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Association of cerebrospinal fluid α -synuclein with total and phospho-tau₁₈₁ protein concentrations and brain amyloid load in cognitively normal subjective memory complainers stratified by Alzheimer's disease biomarkers

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Conflict of interests: H.Z. and K.B. are cofounders of Brain Biomarker Solutions in Gothenburg AB, a GU Ventures-based platform company at the University of Gothenburg. H.Z. has served at advisory boards of Eli Lilly and Roche Diagnostics and has received travel support from Teva. K.B. has served as a consultant or at advisory boards for Alzheon, BioArctic, Biogen, Eli Lilly, Fujirebio Europe, IBL International, Merck, Novartis, Pfizer, and Roche Diagnostics. M.O.H. has received consultant's honoraria from GE Healthcare, AVID-LILLY, and PIRAMAL. B.D. reports personal fees from Eli Lilly and company. S.L. received lecture honoraria from Roche. H.H. serves as Senior Associate Editor for the Journal Alzheimer's & Dementia; he received lecture fees from Biogen and Roche, research grants from Pfizer, Avid, and MSD Avenir (paid to the institution), travel funding from Functional Neuromodulation, 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, Oryzon Genomics, and Functional Neuromodulation, and participated in scientific advisory boards of Functional Neuromodulation, Axovant, Anavex, Eli Lilly and company, Cytox Ltd., GE Healthcare, Takeda and Zinfandel, Oryzon Genomics, and Roche Diagnostics. H.H. is co-inventor in the following patents as a scientific expert and has received no royalties: 1) *In Vitro* Multiparameter Determination Method

for The Diagnosis and Early Diagnosis of Neurodegenerative Disorders Patent Number: 8916388, 2) *In Vitro* Procedure for Diagnosis and Early Diagnosis of Neurodegenerative Diseases Patent Number: 8298784, 3) Neurodegenerative Markers for Psychiatric Conditions Publication Number: 20120196300, 4) *In Vitro* Multiparameter Determination Method for The Diagnosis and Early Diagnosis of Neurodegenerative Disorders Publication Number: 20100062463, 5) *In Vitro* Method for The Diagnosis and Early Diagnosis of Neurodegenerative Disorders Publication Number: 20100035286, 6) *In Vitro* Procedure for Diagnosis and Early Diagnosis of Neurodegenerative Diseases Publication Number: 20090263822, 7) *In Vitro* Method for The Diagnosis of Neurodegenerative Diseases Patent Number: 7547553, 8) CSF Diagnostic *In Vitro* Method for Diagnosis of Dementias and Neuroinflammatory Diseases Publication Number: 20080206797, 9) *In Vitro* Method for The Diagnosis of Neurodegenerative Diseases Publication Number: 20080199966, and 10) Neurodegenerative Markers for Psychiatric Conditions Publication Number: 20080131921. The other authors report that they have no conflict of interest to disclose.

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Abstract

Introduction: Several neurodegenerative brain proteinopathies, including Alzheimer's disease (AD), are associated with cerebral deposition of insoluble aggregates of α -synuclein. Previous studies reported a trend toward increased cerebrospinal fluid (CSF) α -synuclein (α -syn) concentrations in AD compared with other neurodegenerative diseases and healthy controls.

Methods: The pathophysiological role of CSF α -syn in asymptomatic subjects at risk of AD has not been explored. We performed a large-scale cross-sectional observational monocentric study of preclinical individuals at risk for AD (INSIGHT-preAD).

Results: We found a positive association between CSF α -syn concentrations and brain β -amyloid deposition measures as mean cortical standard uptake value ratios. We demonstrate positive correlations between CSF α -syn and both CSF t-tau and p-tau₁₈₁ concentrations.

Discussion: Animal models presented evidence, indicating that α -syn may synergistically and directly induce fibrillization of both tau and β -amyloid. Our data indicate an association of CSF α -syn with AD-related pathophysiological mechanisms, during the preclinical phase of the disease.

