

**Association of CSF  $\alpha$ -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**

Andrea Vergallo<sup>a,b,c,d,§</sup>, René-Sosata Bun<sup>a,b,c,d,§,\*</sup>, Nicola Toschi<sup>e,f,g</sup>, Filippo Baldacci<sup>a,b,c,d,h</sup>,  
Henrik Zetterberg<sup>i,j,k,l</sup>, Kaj Blennow<sup>i,j</sup>, Enrica Cavedo<sup>a,b,c,d,m</sup>, Foudil Lamari<sup>n</sup>,  
Marie-Odile Habert<sup>o,p,q</sup>, Bruno Dubois<sup>b,c,d</sup>, Roberto Floris<sup>e</sup>, Francesco Garaci<sup>e,r</sup>,  
Simone Lista<sup>a,b,c,d</sup>, and Harald Hampel<sup>a,b,c,d</sup>

and the INSIGHT-preAD study group<sup>\*\*</sup>

**for the Alzheimer Precision Medicine Initiative (APMI)**

<sup>a</sup> AXA Research Fund & Sorbonne Université Chair, Paris, France

<sup>b</sup> Sorbonne Université, AP-HP, GRC n° 21, Alzheimer Precision Medicine (APM), Hôpital de la Pitié-Salpêtrière, Boulevard de l'hôpital, F-75013, Paris, France

<sup>c</sup> Institut du Cerveau et de la Moelle Épinière (ICM), INSERM U 1127, CNRS UMR 7225, Boulevard de l'hôpital, F-75013, Paris, France

<sup>d</sup> Institut de la Mémoire et de la Maladie d'Alzheimer (IM2A), Département de Neurologie, Hôpital de la Pitié-Salpêtrière, AP-HP, Boulevard de l'hôpital, F-75013, Paris, France

<sup>e</sup> Department of Biomedicine and Prevention, University of Rome "Tor Vergata", Rome, Italy

<sup>f</sup> Department of Radiology, "Athinoula A. Martinos" Center for Biomedical Imaging, Boston, MA, USA

<sup>g</sup> Harvard Medical School, Boston, MA, USA

<sup>h</sup> Department of Clinical and Experimental Medicine, University of Pisa, Pisa, Italy

<sup>i</sup> Institute of Neuroscience and Physiology, Department of Psychiatry and Neurochemistry, Mölndal, Sweden

<sup>j</sup> The Sahlgrenska Academy at the University of Gothenburg, Mölndal, Sweden

Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Mölndal, Sweden

<sup>k</sup> Department of Molecular Neuroscience, UCL Institute of Neurology, Queen Square, London, UK

<sup>l</sup> UK Dementia Research Institute, London, UK

<sup>m</sup> Laboratory of Alzheimer's Neuroimaging and Epidemiology, IRCCS Centro San Giovanni di Dio Fatebenefratelli, Brescia, Italy

<sup>n</sup> AP-HP, UF Biochimie des Maladies Neuro-métaboliques, Service de Biochimie Métabolique, Groupe Hospitalier Pitié-Salpêtrière, Paris, France

<sup>o</sup> Sorbonne Université, CNRS, INSERM, Laboratoire d'Imagerie Biomédicale, F-75013, Paris, France

<sup>p</sup> Centre pour l'Acquisition et le Traitement des Images ([www.cati-neuroimaging.com](http://www.cati-neuroimaging.com)),  
France

<sup>q</sup> AP-HP, Hôpital Pitié-Salpêtrière, Département de Médecine Nucléaire, F-75013, Paris,  
France

<sup>r</sup> Casa di Cura "San Raffaele Cassino", Cassino, Italy

**\*Corresponding author:**

René-Sosata Bun, PharmD  
Interne en Pharmacie Hospitalière

AXA Research Fund & Sorbonne Université Chair  
Sorbonne Université  
Département de Neurologie,  
Institut de la Mémoire et de la Maladie d'Alzheimer,  
Institut du Cerveau et de la Moelle Épinière (ICM),  
Pavillon François Lhermitte,  
Hôpital Pitié-Salpêtrière,  
47 Boulevard de l'hôpital, 75651 Paris CEDEX 13, France  
Tel.: +33 1 42 16 19 93  
Fax: +33 1 42 16 75 16  
E-Mail: [rene.bun@aphp.fr](mailto:rene.bun@aphp.fr) (René-Sosata Bun)

Sorbonne Université Groupe de Recherche Clinique (GRC n°21)  
"Alzheimer Precision Medicine (APM)"  
Établissements Publics à caractère Scientifique et Technologique (E.P.S.T.)

