Association of CSF α-synuclein and tau protein concentrations with amyloid mean cortical standard uptake value ratios in preclinical subjective memory complainers stratified by Alzheimer’s disease biomarkers: the INSIGHT-preAD study

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** The full list of members of the INSIGHT-preAD study group is reported in the Acknowledgement section.

**Abbreviations:**

α-syn, alpha-synuclein; Aβ, amyloid-beta; Aβ₁₋₄₂, 42-amino acid-long amyloid beta peptide; Aβ PET, amyloid positron emission tomography; AD, Alzheimer’s disease; APMI, Alzheimer Precision Medicine Initiative; APMI-CP, Alzheimer Precision Medicine Initiative Cohort Program; APOE, Apolipoprotein E; CBS, corticobasal syndrome; CDR, Clinical Dementia Rating scale; CSF, cerebrospinal fluid; DLB, dementia with Lewy bodies; FCSRT, Free and Cued Selective Rating Test; ¹⁸F-FDG-PET, ¹⁸F-2-fluoro-2-deoxy-D-glucose PET; HC, healthy controls; LB, Lewy bodies; LP, lumbar puncture; MCI, mild cognitive impairment; MMSE, Mini-Mental State Examination; MRI, magnetic resonance imaging; MSA, multiple system atrophy; ND, neurodegenerative diseases; NIA-AA, National Institute on Aging-
Alzheimer’s Association; p-tau$_{181}$, hyperphosphorylated tau at Threonine site 181; PD, Parkinson disease; PDD, PD with dementia; SMC, subjective complaint of memory dysfunction; SUVR, standard uptake value ratios; t-tau, total tau.
ABSTRACT

Alpha-synuclein (α-syn) plays a key role in regulating the synaptic vesicles pool size, trafficking, and membrane dynamics. Misfolded forms of α-syn undergo post-translational modifications showing high tendency towards aggregation and deposition into insoluble inclusion bodies, Lewy bodies (LB).

Several brain proteinopathies with neurodegeneration, including Alzheimer’s disease (AD), typically exhibit deposition and spreading of LB in a prion-like fashion.

Studies performed to assess α-syn diagnostic accuracy in cerebrospinal fluid (CSF) have reported a trend towards increased CSF α-syn concentrations in AD versus other neurodegenerative diseases (ND) and healthy controls (HC).

The potential role of CSF α-syn in asymptomatic subjects at risk of AD has not been explored. We performed a cross-sectional study in a large-scale monocentric preclinical at risk cohort (INSIGHT-preAD). We found a positive association between CSF α-syn concentrations and amyloid mean cortical standard uptake value ratios (SUVR), even after adjusting for confounders. There were positive associations of CSF α-syn and CSF t-tau and p-tau_{181} proteins. Furthermore, we found a trend to statistical significance of increased CSF α-syn concentrations in Aβ PET-positive compared with Aβ PET-negative subjects.

Animal models have shown that α-syn may synergistically and directly induce fibrillization of both tau and Aβ. Therefore, the main findings of this study indicate an association of CSF α-syn with AD-related pathophysiological mechanisms, during the preclinical phase of the disease.

Longitudinal studies with larger sample size are needed to assess whether increased CSF α-syn concentrations could depict longitudinal trajectories of asymptomatic subjects at risk for AD.

KEY WORDS

α-synuclein, Alzheimer’s disease, cerebrospinal fluid, subjective memory complainers, preclinical, monocentric, amyloid PET, tau protein, synergistic, SUVR.
INTRODUCTION

Alpha-synuclein (α-syn) is a protein assumed to play a role in the pre-synaptic modulation of cell vesicle trafficking (Wong and Krainc, 2017; Fang et al., 2017). In particular, α-syn binds to specific presynaptic proteins directly involved in the release of neurotransmitters and it preserves the synaptic terminals, both at structural and at functional level (Wong and Krainc, 2017; Fang et al., 2017). Hyperphosphorylated misfolded α-syn proteins, deposited in the brain as insoluble fibrillary aggregates, generate neuronal cytoplasmic inclusions, namely Lewy bodies (LB) (Colom-Cadena et al., 2017) which are pathophysiological hallmarks of several brain proteinopathies with neurodegeneration – including Parkinson disease (PD), PD with dementia (PDD), dementia with Lewy bodies (DLB) – and oligodendroglial cytoplasmic inclusions – typically found in multiple system atrophy (MSA) – all belonging to the synucleinopathy spectrum (Goedert, 2015; Kordower et al., 2008).

