cleaving enzyme-1; BNT: Boston naming test; CDR: clinical dementia rating; CSF: cerebrospinal fluid; ELISA: enzyme-linked immunosorbent assay; FWHM: full-width half maximum; IL-6: Interleukin-6; LVF: letter verbal Fluency; MCI: mild cognitive impairment; MCP-1: monocyte chemotactic protein-1; MIP-1β: macrophage inflammatory protein 1-β; MMSE: mini-mental state examination; MNI: Montreal neurological institute; MRI: magnetic resonance imaging; PALPA: psycholinguistic assessment of language processing in aphasia; PET: positron emission tomography; p-tau_{181}: phosphorylated-tau at threonine 181; PVC: partial volume correction; RPM = Raven's progressive matrices; SUVR: standardized uptake value ratio; Tau: tubulin-associated unit; VILIP-1: visinin-like protein 1; VOI: volume of interest; YKL-40: chitinase-3-like protein 1.

INTRODUCTION

The onset of cognitive impairment in patients with Alzheimer's disease (AD) is preceded by a long asymptomatic phase of 10 to 30 years (Bateman *et al.* 2012; Jack *et al.* 2013; Jansen *et al.* 2015). The asymptomatic phase of AD is defined as evidence of brain amyloid load, assessed by reduced cerebrospinal fluid (CSF) levels of amyloid-β 1-42 ($A\beta_{1-42}$) or through a positive amyloid positron emission tomography (PET) scan (Sperling *et al.* 2011; Sperling *et al.* 2014). [^{18}F]-flutemetamol (Vizamyl, GE Healthcare) is a widely used amyloid-PET ligand which predominantly binds to neuritic amyloid plaques (Salloway *et al.* 2017)(Salloway *et al.* 2017). In a subset of asymptomatic subjects, neurodegeneration is present, as measured by volumetric magnetic resonance imaging (MRI), [^{18}F]-fluoro-deoxy-glucose ([^{18}F]-FDG)-PET or by means of CSF levels of t-tau and phosphorylated tau (p-tau) (Sperling *et al.* 2011; Mattsson *et al.* 2017; Gordon *et al.* 2018; Sperling *et al.* 2014). Biomarkers that could trace neurodegeneration up to the level of synaptic integrity are of great potential interest as outcome measures of clinical trials for AD. In recent years, the target population of clinical trials has gradually shifted towards inclusion of cognitively intact older subjects who are carriers of the main genetic risk factor of sporadic AD: apolipoprotein E (APOE) ε4 (Sperling *et al.* 2014a) or have a positive amyloid status on CSF and/or PET. Several candidate biomarkers for tracking synaptic and neuronal integrity have recently been proposed. These include, among others, visinin-like protein 1 (VILIP-1), neurogranin, α-synuclein and β-secretase amyloid precursor protein (APP) cleaving enzyme-1 (BACE1). The neuronal injury biomarker VILIP-1 is a neuronal calcium sensor protein involved in calcium homeostasis (Braunewell 2012). CSF levels of VILIP-1 are increased in the asymptomatic phase of autosomal dominant AD mutation carriers as well as in cognitively intact individuals at risk for sporadic AD at later age (Fagan *et al.* 2014; Sutphen *et al.* 2015). In patients with mild cognitive impairment (MCI), CSF VILIP-1 levels predict cognitive decline and conversion to AD dementia (Kester *et al.* 2015b; Tarawneh *et al.* 2012). Neurogranin, a dendritic post-synaptic protein, is involved in calcium signalling and in synaptic plasticity (Kester *et al.* 2015a). Higher CSF levels of neurogranin are positively correlated with neurodegeneration as assessed by MRI volume

loss and [^{18}F]-FDG-PET hypometabolism in amyloid-positive subjects (Mattsson *et al.* 2016, Pereira *et al.* 2017). High baseline neurogranin levels predict conversion to AD dementia in amyloid-positive MCI patients (Kester *et al.* 2015a). α-synuclein, a synaptic marker expressed at the pre-synaptic compartment, is involved in synaptic activity and in neurotransmitter release (Burré *et al.* 2010). Elevated CSF levels of α-synuclein correlate with lower cognitive performance in patients with MCI or AD dementia (Korff *et al.* 2013). CSF α-synuclein is suggested to be a better correlate of AD-associated cognitive impairment than the classical CSF AD biomarkers $A\beta_{1-42}$ and t-tau (Larson and Lesné 2012). BACE1 is enriched at the synapse and is involved in amyloidogenic processing (Kandalepas *et al.* 2013). CSF levels of BACE1 are higher at the MCI stage than at the asymptomatic or dementia stages of AD (De Vos *et al.* 2016; Ewers *et al.* 2008; Zhong *et al.* 2007; Barao *et al.* 2013). The ratio of neurogranin over BACE1 correlates with cognitive decline in patients with MCI or clinically probable AD (De Vos *et al.* 2016).

Besides markers for neurodegeneration, markers for neuroinflammation such as Chitinase-3-like protein 1 (YKL-40), monocyte chemotactic protein-1 (MCP-1), eotaxin-3, macrophage inflammatory protein 1β (MIP-1β), and interleukin-6 (IL6) are higher in CSF of MCI and AD patients than in cognitively intact older adults (Richens *et al.* 2014; Brosseron *et al.* 2014; Westin *et al.* 2012; Blum-Degena *et al.* 1995). In cognitively intact older adults, increased CSF YKL-40 levels correlate with cognitive decline (Sala-Llonch *et al.* 2017). CSF YKL-40 levels negatively correlate with cortical thickness in individuals with low CSF $A\beta_{1-42}$ levels while CSF MCP-1 levels positively correlate with white matter microstructural alterations typically related to AD pathology (Alcolea *et al.* 2015; Melah *et al.* 2016; Kester *et al.* 2015b). MCP-1 co-localizes, in particular, with activated astrocytes in the surroundings of amyloid-β plaques (Sokolova *et al.* 2009; Westin *et al.* 2012). Overexpression of MIP-1β is also associated with amyloid-β pathology (Xia *et al.* 1998; Zhu *et al.* 2014; Smits *et al.* 2002). In cognitively intact middle aged and older adults, higher CSF levels of MIP-1β correlate with higher CSF levels of t-tau and p-tau (Bettcher *et al.* 2018). Increased CSF levels of IL-6 are observed in patients with clinically probable AD (Blum-Degena *et al.* 1995; Fan *et al.* 2017). This elevation of

neuroinflammatory markers is suggested to play a role in the activation of glia cells to counteract amyloid burden (Heneka *et al.* 2010).

Following these biomarker-based studies, the first objective of this cross-sectional study was to investigate in cognitively intact older subjects whether CSF biomarkers of amyloidogenic processing, synaptic and neuronal integrity, neurofibrillary tangle formation and neuroinflammation processes shared a common variance. We hypothesized that CSF biomarkers of synaptic integrity (VILIP-1, neurogranin, α-synuclein and BACE1) would cluster together in particular with t-tau and that neuroinflammatory markers (YKL-40, MCP-1, eotaxin-3, MIP-1β and IL6) would cluster together with AD pathology ($A\beta_{1-42}$, p-tau) and amyloidogenic processing ($A\beta_{1-38}$ and $A\beta_{1-40}$) markers.