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**Abbreviations:**

$\alpha$ -syn, alpha-synuclein; A $\beta$ , amyloid-beta; A $\beta$ <sub>1-42</sub>, 42-amino acid-long amyloid beta peptide; A $\beta$  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; <sup>18</sup>F-FDG-PET, <sup>18</sup>F-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<sub>181</sub>, 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 ( $\alpha$ -syn) plays a key role in regulating the synaptic vesicles pool size, trafficking, and membrane dynamics. Misfolded forms of  $\alpha$ -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  $\alpha$ -syn diagnostic accuracy in cerebrospinal fluid (CSF) have reported a trend towards increased CSF  $\alpha$ -syn concentrations in AD *versus* other neurodegenerative diseases (ND) and healthy controls (HC).

The potential role of CSF  $\alpha$ -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  $\alpha$ -syn concentrations and amyloid mean cortical standard uptake value ratios (SUVR), even after adjusting for confounders. There were positive associations of CSF  $\alpha$ -syn and CSF t-tau and p-tau<sub>181</sub> proteins. Furthermore, we found a trend to statistical significance of increased CSF  $\alpha$ -syn concentrations in A $\beta$  PET-positive compared with A $\beta$  PET-negative subjects.

Animal models have shown that  $\alpha$ -syn may synergistically and directly induce fibrillization of both tau and A $\beta$ . Therefore, the main findings of this study indicate an association of CSF  $\alpha$ -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  $\alpha$ -syn concentrations could depict longitudinal trajectories of asymptomatic subjects at risk for AD.

## KEY WORDS

$\alpha$ -synuclein, Alzheimer's disease, cerebrospinal fluid, subjective memory complainers, preclinical, monocentric, amyloid PET, tau protein, synergistic, SUVR.

## INTRODUCTION

Alpha-synuclein ( $\alpha$ -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,  $\alpha$ -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  $\alpha$ -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,  $\alpha$ -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  $\alpha$ -syn suggest a potential role as surrogate biomarker of synaptic loss also in non-synucleopathic 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  $\alpha$ -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  $\alpha$ -syn assessment, and different recruitment criteria. Previous studies exploring CSF  $\alpha$ -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  $\alpha$ -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 ( $A\beta$ ), 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  $\alpha$ -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,  $\geq 27$ ), Clinical Dementia Rating scale (CDR = 0), and Free and Cued Selective Rating Test (FCSRT, total recall score  $\geq 41$ ).

$A\beta$  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 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 A $\beta$  and A $\beta$ -PET have comparable diagnostic performance in detecting cerebral A $\beta$  deposition, at preclinical or prodromal stages of AD (Palmqvist et al., 2015). Thus, CSF A $\beta$  was not included in our analyses due to 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<sub>181</sub>) and A $\beta$ <sub>1-42</sub> concentrations, were measured using established sandwich ELISA methods, namely the INNOTEST hTAU-Ag, INNOTEST Phospho-Tau[181P] (Fujirebio Europe NV, Gent, Belgium) and INNOTEST  $\beta$ -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 $\alpha$ -syn**

All CSF  $\alpha$ -syn analyses were performed at the Clinical Neurochemistry Laboratory at the Sahlgrenska University Hospital, Mölndal, Sweden. CSF  $\alpha$ -syn protein concentration was measured using the U-PLEX Human  $\alpha$ -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 ( $\pm$  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 mm<sup>3</sup>. 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>).

### **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 A $\beta$  positive or A $\beta$  negative according to a method previously described (Habert et al., 2017).

### **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 $\beta$ -PET positive and negative groups in terms of CSF concentrations of core feasible biomarkers and  $\alpha$ -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 A $\beta$ -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 $\beta$ -PET global SUVR on  $\alpha$ -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  $\alpha$ -syn with t-tau + SUVR + covariates; model 3b,  $\alpha$ -syn with p-tau<sub>181</sub>+ 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-tau<sub>181</sub>, and  $\alpha$ -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 A $\beta$ -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 $\beta$ -PET positive participants scored an educational level higher than those with negative A $\beta$ -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-tau<sub>181</sub> and t-tau were significantly different between positive and negative A $\beta$ -PET individuals, with the former showing increased concentrations of both p-tau<sub>181</sub> and t-tau ( $p=0.003$  and  $p=0.005$  respectively) (see **Table 1**).