Hence, α-syn concentrations have been assessed in cerebrospinal fluid (CSF), especially as potential surrogate of cerebral LB deposition, to discriminate in vivo among healthy controls (HC), PD, and atypical parkinsonian syndromes (Eusebi et al., 2016; Hong et al., 2010; Kasuga et al., 2010; Mackin et al., 2015; Zhou et al., 2015).

Moreover, the cellular localization and function of α-syn suggest a potential role as surrogate biomarker of synaptic loss also in non-synucleophatic neurodegenerative diseases (ND), such as AD. However, in spite of numerous research efforts, a general consensus on the relevance of this biomarker candidate in the diagnostic/prognostic workflow of ND is still under debate (Slaets et al., 2014; Wang et al., 2015).

Several studies reported higher CSF α-syn concentrations in AD patients versus both individuals suffering from other ND and HC. However, these results are conflicting, probably due to substantial inter-site methodological differences (Hansson et al., 2014; Mattsson et al., 2013; Slaets et al., 2014; Wang et al., 2015). These include different pre-intra-analytical procedures, performed for CSF α-syn assessment, and different recruitment criteria. Previous studies exploring CSF α-syn concentrations in AD were performed in dementia patients or subjects with prodromal (mild cognitive impairment [MCI]) forms of the disease (Hansson et al., 2014; Slaets et al., 2014; Wang et al., 2015).

To the best of our knowledge, no studies examined the potential role of CSF α-syn in the asymptomatic preclinical phase of AD (Dubois et al., 2016).

Individuals with subjective complaints of memory dysfunction (SMC), together with evi-
dence of cerebral deposition of amyloid beta (Aβ), are considered asymptomatic individuals at risk of developing AD (Dubois et al., 2016). Hence, the aim of the study was to cross-sectionally investigate the variations of CSF α-syn concentrations in relation to the pathophysiological mechanisms of AD in a subset of a preclinical cohort.

**MATERIALS AND METHODS**

**Study participants**

This research is designed as a monocenter, cross-sectional study in a subset of 36 participants with SMC recruited from the “INveStIGation of AlzHeimer’s PredicTors in Subjective Memory Complainers” (INSIGHT-preAD) study, a French mono-centric academic university-based cohort which is part of the Alzheimer Precision Medicine Initiative Cohort Program (APMI-CP), (Hampel H et al., 2017). Participants were enrolled at the Institute of Memory and Alzheimer’s disease (Institut de la Mémoire et de la Maladie d’Alzheimer, IM2A) at the Pitié-Salpêtrière University Hospital in Paris, France. The main goal of the INSIGHT-preAD study is to investigate the earliest preclinical stages of AD and its development, including influencing factors and biomarkers of progression.

The INSIGHT-preAD study includes 318 cognitively normal Caucasian individuals, recruited from the community in the wider Paris area, France, aged 70 to 85, with SMC. The status of SMC is confirmed as follows: (I) participants gave an affirmative answer (“YES”) to both questions: “Are you complaining about your memory?” and “Is it a regular complaint that has lasted now more than 6 months?”; (II) participants presented intact cognitive functions based on Mini-Mental State Examination score (MMSE, ≥ 27), Clinical Dementia Rating scale (CDR = 0), and Free and Cued Selective Rating Test (FCSRT, total recall score ≥ 41).

Aβ positron emission tomography (Aβ-PET) investigation is performed at baseline visit, as mandatory study inclusion criterion. Thus, all subjects enrolled into the study have SMC and are stratified as either positive or negative for cerebral Aβ deposition.

Briefly, exclusion criteria are represented by the absence of history of neurological or psychiatric diseases.