The second objective was to investigate the relationship of these CSF biomarker levels with gray matter volume on one hand and with brain amyloid load on the other hand.

METHODS

Participants

The study cohort of 38 subjects was consecutively recruited between 10/09/2012 and 04/04/2014, as part of a larger longitudinal community-recruited study cohort of 180 cognitively intact older individuals, which has been preregistered under EudraCT 2009-014475-45 on https://eudract.ema.europa.eu/. Participants were included via advertisement in newspapers and online asking for volunteers for scientific research including brain imaging (sic). The inclusion criteria for the full cohort were age between 65 and 80 years, a Mini Mental State Examination (MMSE) score greater than or equal to 27, a Clinical Dementia Rating (CDR) global score of zero and scores on a standard neuropsychological examination within published norms (Adamczuk *et al.* 2014; Adamczuk *et al.* 2013). The exclusion criteria were a significant neurological or psychiatric history, focal brain lesions based on MRI, a history of cancer, a counter indication for MRI (pacemaker, metal implants or severe claustrophobia) or exposure to radiation for research procedures within the year prior to the [^{18}F]-flutemetamol amyloid-PET scan. To enrich the full cohort with individuals at risk for

AD, subjects were stratified at inclusion based on their APOE ε4 genotype such that the proportion of APOE ε4 carriers included in the full cohort was 50% (Adamczuk *et al.* 2014; Adamczuk *et al.* 2013). At baseline, all participants of the full cohort of 180 subjects underwent an extensive neuropsychological examinations, structural MRI and [^{18}F]-flutemetamol amyloid-PET (Fig. 1). A subgroup these 180 subjects, i.e. 38 consecutively recruited subjects (Table 1) underwent additionally a lumbar puncture for CSF biomarker analysis per protocol (Fig. 1). This CSF cohort (n = 38) is identical to that from a previous study comparing CSF AD biomarkers and [^{18}F]-flutemetamol (Adamczuk *et al.* 2015). All participants are currently receiving a two-yearly neuropsychological assessment for a 10-years period.

The local Ethical Committee of the University Hospitals Leuven approved the study (EudraCT: 2009-014475-45). Written informed consent was obtained from all subjects in accordance with the Declaration of Helsinki.

Imaging Procedures

Structural Magnetic Resonance Imaging

A structural T_1-weighted MRI was acquired on a 3T Philips Achieva scanner (Philips, Best, The Netherlands) (3-D turbo field echo sequence, 32-channel Philips sensitivity encoding head coil: coronal inversion recovery prepared 3-D gradient-echo images, inversion time 900 ms, echo time (TE)/repetition time (TR) 4.6/9.6, flip angle 8°, voxel size 0.98×0.98×1.2 mm^3). Pre-processing of the T_1-weighted MRI scans was performed with voxel-based morphometry (VBM8, http://dbm.neuro.uni-jena.de/vbmrunning) (Ashburner and Friston 2000) running on Statistical Parametric Mapping (SPM8, Wellcome Trust Centre for Neuroimaging, London, UK, (http://www.fil.ion.ucl.ac.uk/spm)) implemented in Matlab R2012b (Mathworks, Natick, USA) as described in previous studies (Gillebert *et al.* 2015; Schaeverbeke *et al.* 2017). The resulting modulated gray matter volumes have been adjusted for overall brain size (total intracranial volume)

by using the 'nonlinear only' component in the spatial normalization process for modulation of gray matter voxel intensities (Barnes *et al.* 2010). For subsequent voxel-wise statistical comparisons, gray matter maps were smoothed with an 8 mm isotropic full-width half-maximum (FWHM) Gaussian 3D kernel.

[^{18}F]-flutemetamol amyloid-PET imaging

[^{18}F]-flutemetamol PET was acquired on a 16-slice Siemens Biograph PET/computed tomography (CT) scanner (Siemens, Erlangen, Germany) in all subjects. The tracer was injected as a 180 MBq bolus into an antecubital vein. Scan acquisition started 90 minutes after tracer injection and lasted for 30 minutes. Prior to PET acquisition, a low-dose CT scan of the head was performed for attenuation correction. Random and scatter correction were applied. Images were reconstructed using ordered subsets expectation maximization (4 iterations × 16 subsets). Processing of [^{18}F]-flutemetamol PET images was done as previously described (Adamczuk *et al.* 2016a; Adamczuk *et al.* 2013). Briefly, PET emission frames were individually realigned, summed and rigidly co-registered to the subjects T_1-weighted MRI scan in SPM8 software, running on MatlabR2012b. Both the summed PET images as well as the T_1-weighted MRI segmentations were warped to Montreal Neurological Institute (MNI) template space. Standardized uptake value ratio (SUVR) images with the subject-specific cerebellar gray matter as reference region were generated. The mean SUVR in a composite neocortical volume of interest (VOI) ($SUVR_{comp}$) consisted of 5 bilateral cortical regions (Adamczuk *et al.* 2016a; Adamczuk *et al.* 2013). Concretely, an SUVR value is a proxy for amyloid load as it calculates the mean tracer retention over all voxels of the cortex, divided by the voxel values in a region with no or minimal tracer retention. To have an estimate on how high or low the amyloid load is based on an SUVR value, we compared this value with a cut-off for brain amyloid-PET positivity. Brain amyloid-PET positivity with the [^{18}F]-flutemetamol tracer was defined as $SUVR_{comp} > 1.38$, a threshold derived from an independent cohort with an identical MRI-based volume of interest method as in the current study (Adamczuk *et al.* 2016b). For voxel-wise analyses, normalized [^{18}F]-flutemetamol PET scans were partial volume corrected (PVC), using the information of the MRI scan in a modified

Müller-Gärtner approach (Müller-Gärtner *et al.* 1992). SUVR images were smoothed with a 6 mm isotropic FWHM Gaussian 3D kernel.

Lumbar puncture and immunoassays

Lumbar punctures (LP) were performed according to a standardized protocol by experienced neurologists at the University Hospital of Leuven at a fixed time (10 AM) without prior fasting. A mean delay of 41 days (range 7 to 99 days) and 58 days (range 22 to 106 days) separated the lumbar puncture from the [^{18}F]-flutemetamol amyloid-PET scan and the MRI scan, respectively. LP's were performed while the subject was in sitting position, using a 22G atraumatic needle. Thirteen millilitres (mL) of CSF was collected, discarding the first collected mL and sampled in 4 polypropylene tubes of about 3 mL (cat# 188261, Greiner Bio-one Cellstar, Vilvoorde, Belgium), centrifuged at 2600 rpm for 10 minutes at 4°C and 1.5 mL was aliquoted in low binding tubes (cat#298, Kartell, Noviglio, Italy) and stored at -80°C until analysis.