No significant difference in terms of CSF  $\alpha$ -synuclein concentrations was found between A $\beta$  PET-positive and A $\beta$  PET-negative subjects.

### Univariate linear regression analysis of CSF $\alpha$ -syn predictive factors:

The univariate linear regression models including age and sex as covariates showed that CSF t-tau, CSF p-tau<sub>181</sub>, and global SUVR were all significantly associated with CSF  $\alpha$ -syn ( $\beta=0.72$  (0.14),  $p<0.001$ ;  $\beta=0.52$  (0.17),  $p=0.004$ ;  $\beta =1.31(0.37)$ ,  $p=0.001$ , respectively) (for more details, see **Table 2** and **Figure 1**).

### Multivariate linear regression analysis of CSF $\alpha$ -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<sub>181</sub> instead of CSF t-tau, resulted into a model in which an increase of one unit of CSF p-tau<sub>181</sub> 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<sub>181</sub> 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<sub>181</sub> (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  $\alpha$ -syn concentrations – alone or in combination with the CSF core feasible biomarkers  $A\beta_{1-42}$ , t-tau, and p-tau<sub>181</sub> –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  $\alpha$ -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  $\alpha$ -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  $\alpha$ -syn and CSF t-tau and p-tau<sub>181</sub>, 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  $\alpha$ -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  $\alpha$ -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  $\alpha$ -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  $\alpha$ -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 <sup>18</sup>F-2-fluoro-2-deoxy-D-glucose PET (<sup>18</sup>F-FDG-PET) (Hansson et al., 2014; Mattsson et al., 2013; Slaets et al., 2014; Wang et al., 2015). Notably, since  $\alpha$ -syn is involved in glutamatergic neuronal transmission, the hippocampal atrophy, an early feature of AD pathophysiology, might explain the increased concentrations of CSF  $\alpha$ -syn in AD patients (Ohrfelt et al., 2009; Toledo et al., 2016; Wong and Krainc, 2017; Goedert, 2015). Finally, a possible synergistic link between  $\alpha$ -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  $\alpha$ -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  $\alpha$ -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  $\alpha$ -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  $\alpha$ -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 (Hempel et al., 2017, 2016, 2018, 2018). Supplementary investigations will be essential to establish whether CSF  $\alpha$ -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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## CONTRIBUTORS TO THE ALZHEIMER PRECISION MEDICINE INITIATIVE WORKING GROUP (APMI-WG):

Aguilar LF (Montréal), Babiloni C (Rome), Baldacci F (Pisa), Benda N (Bonn), Black KL (Los Angeles), Bokde ALW (Dublin), Bonuccelli U (Pisa), Broich K (Bonn), Bun RS (Paris), Cacciola F (Siena), Castrillo J† (Derio), Cavedo E (Paris), Ceravolo R (Pisa), Chiesa PA (Paris), Colliot O (Paris), Coman CM (Paris), Corvol JC (Paris), Cuello AC (Montréal), Cummings JL (Las Vegas), Dubois B (Paris), Duggento A (Rome), Durrleman S (Paris), Escott-Price V (Cardiff), Federoff H (Irvine), Ferretti MT (Zürich), Fiandaca M (Irvine), Frank RA (Malvern), Garaci F (Rome), Genthon R (Paris), George N (Paris), Giorgi FS (Pisa), Graziani M (Roma), Haberkamp M (Bonn), Habert MO (Paris), Hampel H (Paris), Herholz K (Manchester), Karran E (Cambridge), Kim SH (Seoul), Koronyo Y (Los Angeles), Koronyo-Hamaoui M (Los Angeles), Lamari F (Paris), Langevin T (Minneapolis-Saint Paul), Lehericy S (Paris), Lista S (Paris), Lorenceau J (Paris), Mapstone M (Irvine), Neri C (Paris), Nisticò R (Rome), Nyasse-Messene F (Paris), O’Bryant SE (Fort Worth), Perry G (San Antonio), Ritchie C (Edinburgh), Rojkova K (Paris), Rossi S (Siena), Santarnecchi E (Siena), Schneider LS (Los Angeles), Sporns O (Bloomington), Toschi N (Rome), Verdooner SR (Sacramento), Vergallo A (Paris), Villain N (Paris), Welikovitch L (Montréal), Woodcock J (Silver Spring), Younesi E (Esch-sur-Alzette).