At the point of study inclusion, several data are collected such as, demographic data and Apolipoprotein E (APOE) genotype as well.
The study was conducted in accordance with the tenets of the Declaration of Helsinki of 1975 and approved by the local Institutional Review Board at the participating center. All participants or their representatives gave written informed consent for the use of their clinical data for research purposes.

For the present study, we included 36 subjects that volunteered for the lumbar puncture (LP) at baseline. It has been previously reported that CSF Aβ and Aβ-PET have comparable diagnostic performance in detecting cerebral Aβ deposition, at preclinical or prodromal stages of AD (Palmqvist et al., 2015). Thus, CSF Aβ was not included in our analyses due to the its high degree of intercorrelation with PET data.

**CSF sampling**

A LP was performed at baseline in all 36 participants of the cohort subset. All CSF samples included were collected in polypropylene tubes and centrifuged at 1,000 g for 10 min at +4°C. The collected supernatant was aliquoted and stored at –80°C pending biochemical analysis.

**Immunoassays for CSF core biomarkers**

CSF analyses of the core feasible biomarkers were performed at the Laboratory of Biochemistry, Unit of Biochemistry of Neurometabolic diseases, Pitié-Salpêtrière University Hospital of Paris. CSF total tau (t-tau), tau phosphorylated at Threonine site 181 (p-tau$_{181}$) and Aβ$_{1-42}$ concentrations, were measured using established sandwich ELISA methods, namely the INNOTEST hTAU-Ag, INNOTEST Phospho-Tau[181P] (Fujirebio Europe NV, Gent, Belgium) and INNOTEST β-AMYLOID(1-42), respectively (Blennow et al., 1995; Vanmechelen et al., 2000; Vanderstichele et al., 2000) All CSF analyses were performed by board-certified laboratory technicians blinded to clinical information.

**Immunoassay for CSF α-syn**

All CSF α-syn analyses were performed at the Clinical Neurochemistry Laboratory at the Sahlgrenska University Hospital, Mölndal, Sweden. CSF α-syn protein concentration was measured using the U-PLEX Human α-syn Singleplex immunoassay kit (Meso Scale Discovery, Rockville, MD, US), according to the manufacturer’s instructions (available at https://www.mesoscale.com/en/products/u-plex-human-alpha-synuclein-kit-k151wkk/). The assay consists of a rabbit monoclonal capture antibody coupled with a mouse monoclonal antibody for detection. The lower limit of quantification was 84 pg/mL. All CSF analyses
were performed on one occasion with randomized samples using one batch of reagents by board-certified laboratory technicians blinded to clinical information to avoid bias.

**PET acquisition**

All Florbetapir-PET scans are acquired in a single session on a Philips Gemini GXL CT-PET scanner 50 (± 5) minutes after injection of approximately 370 MBq (333-407 MBq) of Florbetapir. PET acquisition consists of 3 x 5 minutes frames, a 128 x 128 acquisition matrix and a voxel size of 2 x 2 x 2 mm³. Images are then reconstructed using iterative LOR-RAMLA algorithm (10 iterations), with a smooth post-reconstruction filter. All corrections (attenuation, scatter, and random coincidence) are integrated in the reconstruction. Lastly, frames are realigned, averaged and quality-checked by the CATI team. CATI is a French neuroimaging platform funded by the French Plan Alzheimer (available at [http://cati-neuroimaging.com](http://cati-neuroimaging.com)).

**PET data processing**

Reconstructed PET images are analyzed with a pipeline developed by CATI. A standard uptake value ratio (SUVR) with a threshold of 0.7918 has been used to categorize our population in Aβ positive or Aβ negative according to a method previously described (Habert et al., 2017).

**Statistical analysis**

Demographic characteristics, baseline CSF and imaging characteristics, and scores on neuropsychological tests of the analyzed participants are provided in Table 1. Continuous variables were described by the median and interquartile ranges.

Differences between the Aβ-PET positive and negative groups in terms of CSF concentrations of core feasible biomarkers and α-syn were explored assuming non-normal distribution. Thus, a Wilcoxon-Mann-Whitney pairwise comparison test was performed.