Immunoassays

The CSF protein levels of neurogranin, α-synuclein, BACE1, $A\beta_{1-42}$, $A\beta_{1-40}$, $A\beta_{1-38}$, t-tau and phosphorylated tau ($p\text{-}tau_{181}$) were assessed at ADx NeuroSciences (Ghent, Belgium) using commercially available assays (neurogranin, BACE1, $A\beta_{1-42}$, $A\beta_{1-40}$, $A\beta_{1-38}$, t-tau and $p\text{-}tau_{181}$ (Euroimmun, Luebeck, Germany)) or a qualified immunoassay for α-synuclein (detailed cat# for all assays: see in Supplementary Table 1). The cut-off for CSF $A\beta_{1-42}$ to predict amyloid-PET positivity was 745 pg/mL and was previously defined on the same cohort (Adamczuk *et al.* 2015). The CSF $A\beta_{1-42}$ cut-off of 745 pg/mL was derived by calculating the Area Under the Curve (AUC) in a Receiver Operating Characteristics (ROC) approach for which the Youden criterium was applied to select the optimal cut-off, i.e. the best compromise between high sensitivity and high specificity (AUC: 0.908, 100% sensitivity and 74.19% specificity) (Adamczuk *et al.* 2015). The standard of truth for the ROC

analysis in that study was amyloid-positivity based on an autopsy validated amyloid-PET SUVRcomp cut-off of 1.57 (Adamczuk *et al.* 2015).

CSF levels of VILIP-1 were assessed with an in-house developed electrochemiluminescent immunoassay, which can be provided on reasonable request. Briefly, high-binding Meso Scale Discovery 96-well plates were coated with a capture polyclonal antibody, followed by incubation with a detection polyclonal antibody. A SULFO-tagged secondary antibody against the detection antibody was used to generate electrochemiluminescence that was quantified with the MESO QuickPlex SQ 120 platform. The recombinant VILIP-1 protein (cat# MBS143824, Mybiosource.com, California, United-States) was serially diluted in blocking buffer to generate a four-parameter logistic regression standard curve to assign a concentration to every electrochemiluminescence signal. CSF levels of the neuroinflammatory markers MCP-1, MIP-1β, eotaxin-3 and IL-6 were determined with the electrochemiluminescent V-PLEX multiplex assays purchased from Meso Scale Discovery (cat# K15047G and K15049G, Maryland, United-States). CSF levels of YKL-40 were measured with the MicroVue Enzyme-Linked ImmunoSorbent Assay (ELISA) from Quidel (cat# 8020 San Diego, United-States). Total protein levels were measured with the Roche Cobas c702 employing the benzethonium chloride method (cat# 05171954190 Roche Diagnostics, Basel, Switzerland); albumin and immunoglobulin G levels were measured in the CSF samples by Nephelometry using the Beckam Coulter Image 800 (cat# 447600 and 446400 Beckman Coulter, California, United States). Analytical performances of the individual immunoassays are listed in Supplementary Table 1. Immunoassays were performed blinded to study information.

Statistical Analysis

Primary outcome analyses

As a primary outcome analysis, we evaluated the latent structure of the CSF biomarkers within the dataset by means of a factor analysis as well as the correlation of these factor scores with imaging measures of gray matter volume and amyloid load. No sample calculation was performed a priori.

Factor analysis

Normality of the CSF biomarker values was first assessed with the Agostino-Pearson's test. In case CSF biomarker values deviated from normality ($\alpha < 0.05$), a log-transformation was performed to limit skewed distribution and to approach normality. The dataset was then statistically assessed for suitability for factor analysis based on the Kaiser-Meyer-Olkin's test criterion (> 0.6) and for sphericity with the Bartlett's test ($p < 0.001$). A factor analysis was conducted using the principal axis factoring method as previously described (Vandenbulcke *et al.* 2005; Molenberghs *et al.* 2009; Nelissen *et al.* 2010) in Statistical Package for the Social Sciences software version 23 (SPSS, IBM, Armonck, USA). The principal axis factoring method was applied in order to extract factors and only retain factors with an eigenvalue > 1.0 (Kaiser's criterion) (Cerny and Kaiser 1977). The eigenvalue of each factor corresponds to the proportion of variance explained by that factor. The factors were rotated with a VARIMAX orthogonal rotation to obtain interpretable and uncorrelated factor loadings. The factor loading for a given variable can be interpreted as a standardized regression coefficient: a high and positive factor loading means that that variable has a strong and positive correlation with that factor. Only the factor loadings greater than 0.7 were retained based on the cut-off by Hair et al. (2010) (Hair *et al.* 2010). In a next step, for every subject, individual weighted factor scores were derived for each factor using a regression based approach taking into account weighting of each variable to a given factor. Weighting refers here to the factor loading of that variable. Factor scores are a weighted sum of the variables, with weights comprised between -1 and 1. Individual weighted factor scores for one subject could not be calculated because of an out of range value for eotaxin-3 CSF levels.

- **Whole-brain voxel-wise analyses of factor scores**

For each extracted factor, a whole-brain voxel-wise linear regression analysis was conducted with the modulated gray matter volume images or PVC [^{18}F]-flutemetamol SUVR images as the dependent variable and with the individual weighted factor scores for that factor as the independent variable.

Age and gender were added as nuisance variables. The significance threshold for the whole-brain voxel-wise linear regression analysis was set at a voxel-level uncorrected $p < 0.001$ combined with a cluster level family-wise error (FWE) correction at $p < 0.05$ (Poline *et al.* 1997). For each significant cluster, the mean voxel value in that cluster was calculated for each individual subject solely to visualize the correlation and directionality between the variables.

Secondary outcome analyses

- **Whole-brain voxel-wise analyses of individual biomarkers**

For factor 1 and 2, we also evaluated the correlation between the individual markers composing the factor, on the one hand, and gray matter volume and amyloid load on the other hand. In order to further explore the association of individual CSF biomarker alterations to gray matter volume, we conducted for the biomarkers clustering in factor 1 and 2 (n = 10) a whole-brain voxel-wise linear regression analysis with the modulated gray matter volume maps as the dependent variable and with the CSF values of that biomarker as the independent variable. Age and gender were added as nuisance variables. This secondary analysis was performed in a more exploratory fashion to evaluate which CSF biomarkers were mainly driving the correlation for a given factor.

- **Additional standard statistical analyses**

The primary outcome analyses have been repeated with addition of APOE ε4 status and education as nuisance variables. APOE ε4 was treated as a binary variable, education as a continuous variable. We additionally assessed the effect of time between LP and imaging (MRI or PET) by incorporating time (in days) as nuisance variable in each of the voxel-wise regression models used in the primary outcome analyses. Correlations between CSF biomarkers were assessed with the Spearman's ranking test at a Bonferroni adjusted significance level of 0.004, corresponding to an α of 0.05 divided by the

number of correlations (n = 13) for each biomarker. Correlations with eotaxin-3 were not performed because of an out of range value for one patient. Effects of gender on CSF biomarker levels were assessed using a Mann-Whitney U test ($\alpha < 0.05$). Potential correlations between CSF biomarker levels and age at LP, or between CSF biomarker levels and protein concentration, albumin content and immunoglobulin G levels in CSF were assessed using Spearman's ranking tests ($\alpha < 0.05$). To assess the effect of the APOE ε4 genotype on individual weighted factor scores and on [^{18}F]-flutemetamol $SUVR_{comp}$ values, subjects were divided into two groups (APOE ε4 carriers vs. non-carriers) and separate Mann-Whitney *U* tests were performed ($\alpha < 0.05$).