Keywords: α -Synuclein; Alzheimer's disease; Cerebrospinal fluid; Subjective memory complainers; Preclinical; Monocentric; Amyloid PET; Tau protein; Synergistic; SUVR

1. Introduction

α -Synuclein (α -syn) is a protein assumed to play a role in the presynaptic modulation of cell vesicle trafficking [1,2]. In particular, α -syn binds to specific presynaptic proteins directly involved in the release of neurotransmitters and preserves the synaptic terminals, both at structural and at functional level (Wong and Krainc [1]; Fang et al., [2]). Hyperphosphorylated misfolded α -syn proteins, deposited in the brain as insoluble fibrillary aggregates, generate neuronal cytoplasmic inclusions, namely Lewy bodies (LBs) [3] which are pathophysiological hallmarks of several brain proteinopathies with neurodegeneration—including Parkinson disease (PD), PD with dementia, and dementia with Lewy bodies—and oligodendroglial cytoplasmic inclusions—typically found in multiple system atrophy, all belonging to the synucleinopathy spectrum [4,5]. However, Lewy body pathology is also found in most cases with familial Alzheimer's disease (AD) harboring presenilin (PSEN 1 and 2) mutations [6].

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 (HCs), PD, and atypical parkinsonian syndromes [7–11]. In summary, most studies show a minor reduction in CSF total α -syn in PD and LBD.

Moreover, the cellular localization and function of α -syn suggest a potential role as surrogate biomarker of synaptic loss also in non-synucleinopathy neurodegenerative diseases (NDs), such as AD. However, despite numerous research

efforts, a general consensus on the relevance of this biomarker candidate in the diagnostic/prognostic workflow of ND is still under debate [12,13].

Several studies reported higher CSF α -syn concentrations in patients with AD *versus* both individuals suffering from other NDs and HCs. However, these results are conflicting, probably due to substantial intersite methodological differences [12–15]. These include different pre-intra-analytical procedures and assays, 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) forms of the disease [12–14].

To the best of our knowledge, no studies examined the potential pathophysiological role of CSF α -syn in the asymptomatic preclinical phase of AD [16].

Individuals with subjective complaints of memory dysfunction (SMC), together with evidence of cerebral deposition of amyloid β (A β), are considered asymptomatic individuals at risk of developing AD [16]. 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.

2. Materials and methods

2.1. 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 Pre-dicTors in Subjective Memory Complainers” (INSIGHT-preAD) study, a French monocentric academic university-based cohort which is part of the Alzheimer Precision Medicine Initiative Cohort Program [17]. Participants were enrolled at the Institute of Memory and AD (*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 pre-clinical 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 years, with SMC. The status of SMC is confirmed as follows: (1) 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?”; (2) participants presented intact cognitive functions based on Mini-Mental State Examination score (≥ 27), Clinical Dementia Rating scale = 0, and Free and Cued Selective Rating Test (total recall score ≥ 41).

β -Amyloid positron emission tomography ($A\beta$ -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 $A\beta$ deposition.

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

At the point of study inclusion, several data, such as demographic data and apolipoprotein E genotype (APOE), are collected.

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 at baseline. It has been previously reported that CSF $A\beta$ and $A\beta$ -PET have comparable diagnostic performance in detecting cerebral $A\beta$ deposition, at preclinical or prodromal stages of AD [18]. Thus, CSF $A\beta$ was not included in our analyses due to its high degree of intercorrelation with PET data.

2.2. CSF sampling

A lumbar puncture was performed at baseline in all 36 participants of the cohort subset. All CSF samples included were collected in polypropylene tubes and centrifuged at 1000 g for 10 min at 4°C. The collected supernatant was aliquoted and stored at -80°C pending biochemical analysis.

2.3. 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-tau₁₈₁), and $A\beta$ fraction 1-42 ($A\beta_{1-42}$) concentrations were measured using established sandwich enzyme-linked immunosorbent assay methods, namely the INNOTEST hTAU-Ag, INNOTEST Phospho-Tau[181P], and INNOTEST β -AMYLOID(1-42), respectively (Fujirebio Europe NV, Gent, Belgium) [19–21]. All CSF analyses were performed by board-certified laboratory technicians blinded to clinical information.

2.4. 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-k151wk/>). 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.

2.5. PET acquisition

All florbetapir-PET scans are acquired in a single session on a Philips Gemini GXL computed tomography-PET scanner 50 (± 5) minutes after injection of approximately 370 MBq (333–407 MBq) of florbetapir. PET acquisition consists of 3×5 minutes frames, a 128×128 acquisition matrix and a voxel size of $2 \times 2 \times 2 \text{ mm}^3$. Images are then reconstructed using iterative LOR-RAMLA algorithm (10 iterations), with a smooth postreconstruction filter. All corrections (attenuation, scatter, and random coincidence) are integrated in the reconstruction. Finally, frames are realigned, averaged, and quality-checked by the Centre pour l’Acquisition et le Traitement des Images (CATI) team. CATI is a French neuroimaging platform funded by the French Plan Alzheimer (available at <http://cati-neuroimaging.com>).