We then performed regression analysis preceded by logarithmic transformation of all biomarkers in order to approximate assumptions of normality and hence remain within the assumptions of linear regression. Associations between log-transformed CSF biomarker concentrations and log-transformed Aβ-PET global SUVR values were tested with a series of univariate linear regressions (see Table 2). They were conducted to determine the influence of tau (both total and phosphorylated), Aβ-PET global SUVR on α-syn values including age and sex as covariates.
To follow, and in order to establish the independent contribution of each biomarker to the prediction of group, a multivariate analysis was carried out (with bootstrapped P values included in Table 3). Model 3a approximated α-syn with t-tau + SUVR + covariates; model 3b, α-syn with p-tau181 + SUVR covariates (see Table 3). Finally, a binary logistic regression was executed setting the PET status as the outcome variable and CSF t-tau, p-tau181, and α-syn as predictive factors (see Table 4).

All tests performed were two tailed and with a significance set at P < 0.05.

All statistics are performed using R (v. 3.2.3, The R Foundation for Statistical Computing).

RESULTS

Comparisons between groups according to the PET status
The median (range) age was 76 (72.5-77) years and the sex ratio was well balanced (18:18) in the whole subset (see Table 1). Subjects were dichotomized according to the Aβ-PET status, either positive (N=8) or negative (N=28), which was identified as the primary outcome. Demographic and clinical data of subjects are shown in Table 1. Hence, we performed comparisons between the two groups. Notably, Aβ-PET positive participants scored an educational level higher than those with negative Aβ-PET (see Table 1). A significant difference was also found when comparing the two groups for sex ratio (see Table 1).

CSF concentrations of p-tau181 and t-tau were significantly different between positive and negative Aβ-PET individuals, with the former showing increased concentrations of both p-tau181 and t-tau (p=0.003 and p=0.005 respectively) (see Table 1).

No significant difference in terms of CSF α-synuclein concentrations was found between Aβ PET-positive and Aβ PET-negative subjects.

Univariate linear regression analysis of CSF α-syn predictive factors:
The univariate linear regression models including age and sex as covariates showed that CSF t-tau, CSF p-tau181, and global SUVR were all significantly associated with CSF α-syn (β=0.72 (0.14), p<0.001; β=0.52 (0.17), p=0.004; β =1.31(0.37), p=0.001, respectively) (for more details, see Table 2 and Figure 1).

Multivariate linear regression analysis of CSF α-syn predictive factors
The multivariate linear regression model, including both global SUVR and CSF t-tau, showed that an increase of one unit of CSF t-tau concentration resulted in a significant increase of 0.71 (0.08) pg/mL \((p<0.001)\) in CSF \(\alpha\)-syn concentration, after adjusting for age and sex. This model is accurate with an adjusted R\(^2\)-squared of 0.80. (for more details, see Table 3).

At a lesser extent, a similar arrangement, including global SUVR and CSF p-tau\(_{181}\) instead of CSF t-tau, resulted into a model in which an increase of one unit of CSF p-tau\(_{181}\) concentration lead to a significant increase of 0.48 (0.12) pg/mL \((p<0.01)\) in CSF \(\alpha\)-syn concentration, after adjusting for age and sex (see Table 3).

We decided not to include CSF p-tau\(_{181}\) and CSF t-tau together in the same model given the existence of a high degree of collinearity between the two variables, which notoriously makes model estimation unstable (data not shown).

**Logistic regression analysis for PET status**

The regression for CSF \(\alpha\)-syn was significant with a positive odds ratio indicating that greater values of the marker are more likely to explain an increased cerebral A\(\beta\) load. The same was found for t-tau and p-tau\(_{181}\) (see Table 4).

**DISCUSSION**

Using a cross-sectional study design in a large monocentric cohort (INSIGHT-preAD) – within the framework of the APMI as part of the APMI-CP – we found a positive association between CSF \(\alpha\)-syn concentrations and mean cortical SUVR in asymptomatic subjects at risk of AD. This association was confirmed using multivariate analysis after adjusting for age and sex. Emerging evidences from pathological studies suggest that about 10–40\% of AD patients showed concomitant brain LB deposition (Hyman et al., 2012; Rabinovici et al., 2017; Schneider et al., 2009). Additionally, cerebral A\(\beta\) pathology is a common finding in synucleinopathies especially in DLB individuals (Donaghy et al., 2015; Kovacs et al., 2013).