RESULTS

The demographic data of the study cohort are shown in Table 1, and the analytical performances of the immunoassays are listed in Supplementary Table 1. Mean levels of the tested CSF biomarker levels in the study cohort are listed in Table 1. Nine out of 38 subjects (24%) were amyloid-positive on amyloid-PET and another seven out of 38 subjects (18%) had low CSF $A\beta_{1-42}$ levels yet a negative amyloid-PET scan.

Primary outcome analyses

- **Latent structure of the CSF biomarkers**

The CSF biomarker values were suitable for factor analysis given a Kaiser-Meyer-Olkin's test score of 0.827 and a Bartlett's test with a $p < 0.0001$. Four factors reached an Eigenvalue above 1. These factors cumulatively explained 86.8% of the total variance of the CSF biomarker dataset (Table 2). The first factor (eigenvalue = 7.68) explained 54.9% of the total variance. The CSF biomarkers with the highest factor loadings (> 0.70) on factor 1 were t-tau, α-synuclein, p-tau_{181}, neurogranin, BACE1, VILIP-1, YKL-40, $A\beta_{1-40}$ and $A\beta1_{-38}$ (Table 2). These variables correlated positively with factor 1. The second factor (eigenvalue = 1.89) explained 13.5% of the overall variance and contained CSF $A\beta_{1-42}$

(Table 2). CSF $A\beta_{1-42}$ values correlated positively with factor 2. The third (eigenvalue = 1.55) and fourth (eigenvalue = 1.03) factors explained 11.0% and 7.38% of the total variance, respectively, and clustered the CSF biomarkers MCP-1 and IL-6 in factor 3 and MIP-1β in factor 4 (Table 2). The obtained factor loading for eotaxin-3 was below the 0.7 cut-off (Hair, 2010) and could not be assigned to any of the above-mentioned factors.

Correlation with gray matter volume

Voxel-based morphometry revealed a significant inverse correlation between individual weighted factor 1 scores and gray matter volume of the precuneus (897 voxels, $Z = 4.24$, MNI coordinates: 14, -72, 60, voxel-level p uncorrected < 0.001 combined with cluster-level $p_{FWE\text{-}corrected}$ ($p = 0.020$) (Fig. 2a and b): The higher the individual scores on this factor, the lower gray matter volume. This is logical since the variables loading most strongly on factor 1 (Table 2) are known to increase with disease. No significant correlations were obtained between individual weighted scores on any of the other factors (i.e. factor 3 and 4) and gray matter volume.

- **Correlation with brain amyloid load**

The whole-brain voxel-wise analysis revealed that individual weighted factor 1 scores correlated positively with amyloid load in the precuneus, extending into the posterior cingulate (262 voxels, Z = 4.69, MNI coordinates: 2, -66, 14, $p = 0.015$) (Fig. 3a and b). Individual weighted factor 2 scores correlated inversely with amyloid load in bilateral neocortical association areas (right temporal inferior: 3670 voxels, $Z = 5.09$, MNI coordinates: 56, -52, -16, p <0.001; right middle cingulum: 5851 voxels, $Z = 5.06$, MNI coordinates: 6, 28, 32, p <0.001; left insula: 407 voxels, Z = 4.61, MNI coordinates: -36, 10, 10, $p = 0.001$; right frontal operculum: 1906 voxels, $Z = 4.57$, MNI coordinates: -62, -16, -2, p <0.001 and left orbitofrontal cortex: 502 voxels, $Z = 4.20$, MNI coordinates: -36, 58, 0, p <0.001) (Fig. 3c and d). No significant voxel-wise correlations were found between scores on factor 3 or 4 and brain amyloid load.

Secondary outcome analyses

- **Correlation with gray matter volume, corrected for APOE ε4, education or time interval**

For the inverse correlation between factor 1 scores and gray matter volume, addition of APOE ε4 status and education or for the number of days between LP and MRI as nuisance variables essentially provided the same results.

- **Correlation with brain amyloid load, corrected for APOE ε4, education or time interval**

For the positive correlation between factor 1 scores and amyloid load, additional corrections for APOE ε4 status and education revealed the same cluster in the precuneus, extending into the posterior cingulate, however at a lower significance threshold of voxel-level uncorrected $P<0.001$. For the inverse correlation between factor 2 scores and amyloid load, the same neocortical clusters were obtained after addition of APOE ε4 status and education as nuisance variables, though slightly diminished in size. Correcting for the number of days between LP and MRI as a nuisance variable essentially provided the same results as well.

- **Correlation between individual CSF biomarkers and gray matter volume**

Voxel-based morphometry revealed an inverse correlation between CSF levels of VILIP-1, neurogranin, BACE1, $A\beta_{1-40}$, $A\beta_{1-38}$ and YKL-40 and gray matter volume in clusters containing the precuneus (Fig. 4a, Table 3 and Supplementary 2). At a lower significance level (voxel-level uncorrected $p < 0.001$), additional inverse correlations were obtained between individual CSF levels of α-synuclein, t-tau or p-tau_{181} and gray matter volume in the precuneus.

- **Correlation between t-tau and p-tau and brain amyloid load**

Significant positive voxel-wise correlations were obtained between CSF levels of t-tau and focally elevated amyloid load in the precuneus. CSF p-tau$_{181}$ levels correlated positively with amyloid load in a more extensive cluster of amyloid load in neocortical association regions (Fig. 4b, Table 3 and Supplementary 2). No other significant correlations were obtained at the pre-set threshold (voxel-level *p* uncorrected < 0.001 combined with cluster-level $p_{FWE\text{-}corrected}$).

- **Correlations between individual CSF biomarkers**

The correlations between the individual biomarkers are listed in Table 4. Correlations were in line with the aforementioned factor analyses. There was a significant inverse correlation between CSF levels of A$\beta_{1\text{-}42}$ and levels of most markers (except for tau, p-tau and YKL-40) included in factor 1.

Effect of APOE ε4 genotype

APOE ε4 carriers had significantly lower weighted factor 2 scores and higher [^{18}F]-flutemetamol SUVR$_{comp}$ values compared to non-APOE ε4 carriers (Unpaired *t*-test $p = 0.02$ and Mann-Whitney test $p = 0.0007$, respectively). APOE ε4 genotype status did not have an effect on factor 1 scores.

Effect of gender

A gender difference was only observed for eotaxin-3, for which CSF levels were significantly higher in male subjects (Mann-Whitney test $p = 0.027$) (Table 1).

DISCUSSION

This study reveals discovered a latent variable that explains shared variances across several commonly assessed biomarkers (CSF levels of t-tau, α-synuclein, p-tau$_{181}$, neurogranin, BACE1, VILIP-1, Aβ$_{1-40}$, Aβ$_{1-38}$ and YKL-40). This is important because these analytes are often investigated individually, and mechanistic inferences are drawn from the results, yet the levels in these analytes probably reflect an underlying shared biology. Another strength of this study is the combination of MRI and PET imaging with CSF (multiple analytes), which showed that this shared latent structure of CSF biomarkers correlated with reduced gray matter volume and elevated amyloid load in the precuneus in cognitively intact older subjects.