2.6. 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 as $A\beta$ positive or $A\beta$ negative according to a method previously described [22].

Table 1

Demographic and clinical data of subjects stratified by amyloid PET status

Clinical data	Total sample	PET negative	PET positive	Statistic test, P value
Sex (M/F)	36 (18/18)	28 (10/18)	8 (8/0)	χ^2 , $P = .005^*$
Age at time of CSF collection (yrs)	76.0 [72.5–77]	75.5 [72–77]	76.0 [75.3–77.3]	W, $P = .49$
Education (/8)	8.0 [5.0–8.0]	8.0 [7.0–8.0]	4.5 [3.8–6.0]	W, $P = .003^*$
CSF biomarkers				
p-tau ₁₈₁ (pg/mL)	55 [39–64]	48.25 [35.50–58.25]	68.0 [59.25–85.25]	W, $P = .003^*$
t-tau (pg/mL)	332 [259–411]	304.5 [227.0–377.0]	510.5 [334.2–597.5]	W, $P = .005^*$
A β 1-42 (pg/mL)	888 [663–1596]	975.5 [690.5–1151]	659.0 [545.5–680.5]	W, $P = .002^*$
α -syn (pg/mL)	460 [363–566]	451.5 [333.5–524.8]	555.0 [456.8–625.0]	W, $P = .08$
APOE ϵ 4, n (0/1)	36 (27/9)	28 (23/5)	8 (4/4)	χ^2 , $P = .16$
Global SUVR	0.71 [0.68–0.83]	0.700 [0.668–0.720]	0.970 [0.950–1.040]	W, $P < .001^*$

Abbreviations: α -syn, α -synuclein; A β 1-42, 42-amino acid-long amyloid β peptide; CSF, cerebrospinal fluid; M, male; F, female; PET, positron emission tomography; t-tau, total tau; p-tau₁₈₁, hyperphosphorylated tau at Threonine site 181; APOE ϵ 4, apolipoprotein E ϵ 4 carrier; SUVR, mean standardized uptake value ratio; χ^2 , chi-squared test; W, Wilcoxon-Mann-Whitney pairwise comparison.

NOTE. 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 $P < .05$, two tailed. The * symbol refers to the presence of statistical significance.

2.7. Statistical analysis

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

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

We then performed regression analysis preceded by logarithmic transformation of all biomarkers to approximate assumptions of normality and hence remain within the assumptions of linear regression. Associations between log-transformed CSF biomarker concentrations and log-transformed A β -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), A β -PET global SUVR on α -syn values including age and sex as covariates.

To follow and 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 $P < .05$.

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

3. Results

3.1. 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 A β -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, A β -PET positive participants scored an educational level higher than those with negative A β -PET (see Table 1). A significant difference was also found when comparing the two groups for sex ratio (see Table 1).

Table 2

Univariate linear regression analysis with predictive factors of the CSF α -synuclein concentrations

Covariate by model (adjusted R^2 value)	Estimate β	Standard error	P value
Model 1 (=0.297)			
Intercept	3.662	1.318	.009*
Log global SUVR	1.312	0.368	.001*
Model 2a (=0.248)			
Intercept	2.457	1.394	.088
Log CSF p-tau ₁₈₁	0.523	0.167	.004*
Model 2b (=0.462)			
Intercept	1.773	1.194	.147
Log CSF t-tau	0.715	0.139	<.001*

Abbreviations: CSF, cerebrospinal fluid; Log, logarithmic transformation; t-tau, total tau; p-tau₁₈₁, hyperphosphorylated tau at Threonine site 181; SUVR, mean standardized uptake value ratio.

NOTE. Logarithmic transformation of CSF variables was used to reduce the skewness of distribution. P value: significant level $P < .05$, two tailed. The * symbol refers to the presence of statistical significance. Each model is adjusted for age and sex.

Table 3

Predictive factors of the CSF α -synuclein concentration: a multivariate analysis

Covariate by model (adjusted R ² value)	Estimate β (95% CI)	Standard error	<i>P</i> value	Bootstrapped CI 95%	Bootstrapped <i>P</i> value
Model 3a (=0.8001)					
Intercept	1.825	0.569	.003	1.491; 2.186	.002
Log CSF t-tau	0.705	0.077	.000*	0.644; 0.758	.000
Log global SUVR	-0.064	0.188	.734	-0.209; 0.078	.766
Model 3b (=0.5085)					
Intercept	2.638	0.872	.005	2.241; 2.975	.002
Log CSF p-tau ₁₈₁	0.479	0.119	.000*	0.33; 0.733	.009
Log global SUVR	0.296	0.285	.307	0.033; 0.462	.344

Abbreviations: 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, logarithmic transformation.