Recently, the existence of an anti-A\(\beta\) deposition effect of \(\alpha\)-syn has been proposed in a mouse model of AD (Bachhuber et al., 2015). This observation, if confirmed in humans, might provide novel insights on potential targets for precise pathomechanistic therapies of AD and synucleinopathies.

Although we found a trend of increased CSF \(\alpha\)-syn concentrations in A\(\beta\) PET-positive compared with A\(\beta\) PET-negative subjects, these values did not reach statistical significance likely due to the relatively small sample size. To our knowledge, this is a novel finding in
asymptomatic at risk subjects for AD. Previous studies also explored the diagnostic value of CSF α-syn concentrations – alone or in combination with the CSF core feasible biomarkers Aβ1-42, t-tau, and p-tau181 –differentiating a large spectrum of ND, including AD (Hansson et al., 2014; Mattsson et al., 2013; Slaets et al., 2014; Wang et al., 2015). Although some results are still controversial, the majority of the studies reported increased CSF α-syn concentrations in AD compared with other ND and HC (Slaets et al., 2014; Wang et al., 2015). Discrepancies emerging from these data might be attributable to a high degree of inter-site variability and to analytical and methodological differences, such as the CSF measurement of either the full-size protein or specific oligomers of α-syn (Slaets et al., 2014; Wang et al., 2015; Eusebi P et al., 2016). Furthermore, most of the investigations lack of a reliable HC group (Slaets et al., 2014; Wang et al., 2015; Eusebi P et al., 2016).

Furthermore, we disclosed a positive association between CSF α-syn and CSF t-tau and p-tau181, both using univariate and in multivariate analyses. This finding is consistent with those emerging from investigations performed in mouse models and in humans. In general, the brain extracellular increase of both tau and α-syn concentrations is related to the concomitant neuronal loss and the increased level of phosphorylation preceding the aggregation process leading to LB and neurofibrillary tangles, respectively (Wong and Krainc, 2017). Indeed, hyperphosphorylation is a post-transcriptional modification mechanism common to several misfolded proteins accumulating in the brain, including α-syn (Gassowska et al., 2014; Hebron et al., 2013). In particular, phosphorylation at S129 (pS129) is the most common alteration characterizing this protein in its fibrillar aggregates. Interestingly, the increase of both CSF α-syn and tau protein concentrations might be considered an early biomarker reflecting different pathophysiological mechanisms leading to neurodegeneration, in particular synaptic degeneration and neuronal death, respectively. In this regard, CSF α-syn concentrations in AD are also tightly associated with other neurodegeneration surrogates such as grey matter atrophy and cerebral hypometabolism, measured using magnetic resonance imaging (MRI) and 18F-2-fluoro-2-deoxy-D-glucose PET (18F-FDG-PET) (Hansson et al., 2014; Mattsson et al., 2013; Slaets et al., 2014; Wang et al., 2015). Notably, since α-syn is involved in glutamatergic neuronal transmission, the hippocampal atrophy, an early feature of AD pathophysiology, might explain the increased concentrations of CSF α-syn in AD patients (Ohrfelt et al., 2009; Toledo et al., 2016; Wong and Krainc, 2017; Goedert, 2015). Finally, a possible synergistic link between α-syn and tau protein byproducts on neurodegeneration has been suggested (Ciaccioli et al., 2013; Daniele et al., 2017). Such an interaction is supposed to facilitate the spreading of LB and the deposition of neurofibrillary tangles activated by an imbalance between brain ki-
nases and phosphatases (Ciaccioli G et al., 2013; Gąssowska M et al., 2014; Wong and Krainc, 2017)

This study presents some caveats. First of all, the sample size is relatively limited. Second, given that this is a cross-sectional study and longitudinal data are not yet available, it is not possible to state whether increased CSF α-syn concentrations predict AD or other ND, such as, DLB onset. Moreover, structural MRI analyses, which are useful to confirm the presence of direct cerebral evidences of neurodegeneration, were not reported.