CSF biomarkers of pathophysiological pathways in AD

Previous studies describe correlations between CSF levels of biomarkers reflecting neuronal loss, loss of synaptic integrity and amylodoigenic processing using standard statistical correlational methods (Wang *et al.* 2018; Barao *et al.* 2013; Luo *et al.* 2013; De Vos *et al.* 2016). The current study revealed the latent structure within a set of CSF biomarkers involved in pathophysiological processes of AD by means of a factor analysis, a statistical method that assesses the underlying structure in a dataset based on its variance. The latent structure of these biomarkers in CSF could possibly reflect latent relationships between pathophysiological processes including neuronal and synaptic loss, specific neuroinflammatory processes and amyloid processing, which are already ongoing in cognitively healthy individuals at risk for AD. In the current dataset, CSF Aβ$_{1-42}$ belonged to a factor separate from the neuronal and synaptic injury markers and separate from neuroinflammatory markers. This indicates only a limited common variance between CSF Aβ$_{1-42}$ and the other pathophysiological markers. This finding is in line with previous work that shows no association between CSF neurogranin and Aβ$_{1-42}$ in AD samples (Hellwig *et al.* 2015) or between CSF BACE1 enzymatic activity and Aβ$_{1-42}$ levels in cognitively intact older subjects (Mulder *et al.* 2010; Tsolakidou *et al.* 2013; Perneczky *et al.* 2014; Savage *et al.* 2015). The latent relationship that we have

demonstrated between biomarkers may be stage-dependent as for instance in advanced AD, t-tau and p-tau do not correlate anymore with YKL-40 levels (Hellwig *et al.* 2015). Furthermore, certain biomarkers such as VILIP-1 or YKL-40 might have different rates of change depending of the disease stage and disease progression (Kester *et al.* 2015b). Others have recently suggested that the longitudinal progression of α-synuclein levels in CSF remains unchanged over the different clinical stage of AD (Wang *et al.* 2018). In addition, one should not exclude the importance of comorbidities of pathology, which are reflected in biomarker levels. The majority of patients with AD display multiple pathologies that will play an important role in the ongoing neuropathological changes and thus on biomarker levels (Rabinovici *et al.* 2017). The pattern of longitudinal progression of the different biomarkers will need to be investigated further. Additionally, it remains to be demonstrated in larger cohorts of asymptomatic individuals whether the levels of these biomarkers can be used as predictive markers of cognitive decline and conversion to the MCI stage over time.

CSF biomarkers correlate with gray matter volume in the precuneus

A negative correlation was found between the first factor (clustering markers of neuronal and synaptic loss, measures of innate immunity (YKL-40) and amyloid processing) and gray matter volume in the precuneus. The variables loading most heavily on factor 1 increase with pathology, hence the directionality of the correlation with higher values of these variables being associated with lower volumes. Atrophy of the precuneus, posterior cingulate cortex and the fusiform gyrus has been reported in asymptomatic subjects who are amyloid-positive (considered as preclinical AD according to (Sperling *et al.* 2011)) (Petrie *et al.* 2009; Jacobs *et al.* 2013; Reiman *et al.* 2012). Atrophy in these regions can already be present in asymptomatic subjects and in patients with amnestic MCI three to five years prior to the diagnosis of AD (Whitwell *et al.* 2007; Pegueroles *et al.* 2017; Gordon *et al.* 2018). Gray matter volume of the precuneus seems to be a robust early

biomarker as it has been shown to predict subsequent whole-brain gray matter volume loss in cognitively healthy individuals over a 6 year follow-up period (Taki *et al.* 2011).

Correlation with gray matter volume and individual CSF marker levels

In a secondary analysis, we assessed the correlation between the individual CSF biomarker values and gray matter volume. We found inverse correlations between gray matter volume in the precuneus and CSF levels of VILIP-1, neurogranin, BACE1, $A\beta_{1-38}$, $A\beta_{1-40}$ and YKL-40 and at a lower significance level for CSF levels of α-synuclein, t-tau and p-tau. Previous studies on VILIP-1 described that cognitively intact individuals with elevated VILIP-1 CSF levels had a higher rate of whole-brain, hippocampal and entorhinal atrophy (Tarawneh *et al.* 2011; Tarawneh *et al.* 2015). CSF VILIP-1 levels are increased at the MCI stage compared to controls and reach a plateau as the disease progresses (Babić Leko *et al.* 2016; Kester *et al.* 2015b). These findings suggest that an earlier assessment at the asymptomatic stage might be most informative to track early neurodegeneration in individuals at risk for AD. CSF levels of neurogranin are also indicative of ongoing neurodegeneration as demonstrated by cortical thinning of the precuneus in CSF $A\beta_{1-42}$-positive asymptomatic older individuals (Pereira *et al.* 2017; Höglund *et al.* 2017). Moreover, both high CSF levels of neurogranin and VILIP-1 can predict conversion from MCI to AD (Kester *et al.* 2015a; Kester *et al.* 2015b). Our findings reveal that biomarker CSF levels correlate with regions of interest early affected in AD, including the precuneus, albeit these regions are integrated in an alternative trajectory than proposed in the classical Braak staging scheme (Braak and Braak 1991). As our study indicates that the gray matter volume in the precuneus correlates with CSF biomarkers related to *pathophysiological* processes occuring in AD, it warrants follow-up studies on the gray matter volume loss in the precuneus as an indicator of incipient AD. It is however interesting to note that of these *pathophysiological biomarkers,* the synaptic biomarker: neurogranin (a synaptic plasticity protein), the APP processing biomarkers BACE1, $A\beta_{1-38}$ and, $A\beta_{1-40}$ (involved in synaptic signaling) as

well as the neuronal calcium sensor protein VILIP-1 are all associated with decreased gray matter volume of the precuneus (Fig. 4a). Based on studies assessing brain network alterations (Jones *et al.* 2016), the precuneus might be characterized by high synaptic activity and receives synaptic input from widespread brain regions. Correlation of these synaptic markers with precuneus volume might indicate that synaptic failure eventually may cause changes in synaptic plasticity, which ultimately results in gray matter volume loss. It is also peculiar that the classical AD biomarkers t-tau and p-tau only correlated at a lower significance level, potentially indicating that the changes in gray matter volume might not yet be as severe and widespread as reflected by highly increased CSF t-tau and p-tau levels seen in clinically probable AD.