## INSIGHT-preAD Study Group:

Audrain C, Auffret A, Bakardjian H, Baldacci F, Batrancourt B, Benakki I, Benali H, Bertin H, Bertrand A, Boukadida L, Cacciamani F, Causse V, Cavedo E, Cherif Touil S, Chiesa PA, Colliot O, Dalla Barba G, Depaulis M, Dos Santos A, Dubois B, Dubois M, Epelbaum S, Fontaine B, Francisque H, Gagliardi G, Genin A, Genthon R, Glasman P, Gombert F, Habert MO, Hampel H, Hewa H, Houot M, Jungalee N, Kas A, Kilani M, La Corte V, Le Roy F, Lehericy S, Letondor C, Levy M, Lista S, Lowrey M, Ly J, Makiese O, Masetti I, Mendes A, Metzinger C, Michon A, Mochel F, Nait Arab R, Nyasse F, Perrin C, Poirier F, Poisson C, Potier MC, Ratovohery S, Revillon M, Rojkova K, Santos-Andrade K, Schindler R, Servera MC, Seux L, Simon V, Skovronsky D, Thiebaut M, Uspenskaya O, Vlaincu M.

### **INSIGHT-preAD Scientific Committee Members:**

Dubois B, Hampel H, Bakardjian H, Colliot O, Habert MO, Lamari F, Mochel F, Potier MC, Thiebaut de Schotten M.

### **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 PIRAMAL.

**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

- *In Vitro* Method for The Diagnosis and Early Diagnosis of Neurodegenerative Disorders Publication Number: 20100035286

- *In Vitro* Procedure for Diagnosis and Early Diagnosis of Neurodegenerative Diseases Publication Number: 20090263822

- *In Vitro* Method for The Diagnosis of Neurodegenerative Diseases Patent Number: 7547553

- CSF Diagnostic *In Vitro* Method for Diagnosis of Dementias and Neuroinflammatory Diseases Publication Number: 20080206797

- *In Vitro* Method for The Diagnosis of Neurodegenerative Diseases Publication Number: 20080199966

- Neurodegenerative Markers for Psychiatric Conditions Publication Number: 20080131921

**AV, RSB, NT, FB, EC, FL, MOH, RF, and FG** declare no conflict of interest.

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## TABLES

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

	<b>Total sample</b>	<b>PET negative</b>	<b>PET positive</b>	<b>Statistic test, p value</b>
Sex (M/F)	36 (18/18)	28(10/18)	8(8/0)	$\chi^2$ , p = 0.005*
Age at time of CSF collection (yrs)	76.0 [72.5-77]	75.5 [72-77]	76.0 [75.3-77.3]	W, p = 0.49
Education (/8)	8.0 [5.0-8.0]	8.0 [7.0-8.0]	4.5 [3.8-6.0]	W, p = 0.003*
<u>CSF biomarkers</u>				
p-tau <sub>181</sub> (pg/mL)	55 [39-64]	48.25[35.50-58.25]	68.0[59.25-85.25]	W, p = 0.003*
t-tau (pg/mL)	332 [259-411]	304.5[227.0-377.0]	510.5[334.2-597.5]	W, p = 0.005*
A $\beta$ 1-42 (pg/mL)	888 [663-1596]	975.5[690.5-1151]	659.0[545.5-680.5]	W, p = 0.002*
$\alpha$ -syn (pg/mL)	460 [363-566]	451.5[333.5-524.8]	555.0[456.8-625.0]	W, p = 0.08
APOE $\epsilon$ 4, n (0/1)	36 (27/9)	28 (23/5)	8(4/4)	$\chi^2$ , p = 0.16
Global SUVR	0.71 [0.68-0.83]	0.700[0.668-0.720]	0.970[0.950-1.040]	W, p < 0.001*

**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:**  $\alpha$ -syn,  $\alpha$ -synuclein; A $\beta$ 1-42, 42-amino acid-long amyloid beta peptide; CSF, cerebrospinal fluid; M, male; F, female; PET, positron emission tomography; t-tau, total tau; p-tau<sub>181</sub>, hyperphosphorylated tau at Threonine site 181; Apolipoprotein E  $\epsilon$ 4 carrier, APOE  $\epsilon$ 4; SUVR, mean standardized uptake value ratio;  $\chi^2$ : Chi square test; W: Wilcoxon-Mann-Whitney pairwise comparison

**Table 2.** Univariate linear regression analysis with predictive factors of the CSF  $\alpha$ -synuclein concentrations.