In summary, we found that increased CSF α-syn concentrations are potentially associated with early AD pathophysiology – in terms of both amyloid- and tau-related pathophysiological mechanisms – during the asymptomatic stage of the disease. Longitudinal studies with larger sample size are needed to assess whether increased concentrations of CSF α-syn could represent a predictive surrogate outcome of cognitive impairment and neurodegeneration in asymptomatic at risk of AD subjects. This in turn, will allow to depict different longitudinal molecular trajectories underpinning apparently similar phenotypes.

In conclusion, we believe that CSF α-syn could represent an additional molecular candidate biomarker to be integrated in the expanding biomarker array needed to accurately stratify cohorts (biomarker-guided) of individuals according to existing and relevant AD- and other ND-associated pathophysiological pathways. From a translational perspective, this enhanced biomarker guidance is expected to substantially optimize the basis to develop and enhance effective targeted therapeutic strategies for the efficient treatment of the individual subject, in line with the evolving precision medicine paradigm (Hampel et al., 2017, 2016, 2018, 2018). Supplementary investigations will be essential to establish whether CSF α-syn may be utilized as a biological indicator of mechanism of action and/or target engagement or even as a biological marker to predict the progression of cognitive decline in drug development analyses. Indeed, increasingly accurate guideposts are necessary both to identify the disease at its earliest preclinical stages and to commence treatment strategies of specific pathophysiological mechanisms via biomarker-guided targeted therapy trials.
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CONFLICT OF INTERESTS
HZ and KB are co-founders of Brain Biomarker Solutions in Gothenburg AB, a GU Ventures-based platform company at the University of Gothenburg. HZ has served at advisory boards of Eli Lilly and Roche Diagnostics and has received travel support from Teva.
MOH has received consultant’s honoraria from GE Healthcare, AVID-LILLY and PI-RAMAL.
BD reports personal fees from Eli Lilly and company.
SL received lecture honoraria from Roche.
HH serves as Senior Associate Editor for the Journal Alzheimer’s & Dementia; he received lecture fees from Eisai, Biogen and Roche, research grants from Pfizer, Avid, and MSD Avenir (paid to the institution), travel funding from Axovant, Eli Lilly and company, Takeda and Zinfandel, GE-Healthcare and Oryzon Genomics, consultancy fees from Jung Diagnostics, Cytox Ltd., Axovant, Anavex, Takeda and Zinfandel, GE Healthcare and Oryzon Genomics, and participated in scientific advisory boards of Axovant, Eli Lilly and company, Cytox Ltd., GE Healthcare, Takeda and Zinfandel, Oryzon Genomics and Roche Diagnostics.
HH is co-inventor in the following patents and has received no royalties:
• In Vitro Multiparameter Determination Method for The Diagnosis and Early Diagnosis of Neurodegenerative Disorders Patent Number: 8916388
• In Vitro Procedure for Diagnosis and Early Diagnosis of Neurodegenerative Diseases Patent Number: 8298784
• Neurodegenerative Markers for Psychiatric Conditions Publication Number: 20120196300
• In Vitro Multiparameter Determination Method for The Diagnosis and Early Diagnosis of Neurodegenerative Disorders Publication Number: 20100062463
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REFERENCES


Hansson, O., Hall, S., Ohrfelt, A., Zetterberg, H., Blennow, K., Minthon, L., Nagga,


https://doi.org/10.1136/jnnp.2009.197483


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https://doi.org/10.1212/WNL.0000000000001991