AD pathophysiological markers of amyloid load

CSF $A\beta_{1-42}$ levels correlated inversely with [^{18}F]-flutemetamol SUVR in neocortical association areas including the precuneus and posterior cingulate cortex. The inverse correlation between amyloid-PET and CSF $A\beta_{1-42}$ levels is in accordance with a number of previous studies in cognitively healthy elderly subjects (Fagan *et al.* 2009; Landau *et al.* 2013; Leuzy *et al.* 2016). We found that seven out of thirty-eight individuals (18%) cognitively intact individuals with low CSF levels of $A\beta_{1-42}$ were negative on amyloid-PET (i.e. [^{18}F]-flutemetamol $SUVR_{comp} < 1.38$) (Adamczuk *et al.* 2016b). These discordant findings between CSF and amyloid-PET are in agreement with previous findings, describing that 21% of cognitively healthy subjects were discordant on amyloid biomarkers, displaying low CSF levels of $A\beta_{1-42}$ but a negative amyloid-PET scan (Mattsson *et al.* 2015). Our findings additionally show that a cluster of CSF biomarkers reflecting neuronal injury, synaptic loss, amyloidogenic processing, neurofibrillary tangle formation and glial activation (factor 1) correlated with PET-traceable amyloid load in the precuneus, extending to the posterior cingulate cortex, in older cognitively intact individuals at risk for AD. Although most biomarkers loading on factor 1 correlated only weakly with CSF $A\beta_{1-42}$ levels, this cluster of CSF biomarkers did not include $A\beta_{1-42}$. It therefore encourages

further research to position these changes in CSF biomarkers in time, with respect to the $A\beta_{1-42}$ pathological pathway (Bateman *et al.* 2012; Jack *et al.* 2013; Jansen *et al.* 2015). It has been shown that the spatial progression of Alzheimer's disease reflects the healthy brain's intrinsic functional network architecture (Seeley *et al.* 2009; Teipel *et al.* 2016; Warren *et al.* 2013). One of these vulnerable networks is the default mode network, of which the precuneus constitutes an important hub, i.e. a densely connected region (Jones *et al.* 2016). This 'hub' characteristic of the precuneus is suggested to make it a vulnerable region for accumulation and spread of proteinopathy such as β-amyloid to other hubs of high connectivity located mainly in the frontal lobe (Jones *et al.* 2016). As previously mentioned, the precuneus is among the first regions to show amyloid aggregation. This is a highly robust finding which has been replicated by several independent studies (Mintun et al. 2006; Villemagne et al. 2011; Adamczuk et al. 2013; Gordon et al. 2018; Villain et al. 2012). It is hypothesized that the posterior default mode network, to which the precuneus belongs, fails functionally before measurable amyloid plaques occur and appears to initiate a connectivity cascade that continues throughout the disease spectrum (Jones *et al.* 2016). In this perspective, it is relevant to note that the cluster obtained for factor 1 and 2 had different brain locations associated with amyloid load deposition. We speculate that this could possibly relate to the temporal sequence between neurodegeneration and amyloid. If amyloid aggregation precedes neurodegeneration, it is plausible that individual scores on the 'neurodegeneration' factor 1 correlate mostly with the precuneus, given that amyloid load possibly reached already a plateau phase in the precuneus. In that case, we would not expect a correlation with $A\beta_{1-42}$. Amyloid deposition extends in a later phase into the lateral temporal cortex, the occipital lobe and the dorsolateral and medial parts of the prefrontal cortex (Villain *et al.* 2012), a pattern of deposition which was also observed in our cohort, be it cross-sectionally. Hence, it is plausible that neurodegeneration in these regions is not as pronounced yet and that amyloid load has not yet reached a plateau phase in cognitively intact individuals so that a correlation with $A\beta_{1-42}$ can be observed more easily in these regions than in the precuneus.

Analysis of longitudinal data will be needed to examine whether the cluster of those CSF biomarkers could predict cognitive decline, further neurodegeneration and conversion to AD. Furthermore, longitudinal studies will give valuable information on whether this cluster of biomarkers (clustering neuronal and synaptic loss, amyloidogenic processing, tau phosphorylation and glial activation), associated with precuneus volume and amyloid load, could be associated with or could predict the progression of amyloid pathology and of neurodegeneration to other brain regions in later disease stages.

Study limitations

The present study has several limitations: the sample size was relatively small and therefore it was not possible to analyze amyloid-positive and amyloid-negative as separate groups. In fact within the healthy controls, the distribution of amyloid load is more continuous than when a clinical patient group is compared with controls and more individuals have values around the threshold. Also for that reason, it is recommended to treat amyloid load as a continuous variable in this study group rather than a binary variable.

Furthermore, the use of CSF biomarkers does not give further information on how the above-mentioned pathways are influencing each other neurobiologically. Further investigations, in particular longitudinal studies are needed to understand the dynamics of the relationships between these pathophysiological pathways and their predictive value, in asymptomatic individuals for progression to clinically overt AD.

CONCLUSION

The findings in this study describe strong correlations between CSF biomarkers of the main pathophysiological processes of AD in cognitively healthy older adults. These CSF biomarkers are correlated with gray matter volume and amyloid load in regions typically involved early in AD. These CSF markers when used as a cluster are potential promising biomarkers for tracking subtle neurodegeneration at the earliest stages of the disease. This is of particular importance for clinical trials since the modulation of fluid biomarkers that reflect different pathophysiological pathways involved in AD, would give valuable information on the mechanism of action of candidate therapeutics.

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TABLES

Table 1: Demographics, neuropsychological scores and biomarker assessments

	Mean ± SD (range)
n (male/female)	**38** (22/16)
Age (years)	**72** ± 4.7 (65-80)
Education (years)	**13.4** ± 3.1 (8-20)
APOE ε4	**19** (50%)
MMSE (/30)	**29.0** ± 1 (27 – 30)
RPM (/60)	**36.1** ± 9.8 (15 – 53)
AVLT total learning (/75)	**45.8** ± 8.5 (31 – 69)
AVLT % Delayed Recall	**83.7** ± 11.7 (54.5 – 107.7)
Buschke long term storage	**73.9** ± 19.7 (41 – 123)
Buschke total learning	**91.2** ± 13.7 (71 – 129)
Buschke Delayed Recall	**7.1** ± 2.6 (2.0 – 12.0)
BNT (/60)	**53.9** ± 4.3 (41.0 – 60.0)
AVF (#words 1 min)	**23.7** ± 5.6 (14 – 40)
LVF (# words 1 min)	**34.7** ± 11.1 (16 – 64)
PALPA 49	**27.3** ± 1.6 (23 – 30)
VILIP-1 (pg/mL)	**200** ± 65 (91 – 351)
neurogranin trunc p75 (pg/mL)	**424** ± 205 (115 – 937)
α-synuclein (pg/mL)	**2571** ± 814 (1157 – 4141)
BACE1 (pg/mL)	**2363** ± 776 (1068 – 4176)
YKL-40 (ng/mL)	**369** ± 93 (239 – 594)
MCP-1 (pg/mL)	**488** ± 167 (286 – 1302)
MIP-1β (pg/mL)	**14.6** ± 18.2 (5.0 – 121.9)
IL-6 (pg/mL)	**602** ± 1077 (224 – 6684)
Eotaxin-3 (pg/mL) [c, d]	**3.9** ± 1.7 (0.7 – 8.3)

$A\beta_{1\text{-}42}$ (pg/mL) [a]	**996** ± 430 (351 – 1859)
$A\beta_{1\text{-}40}$ (pg/mL)	**8933** ± 2456 (3640 – 13273)
$A\beta_{1\text{-}38}$ (pg/mL)	**2401** ± 654 (1057 – 3505)
t-tau (pg/mL)	**360** ± 134 (126 – 660)
p-tau_{181} (pg/mL)	**56** ± 26 (22 – 132)
[^{18}F]-flutemetamol $SUVR_{comp}$ [b]	**1.342** ± 0.261 (1.089 – 2.265)
CSF Protein content (mg/L)	**486** ± 134 (266 – 850)
CSF Immunoglobulin G (mg/L)	**31** ± 11 (9 – 59)
CSF Albumin (mg/L)	**257** ± 92 (131 – 535)