Covariate by Model (Adjusted R <sup>2</sup> Value)	Estimate $\beta$	Standard error	p value
<b>Model 1 (=0.297)</b>			
Intercept	3.662	1.318	0.009*
Log global SUVR	1.312	0.368	0.001*
<b>Model 2a (=0.248)</b>			
Intercept	2.457	1.394	0.088
Log CSF p-tau <sub>181</sub>	0.523	0.167	0.004*
<b>Model 2b (=0.462)</b>			
Intercept	1.773	1.194	0.147
Log CSF t-tau	0.715	0.139	< 0.001*

**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<sub>181</sub>, hyperphosphorylated tau at Threonine site 181; SUVR, mean standardized uptake value ratio

**Table 3.** Predictive factors of the CSF  $\alpha$ -synuclein concentration: a multivariate analysis.

Covariate by Model (Adjusted R <sup>2</sup> Value)	Estimate $\beta$ (95%CI)	Standard error	p value	Bootstrapped CI 95%	Bootstrapped P- value
Model 3a (=0.8001)					
Intercept	1.825	0.569	0.003	[1.491; 2.186]	0.002
Log CSF t-tau	0.705	0.077	0.000*	[0.644; 0.758]	0.000
Log global SUVR	-0.064	0.188	0.734	[-0.209; 0.078]	0.766
Model 3b (=0.5085)					
Intercept	2.638	0.872	0.005	[2.241; 2.975]	0.002
Log CSF p-tau <sub>181</sub>	0.479	0.119	0.000*	[0.33; 0.733]	0.009
Log global SUVR	0.296	0.285	0.307	[0.033; 0.462]	0.344

**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:**  $\alpha$ -syn,  $\alpha$ -synuclein; CSF, cerebrospinal fluid; PET, positron emission tomography; t-tau, total tau; p-tau<sub>181</sub>, 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.

Covariate	Model 1 OR [95%CI]	p-value
Log CSF $\alpha$ -syn	1.000 [1.000—1.002]	0.005*
Log CSF p-tau <sub>181</sub>	1.474 [1.110—1.957]	0.011*
Log CSF t-tau	1.537 [1.194—1.980]	0.002*

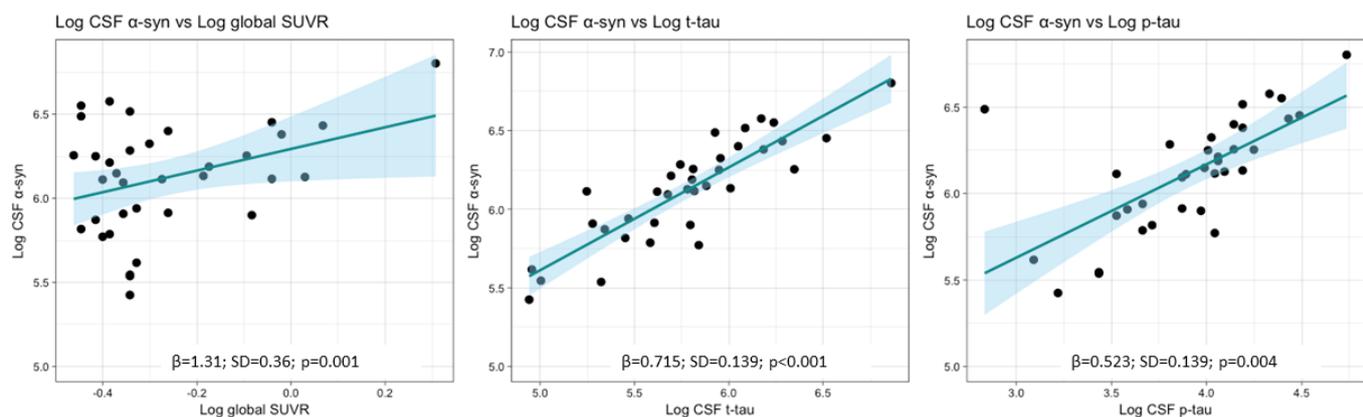
**Notes.** Logarithmic transformation of CSF variables was used to reduce the skewness of distribution.

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:**  $\alpha$ -syn,  $\alpha$ -synuclein; CSF, cerebrospinal fluid; PET, positron emission tomography; t-tau, total tau; p-tau<sub>181</sub>, hyperphosphorylated tau at Threonine site 181; Log: Logarithmic transformation

## FIGURE LEGENDS

**Figure 1.** Plots showing the association between CSF  $\alpha$ -synuclein and global SUVR, CSF  $\alpha$ -synuclein and CSF t-tau, and CSF  $\alpha$ -synuclein and CSF p-tau<sub>181</sub>: the univariate analysis.



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

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