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## TABLES

**Table 1.** Demographic and clinical data of subjects stratified by amyloid PET status.

<table>
<thead>
<tr>
<th></th>
<th>Total sample</th>
<th>PET negative</th>
<th>PET positive</th>
<th>Statistic test, p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (M/F)</td>
<td>36 (18/18)</td>
<td>28 (10/18)</td>
<td>8 (8/0)</td>
<td>χ², p = 0.005*</td>
</tr>
<tr>
<td>Age at time of CSF collection (yrs)</td>
<td>76.0 [72.5-77]</td>
<td>75.5 [72-77]</td>
<td>76.0 [75.3-77.3]</td>
<td>W, p = 0.49</td>
</tr>
<tr>
<td>Education (/8)</td>
<td>8.0 [5.0-8.0]</td>
<td>8.0 [7.0-8.0]</td>
<td>4.5 [3.8-6.0]</td>
<td>W, p = 0.003*</td>
</tr>
<tr>
<td>CSF biomarkers</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p-tau₁₈₁ (pg/mL)</td>
<td>55 [39-64]</td>
<td>48.25 [35.50-58.25]</td>
<td>68.0 [59.25-85.25]</td>
<td>W, p = 0.003*</td>
</tr>
<tr>
<td>t-tau (pg/mL)</td>
<td>332 [259-411]</td>
<td>304.5 [227.0-377.0]</td>
<td>510.5 [334.2-597.5]</td>
<td>W, p = 0.005*</td>
</tr>
<tr>
<td>Aβ₁₋₄₂ (pg/mL)</td>
<td>888 [663-1596]</td>
<td>975.5 [690.5-1151]</td>
<td>659.0 [545.5-680.5]</td>
<td>W, p = 0.002*</td>
</tr>
<tr>
<td>α-syn (pg/mL)</td>
<td>460 [363-566]</td>
<td>451.5 [333.5-524.8]</td>
<td>555.0 [456.8-625.0]</td>
<td>W, p = 0.08</td>
</tr>
<tr>
<td>APOE ε4, n (0/1)</td>
<td>36 (27/9)</td>
<td>28 (23/5)</td>
<td>8 (4/4)</td>
<td>χ², p = 0.16</td>
</tr>
<tr>
<td>Global SUVR</td>
<td>0.71 [0.68-0.83]</td>
<td>0.700 [0.668-0.720]</td>
<td>0.970 [0.950-1.040]</td>
<td>W, p &lt; 0.001*</td>
</tr>
</tbody>
</table>

**Notes.** Quantitative demographic and clinical characteristics (at time of CSF collection) are expressed as median and [interquartile].

* *Statistical tests are presented as type of test performed test, p value: significant level p < 0.05, two tailed. The * symbol refers to the presence of statistical significance.

**Abbreviations:** α-syn, α-synuclein; Aβ₁₋₄₂, 42-amino acid-long amyloid beta peptide; CSF, cerebrospinal fluid; M, male; F, female; PET, positron emission tomography; t-tau, total tau; p-tau₁₈₁, hyperphosphorylated tau at Threonine site 181; Apolipoprotein E ε4 carrier, APOE ε4; SUVR, mean standardized uptake value ratio; χ²: Chi square test; W: Wilcoxon-Mann-Whitney pairwise comparison
Table 2. Univariate linear regression analysis with predictive factors of the CSF \( \alpha \)-synuclein concentrations.

<table>
<thead>
<tr>
<th>Covariate by Model (Adjusted R(^2) Value)</th>
<th>Estimate ( \beta )</th>
<th>Standard error</th>
<th>( p ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1 (=0.297)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td>3.662</td>
<td>1.318</td>
<td>0.009*</td>
</tr>
<tr>
<td>Log global SUVR</td>
<td>1.312</td>
<td>0.368</td>
<td>0.001*</td>
</tr>
<tr>
<td>Model 2a (=0.248)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td>2.457</td>
<td>1.394</td>
<td>0.088</td>
</tr>
<tr>
<td>Log CSF p-tau(_{181})</td>
<td>0.523</td>
<td>0.167</td>
<td>0.004*</td>
</tr>
<tr>
<td>Model 2b (=0.462)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td>1.773</td>
<td>1.194</td>
<td>0.147</td>
</tr>
<tr>
<td>Log CSF t-tau</td>
<td>0.715</td>
<td>0.139</td>
<td>&lt; 0.001*</td>
</tr>
</tbody>
</table>

**Notes.** Logarithmic transformation of CSF variables was used to reduce the skewness of distribution. \( p \) -value: significant level \( p < 0.05 \), two tailed. The * symbol refers to the presence of statistical significance. Each model is adjusted for age and sex.