Significant differences between APOE ε4 carriers and non-carriers ([a]p <0.01; [b]p <0.001). [c]n=37. [d]significant gender difference. **Abbreviations**: Aβ = amyloid-β; AVF = animal verbal fluency; APOE = apolipoprotein E; AVLT = Rey's auditory verbal learning; BNT = Boston naming test; BACE1 = β-secretase amyloid precursor protein cleaving enzyme-1; CSF = cerebrospinal fluid; IL-6 = interleukin-6; LVF = letter Verbal Fluency test; MMSE = mini-mental state examination; MCP-1 = monocyte chemotactic protein-1; MIP-1β = macrophage inflammatory protein-1β; p-tau_{181} = phosphorylated-tau at threonine 181; PALPA = psycholinguistic assessments of language processing in aphasia; RPM = Raven's progressive matrices; $SUVR_{comp}$ = standard uptake value ratio in a composite neocortical region; t-tau = total-tau; VILIP-1 = Visinin-like protein 1; YKL-40 = chitinase-3-like protein 1.

Table 2: Results of the factor analysis on the CSF biomarkers

		Factor 1	Factor 2	Factor 3	Factor 4
	Eigenvalue	7.684	1.885	1.546	1.034
	Variance explained (%)	54.883	13.466	11.040	7.383
	Cumulative Variance explained (%)	54.883	68.349	79.389	86.773
Synaptic, neuronal injury, glial, APP processing markers and p-tau_{181}	t-tau	***0.987***	-0.032	-0.074	-0.006
	α-synuclein	***0.926***	0.318	-0.043	-0.001
	p-tau_{181}	***0.915***	-0.233	-0.157	-0.070
	neurogranin trunc p75	***0.859***	0.370	-0.053	-0.091
	BACE1	***0.830***	0.481	-0.021	-0.120
	VILIP-1	***0.778***	0.511	0.027	0.023
	YKL-40	***0.716***	0.182	0.007	0.075
	$Aβ_{1-38}$	***0.712***	0.649	-0.048	-0.058
	$Aβ_{1-40}$	***0.712***	0.684	-0.043	0.007
Amyloid pathology	$Aβ_{1-42}$	0.250	***0.859***	0.097	0.102
Neuroinflammatory markers	MCP-1	-0.086	-0.048	***0.941***	-0.069
	IL-6	-0.066	-0.113	***0.754***	0.578
	MIP-1β	-0.022	0.040	0.033	***0.799***
	eotaxin-3	-0.006	-0.356	0.093	0.019

Based on 37 individuals due to an out of range value for eotaxin-3 in one individual. **Abbreviations**: Aβ = amyloid-β; BACE1 = β-secretase amyloid precursor protein cleaving enzyme-1; IL-6 = interleukin-6; MCP-1 = monocyte chemotactic protein-1; MIP-1β = macrophage inflammatory protein-1β; p-tau = phosphorylated-tau at threonine 181; t-tau = total-tau; VILIP-1 = visinin-like protein 1; YKL-40 = chitinase-3-like protein 1. The factor loadings can be interpreted as standardized regression coefficients: a high and positive factor loading implies a strong and positive correlation between a variable and that factor.

Table 3: Significant voxel-wise correlations between CSF biomarker levels and imaging measures

	Cluster level		Peak level		MNI coordinates (mm)		
	p	Cluster size	*T*	*Z*	x	y	z
Inverse correlations between CSF biomarkers and GM volume							
VILIP-1	< 0.0001	10233	6.5	5.2	1.5	-52.5	49.5
	< 0.0001	2395	6.08	4.97	-39	-60	-19.5
	0.012	992	5.6	4.68	16.5	-4.5	55.5
	0.004	1255	5.48	4.61	-27	-84	30
	0.019	883	4.51	3.97	4.5	-7.5	22.5
neurogranin	0.032	793	5.19	4.42	-42	-63	-19.5
	0.008	1102	5.05	4.33	18	-4.5	55.5
	0.022	1022	4.67	4.07	3	-54	52.5
BACE1	< 0.0001	6192	6.38	5.14	3	-52.5	52.5
	0.004	1285	5.82	4.82	-40.5	-61.5	-19.5
	0.022	868	5.36	4.53	30	-6	58.5
p-tau$_{181}$	0.017	954	5.1	4.36	-31.5	3	54
Aβ$_{1-40}$	< 0.0001	3003	6.22	5.05	-28.5	-52.5	-18
	< 0.0001	12766	5.98	4.91	22.5	-60	61.5

	0.023	835	4.67	4.08	18	-4.5	57
	0.018	891	4.59	4.02	-27	-82.5	30
$A\beta_{1-38}$	< 0.0001	10528	5.88	4.85	-7.5	-45	72
	< 0.0001	2734	5.84	4.83	-28.5	-54	-18
	0.009	1069	4.61	4.04	-25.5	-75	46.5
YKL-40	0.006	1188	4.36	3.86	0	-48	66
Positive correlations between CSF biomarkers and brain amyloid load							
t-tau	0.0003	579	5.49	4.62	2	-66	14
p-tau_{181}	< 0.0001	11645	7.75	5.84	4	-66	8
	< 0.0001	4501	7.38	5.66	70	-28	-16
	< 0.0001	4132	7.32	5.63	-58	-62	16

Only CSF markers significantly correlating with gray matter (GM) volume or brain amyloid load at a significance threshold of voxel-level uncorrected *P*<0.001 combined with cluster-level family wise error (FWE)-corrected threshold *P*<0.05 are listed in this table. **Abbreviations**: Aβ = Amyloid-β; BACE1 = β-secretase Amyloid Precursor Protein cleaving enzyme-1; MNI = Montreal Neurological Institute; p-tau = phosphorylated-tau at threonine 181; t-tau = total-tau; vilip-1 = Visinin-like protein 1; YKL-40 = Chitinase-3-like protein 1.