NOTE. Log transformation of CSF variables was used to reduce the skewness of distribution.

P value: significant level $P < .05$, two tailed. The * symbol refers to the presence of statistical significance.

The model is adjusted for age and sex.

CSF concentrations of p-tau₁₈₁ and t-tau were significantly different between positive and negative A β -PET individuals, with the former showing increased concentrations of both p-tau₁₈₁ and t-tau ($P = .003$ and $P = .005$, respectively) (see Table 1).

A trend but not a significant difference in terms of CSF α -syn concentrations was found between A β PET-positive and A β PET-negative subjects (data not shown).

3.2. 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 ($\beta = 0.72$ [0.14], $P < .001$; $\beta = 0.52$ [0.17], $P = .004$; $\beta = 1.31$ [0.37], $P = .001$, respectively) (for more details, see Table 2 and Fig. 1).

3.3. 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 ($P < .001$) in CSF α -syn concentration, after adjusting for age and sex. This model is accurate with an adjusted R-squared value of 0.80 (for more details, see Table 3).

At a lesser extent, a similar arrangement, including global SUVR and CSF p-tau₁₈₁ instead of CSF t-tau, resulted into a model in which an increase of one unit of CSF p-tau₁₈₁ concentration lead to a significant increase of 0.48 (0.12) pg/mL ($P < .01$) in CSF α -syn concentration, after adjusting for age and sex (see Table 3).

We decided not to include CSF p-tau₁₈₁ 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).

3.4. Logistic regression analysis for PET status

The regression for CSF α -syn was significant with a positive odds ratio, indicating that greater values of the marker are more likely to explain an increased cerebral A β load. The same was found for t-tau and p-tau₁₈₁ (see Table 4).

4. Discussion

Using a cross-sectional study design in a large monocentric cohort (INSIGHT-preAD)—within the framework of the Alzheimer Precision Medicine Initiative as part of the Alzheimer Precision Medicine Initiative Cohort Program—we found a positive association between CSF α -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 patients with AD showed concomitant brain LB deposition [23–25]. In addition, cerebral A β pathology is a common finding in synucleinopathies, especially in dementia with Lewy bodies individuals [26,27].

Table 4

Predictive factors of the amyloid PET status: a binary logistic regression analysis

Covariate	Model 1 or [95% CI]	<i>P</i> value
Log CSF α -syn	1.000 [1.000–1.002]	.005*
Log CSF p-tau ₁₈₁	1.474 [1.110–1.957]	.011*
Log CSF t-tau	1.537 [1.194–1.980]	.002*

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.

NOTE. Logarithmic transformation of CSF variables was used to reduce the skewness of distribution.

P value: significant level $P < .05$, two tailed. The * symbol refers to the presence of statistical significance. The model is adjusted for age and sex.