**Abbreviations:** \( \alpha \)-syn, \( \alpha \)-synuclein; CSF, cerebrospinal fluid; Log, Logarithmic transformation; t-tau, total tau; \( p \)-tau\(_{181}\), hyperphosphorylated tau at Threonine site 181; SUVR, mean standardized uptake value ratio
Table 3. Predictive factors of the CSF α-synuclein concentration: a multivariate analysis.

<table>
<thead>
<tr>
<th>Covariate by Model (Adjusted R² Value)</th>
<th>Estimate β (95% CI)</th>
<th>Standard error</th>
<th>p value</th>
<th>Bootstrapped CI 95%</th>
<th>Bootstrapped P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 3a (0.8001)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td>1.825</td>
<td>0.569</td>
<td>0.003</td>
<td>[1.491; 2.186]</td>
<td>0.002</td>
</tr>
<tr>
<td>Log CSF t-tau</td>
<td>0.705</td>
<td>0.077</td>
<td>0.000*</td>
<td>[0.644; 0.758]</td>
<td>0.000</td>
</tr>
<tr>
<td>Log global SUVR</td>
<td>-0.064</td>
<td>0.188</td>
<td>0.734</td>
<td>[-0.209; 0.078]</td>
<td>0.766</td>
</tr>
<tr>
<td>Model 3b (0.5085)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td>2.638</td>
<td>0.872</td>
<td>0.005</td>
<td>[2.241; 2.975]</td>
<td>0.002</td>
</tr>
<tr>
<td>Log CSF p-tau₁₈₁</td>
<td>0.479</td>
<td>0.119</td>
<td>0.000*</td>
<td>[0.33; 0.733]</td>
<td>0.009</td>
</tr>
<tr>
<td>Log global SUVR</td>
<td>0.296</td>
<td>0.285</td>
<td>0.307</td>
<td>[0.033; 0.462]</td>
<td>0.344</td>
</tr>
</tbody>
</table>

Notes. Log transformation of CSF variables was used to reduce the skewness of distribution. p value: significant level p < 0.05, two tailed. The * symbol refers to the presence of statistical significance. The model is adjusted for age and sex.

Abbreviations: α-syn, α-synuclein; CSF, cerebrospinal fluid; PET, positron emission tomography; t-tau, total tau; p-tau₁₈₁, hyperphosphorylated tau at Threonine site 181; SUVR, mean standardized uptake value ratio; Log, log transformation
Table 4. Predictive factors of the amyloid PET status: a binary logistic regression analysis.

<table>
<thead>
<tr>
<th>Covariate</th>
<th>Model 1 OR [95% CI]</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Log CSF α-syn</td>
<td>1.000 [1.000—1.002]</td>
<td>0.005*</td>
</tr>
<tr>
<td>Log CSF p-tau₁₈₁</td>
<td>1.474 [1.110—1.957]</td>
<td>0.011*</td>
</tr>
<tr>
<td>Log CSF t-tau</td>
<td>1.537 [1.194—1.980]</td>
<td>0.002*</td>
</tr>
</tbody>
</table>

Notes. Logarithmic transformation of CSF variables was used to reduce the skewness of distribution. p value: significant level p < 0.05, two tailed. The * symbol refers to the presence of statistical significance. The model is adjusted for age and sex.

Abbreviations: α-syn, α-synuclein; CSF, cerebrospinal fluid; PET, positron emission tomography; t-tau, total tau; p-tau₁₈₁, hyperphosphorylated tau at Threonine site 181; Log, Logarithmic transformation

FIGURE LEGENDS

Figure 1. Plots showing the association between CSF α-synuclein and global SUVR, CSF α-synuclein and CSF t-tau, and CSF α-synuclein and CSF p-tau₁₈₁: the univariate analysis.

Notes. For each curve, β slope and standard deviation (SD) are indicated with respective p-value (significant level p < 0.05) adjusted for age and sex.

Abbreviations: α-syn, α-synuclein; CSF, cerebrospinal fluid; p-tau, hyperphosphorylated tau at Threonine site 181; t-tau, total tau; SUVR, standard uptake value ratios; Log, Logarithmic transformation.