Table 4: Correlation matrix of the CSF biomarkers

		Neurogranin trunc p75	α-synuclein	BACE1	VILIP-1	t-tau	p-tau$_{181}$	Aβ$_{1-42}$	Aβ$_{1-40}$	Aβ$_{1-38}$	YKL-40	MCP-1	MIP-1β	IL-6
Synaptic and Neuronal injury markers + p-tau$_{181}$	Neurogranin trunc p75	-	*p*<0.001	*p*<0.001	*p*<0.001	*p*<0.001	*p*<0.001	0.003	*p*<0.001	*p*<0.001	*p*<0.001	n.s.	n.s.	n.s.
	α-synuclein	**0.953**	-	*p*<0.001	*p*<0.001	*p*<0.001	*p*<0.001	0.002	*p*<0.001	*p*<0.001	*p*<0.001	n.s.	n.s.	n.s.
	BACE1	**0.941**	**0.950**	*p*<0.001	*p*<0.001	*p*<0.001	*p*<0.001	*p*<0.001	*p*<0.001	*p*<0.001	*p*<0.001	n.s.	n.s.	n.s.
	VILIP-1	**0.903**	**0.887**	**0.900**	-	*p*<0.001	*p*<0.001	*p*<0.001	*p*<0.001	*p*<0.001	*p*<0.001	n.s.	n.s.	n.s.
	t-tau	**0.918**	**0.91**	**0.862**	**0.820**	-	*p*<0.001	n.s.	*p*<0.001	*p*<0.001	*p*<0.001	n.s.	n.s.	n.s.
	p-tau$_{181}$	**0.832**	**0.827**	**0.764**	**0.691**	**0.928**	-	n.s.	*p*<0.001	*p*<0.001	*p*<0.001	n.s.	n.s.	n.s.
APP processing markers	Aβ$_{1-42}$	**0.468**	**0.477**	**0.533**	**0.631**	0.266	0.064	-	*p*<0.001	*p*<0.001	0.009	n.s.	n.s.	n.s.
	Aβ$_{1-40}$	**0.878**	**0.875**	**0.914**	**0.911**	**0.755**	**0.645**	**0.714**	-	*p*<0.001	*p*<0.001	n.s.	n.s.	n.s.
	Aβ$_{1-38}$	**0.877**	**0.875**	**0.919**	**0.871**	**0.772**	**0.661**	**0.683**	**0.960**	-	*p*<0.001	n.s.	n.s.	n.s.
mmatory markers	YKL-40	**0.689**	**0.745**	**0.733**	**0.703**	**0.674**	**0.556**	**0.418**	**0.678**	**0.688**	-	n.s.	n.s.	n.s.
	MCP-1	-0.161	-0.156	-0.089	-0.064	-0.142	-0.165	0.018	-0.124	-0.124	-0.044	-	n.s.	n.s.

	MIP-1β	0.041	0.127	-0.025	0.018	0.030	-0.002	-0.134	-0.084	-0.130	0.201	0.083	-	n.s.
	IL-6	-0.196	-0.086	-0.149	-0.131	-0.094	-0.085	-0.129	-0.169	-0.226	0.033	0.255	0.247	-

Lower-left: Coefficient of correlation: significant correlations are marked in bold. Top-right: Correlation *p*-value. Bonferroni adjusted-alpha to 0.004 (0.05/13 markers). **Abbreviations**: Aβ = amyloid-β; BACE1 = β-secretase amyloid precursor protein cleaving enzyme-1; IL-6 = interleukin-6; MCP-1 = monocyte chemotactic protein-1; MIP-1β = macrophage inflammatory protein-1β; n.s. = non-significant; p-tau = phosphorylated-tau at threonine 181; t-tau = total-tau; VILIP-1 = visinin-like protein 1; YKL-40 = chitinase-3-like protein 1.

FIGURE LEGENDS

Figure 1: Study design.

Briefly, 180 cognitively healthy individuals were community recruited and underwent extensive neuropsychological examinations, MRI structural imaging and a [^{18}F]-flutemetamol amyloid-PET-scan at inclusion. Among those, 38 individuals were included in an optional substudy and additionally underwent a lumbar puncture.

Figure 2: Factor 1 correlation with gray matter volume in cognitively healthy individuals.

(a). *T-maps overlaid on sagittal slices of an MNI template brain (Rorden et al. 2007) describing the whole-brain voxel-wise correlation between weighted factor 1 scores clustering the CSF biomarkers (t-tau, α-synuclein, p-tau$_{181}$, neurogranin, BACE1, VILIP-1, Aβ$_{1-40}$, Aβ$_{1-38}$ and YKL-40) and modulated gray matter volume maps, with age and gender as nuisance variables. The brighter the colour, the stronger the correlation.* ***(b).*** *The scatterplot shows the inverse correlation between weighted factor 1 scores and the extracted modulated gray matter volume values of the significant cluster in the precuneus depicted in (a). The plot is for illustrative purposes only with the objective to emphasize the origin of the significant results from the whole-brain voxel-wise regression analysis. Number of individuals = 37.* ***Abbreviations****: A.U. = arbitrary unit.*

Figure 3: Correlation between factor 1 and 2 with amyloid load in cognitively healthy individuals.

(a). *T-maps overlaid on sagittal slices of an MNI template brain showing the whole-brain voxel-wise correlation between weighted factor 1 scores clustering the CSF biomarkers (t-tau, α-synuclein, p-tau$_{181}$, neurogranin, BACE1, VILIP-1, Aβ$_{1-40}$, Aβ$_{1-38}$ and YKL-40) and amyloid load as measured on PVC [^{18}F]-flutemetamol SUVR images, corrected for age and gender.* ***(b).*** *The scatterplot shows the positive correlation between weighted factor 1 scores and the extracted [^{18}F]-flutemetamol SUVR values of the significant cluster in the precuneus, extending into the posterior cingulate depicted in (a).* ***(c).*** *T-maps overlaid on sagittal slices of an MNI template brain showing the whole-brain voxel-*

wise correlation between weighted factor 2 scores clustering CSF levels of $A\beta_{1-42}$, and amyloid load as measured on PVC [^{18}F]-flutemetamol SUVR images, corrected for age and gender. ***(d).*** *The scatterplot shows the inverse correlation between the weighted factor 2 scores and the extracted [^{18}F]-flutemetamol SUVR values of the significant cluster depicted in (c).* Significant differences between APOE ε4 carriers and non-carriers (*p* = 0.0072). *The brighter the colour, the stronger the inverse correlation. Number of individuals = 37.* ***Abbreviations****: SUVR = standardized uptake value ratio.*

Figure. 4: Correlation between individual CSF markers and imaging measures in cognitively healthy subjects.

T-maps overlaid on sagittal slices and on a rendered MNI template brain describing the whole-brain voxel-wise correlation between the CSF levels of VILIP-1, neurogranin, BACE1, $A\beta_{1-40}$, $A\beta_{1-38}$, YKL-40, t-tau and p-tau and ***(a)*** *modulated gray matter volume maps or* ***(b)*** *brain amyloid load, corrected for age and gender.* ***Abbreviations****:* Aβ = amyloid-β; *BACE1* = β-secretase Amyloid Precursor Protein cleaving enzyme-1; *t- p-tau = phosphorylated tau at threonine 181; tau = total-tau; VILIP-1 = visinin-like protein 1; YKL-40 =* Chitinase-3-like protein 1.

Graphical Abstract

The cerebrospinal fluid levels of markers reflecting synaptic and neuronal injury, amyloidogenic processing and neuroinflammation were assessed in 38 cognitively healthy subjects at risk for developing Alzheimer's disease (AD). Our study show that by means of a factor analysis, markers reflecting the above-mentioned pathophysiological processes ongoing in AD share a common variance correlating with gray matter volume as assessed with structural magnetic resonance imaging and brain amyloid deposition as assessed with amyloid positron emission tomography imaging in regions typically involved early in AD.

Abbreviations: *Aβ = amyloid-β; BACE1 = β-secretase amyloid precursor protein cleaving enzyme-1; IL-6 = interleukin-6; MCP-1 = monocyte chemotactic protein-1; MIP-1β = macrophage inflammatory protein-1β; MRI = magnetic resonance imaging; PET = positron emission tomography; p-tau = phosphorylated-tau at threonine 181; t-tau = total-tau; VILIP-1 = visinin-like protein 1; YKL-40 = chitinase-3-like protein 1.*