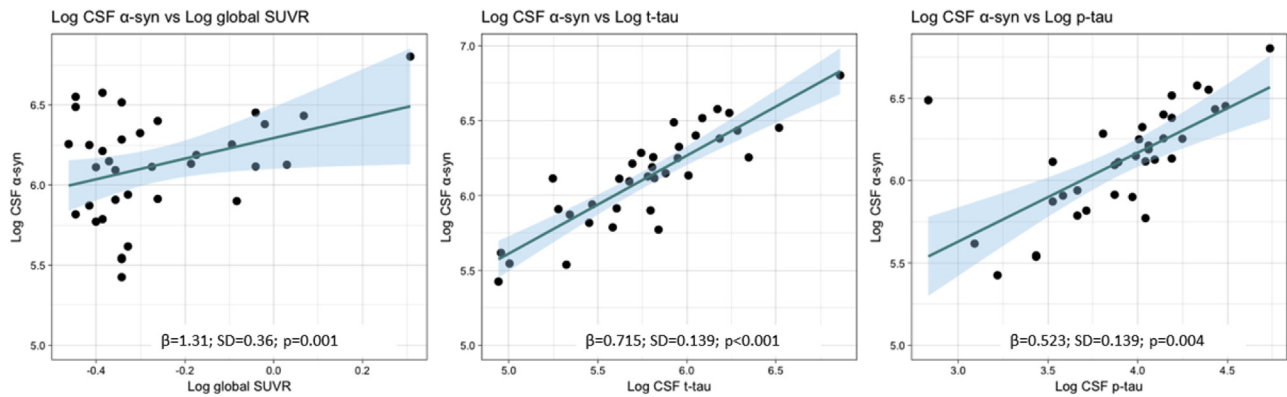


Fig. 1. Plots showing the association between CSF α -synuclein and global SUVR, CSF α -synuclein and CSF t-tau, and CSF α -synuclein and CSF p-tau₁₈₁: the univariate analysis. Notes. For each curve, β slope and standard deviation (SD) are indicated with respective P value (significant level $P < .05$) adjusted for age and sex. Abbreviations: CSF, cerebrospinal fluid; p-tau, hyperphosphorylated tau at Threonine site 181; t-tau, total tau; SUVR, standard uptake value ratios; Log, logarithmic transformation.

Recently, the existence of an anti-A β deposition effect of α -syn has been proposed in a mouse model of AD [28]. This observation, if confirmed in humans, might provide novel insights into potential targets for precise pathomechanistic therapies of AD and synucleinopathies.

Although we found a trend of increased CSF α -syn concentrations in A β PET-positive compared with A β PET-negative subjects, these values did not reach statistical significance likely due to the relatively small sample size. Previous studies also explored the diagnostic value of CSF α -syn concentrations—alone or in combination with the CSF core feasible biomarkers A β ₁₋₄₂, t-tau, and p-tau₁₈₁—differentiating a large spectrum of ND, including AD [12–15]. Although some results are still controversial, most studies reported increased CSF α -syn concentrations in AD compared with other ND and HC [12,13]. Discrepancies emerging from these data might be attributable to a high degree of intersite variability and to analytical and methodological differences, such as the CSF measurement of either the full-size protein or specific oligomers of α -syn [8,12,13]. Furthermore, most of the investigations lack of a reliable HC group [8,12,13].

Furthermore, we disclosed a positive association between CSF α -syn and CSF t-tau and p-tau₁₈₁, using both univariate and 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 [1]. Indeed, hyperphosphorylation is a post-translational modification common to several misfolded proteins accumulating in the brain, including α -syn [29,30]. 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 gray matter atrophy and cerebral hypometabolism, measured using magnetic resonance imaging and ¹⁸F-2-fluoro-2-deoxy-D-glucose PET [12–15]. 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 patients with AD [1,4,31,32]. Finally, a possible synergistic link between α -syn and tau protein byproducts on neurodegeneration has been suggested [33,34]. Such an interaction is supposed to facilitate the spreading of LB and the deposition of neurofibrillary tangles activated by an imbalance between brain kinases and phosphatases [1,29,34].

This study presents some caveats. First, the sample size is relatively limited thus hindering any CSF core biomarker-based stratification of our individuals. Second, due to small sample size, we did not include APOE genotype and education level as additional covariates, preventing the opportunity to exclude that the associations found in this study were partially driven by differences in APOE and education between the high and low amyloid subgroups.

Third, 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 the onset of AD or other ND, such as, dementia with Lewy bodies. Moreover, structural magnetic resonance imaging 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 depicting different longitudinal molecular trajectories underpinning apparently similar phenotypes.

In conclusion, if our results will be confirmed in larger samples, 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 at risk of AD or other ND according to distinctive 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 [17,35–37]. Supplementary investigations will be essential to address the open issues which the present study cannot address due to methodological limit as the total and the A β PET-positive sample size. Throughout removing these potential biases, it will be possible 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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RESEARCH IN CONTEXT

1. Systematic review: Previous studies investigating cerebrospinal fluid (CSF) α -synuclein (α -syn) concentrations in Alzheimer's disease are conflicting, probably due to substantial intersite methodological differences. These include different pre-intra-analytical procedures and assays, performed for CSF α -syn assessment, and different recruitment criteria.

To the best of our knowledge, no studies examined the potential pathophysiological role of CSF α -syn in the asymptomatic preclinical phase of Alzheimer's disease.

We performed a cross-sectional study in a large-scale monocentric preclinical at risk cohort (INSIGHT-preAD).

2. Interpretation: We disclosed a positive association between CSF α -syn concentrations and brain β -amyloid deposition in terms of positron emission tomography standard uptake value ratios. There were also positive correlations between CSF α -syn and both CSF t-tau and p-tau₁₈₁ concentrations.
3. Future directions: It has previously been shown that α -syn may synergistically and directly induce post-translational modifications of both tau and β -amyloid. Therefore, the main findings of this study indicate an association of CSF α -syn with Alzheimer's disease-related pathophysiological mechanisms, during the preclinical phase of the disease.

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