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ORIGINAL COMMUNICATION

Correlates of cerebrospinal fluid levels of oligomeric- and total-α-synuclein in premotor, motor and dementia stages of Parkinson's disease

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Abstract High-oligomeric and low-total-α-synuclein cerebrospinal fluid (CSF) levels have been found in Parkinson's disease (PD), but with inconsistent or limited data, particularly on their clinical and structural correlates in earliest (premotor) or latest (dementia) PD stages. We determined CSF oligomeric- and total-α-synuclein in 77 subjects: 23 with idiopathic REM-sleep behaviour disorder (iRBD, a condition likely to include a remarkable proportion of subjects in the premotor stage of PD) and 41 with PD [21 non-demented (PDND) + 20 demented (PDD)], intended to reflect the premotor–motor–dementia PD continuum, along with 13 healthy controls. The study protocol also included the Unified PD Rating Scale motor-section (UPDRS-III), mini mental state examination (MMSE), neuropsychological cognitive testing, 3T brain MRI for cortical-thickness analyses, CSF τ and CSF Aβ. CSF oligomeric-α-synuclein was higher in PDND than iRBD and in PDD than iRBD and controls, and correlated with UPDRS-III, MMSE, semantic fluency and visuo-perceptive scores across the proposed premotor–motor–dementia PD continuum (iRBD + PDND + PDD). CSF total-α-synuclein positively correlated with age, CSF Aβ, and, particularly, CSF τ, tending towards lower levels in PD (but not iRBD) vs. controls only when controlling for CSF τ. Low CSF total-α-synuclein was associated with dysfunction in phonetic-fluency (a frontal-lobe function) in PD and with frontal cortical thinning in iRBD and PDND independently of CSF τ. Conversely, the associations of high (instead of low) CSF total-α-synuclein with posterior-cortical neuropsychological deficits in PD and with posterior cortical thinning in PDD were driven by high CSF τ. These findings suggest that CSF oligomeric- and total-α-synuclein have different clinical, neuropsychological and MRI correlates across the proposed premotor–motor–dementia PD continuum. CSF total-α-synuclein correlations with CSF τ and Aβ support the hypothesis of an interaction among these proteins in PD, with CSF τ probably influencing the presence of high

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(instead of low) CSF total-α-synuclein and its correlates mostly in the setting of PD-related dementia.

Keywords Parkinson's disease · Premotor · Dementia · Cerebrospinal fluid · α-Synuclein · Cortical thickness · Neuropsychological function

Introduction

The relevance of α-synuclein to intraneuronal Lewy-type inclusions and the close relationship of cerebrospinal fluid (CSF) with brain parenchyma make of α-synuclein determination in CSF a candidate biomarker of Parkinson's disease (PD) [1, 2]. Detecting in vivo α-synuclein abnormalities would be significant for the study of the so-called premotor PD [3], a stage hypothetically more likely to respond to potential early interventions due to more restricted α-synuclein pathology [4]. A reliable α-synuclein biomarker might also be predictive of cognitive impairment, very frequent as PD progresses [5], and shown to correlate with Braak's α-synuclein stages [6].

CSF total-α-synuclein levels in PD vs. controls have ranged in different studies from significant reductions [7–12] to similar levels [13–16]. Blood contamination of CSF has been pointed as a possible explanation for such discrepancies, but it remains unclear why among positive studies, some have needed controlling for CSF haemoglobin levels to find significant CSF total-α-synuclein reductions in PD [7, 11], and some have not [8–10]. Still, the intergroup overlap has been large even in positive studies having controlled for CSF haemoglobin levels [7, 11].

CSF levels of oligomeric-α-synuclein, believed to be a toxic α-synuclein fraction [17], have been found increased [8, 14] or non-significantly different [18] in PD vs. controls, with increased oligomeric/total-α-synuclein ratio having been reported in two of these studies [8, 18]. Allegedly, drop in CSF total-α-synuclein might reflect parenchymal sequestration of α-synuclein in Lewy-type lesions or an attempt of retaining the protein to maintain neuronal physiology, whereas increased clearance to CSF of soluble and toxic oligomers might account for raised CSF oligomeric-α-synuclein [1, 2].

Motor and cognitive impairment and cortical atrophy worsen with PD progression [19–21], but as yet there is no information on the clinical, neuropsychological and MRI correlates of these CSF α-synuclein markers across PD progression ranging from early premotor stages to advanced PD with dementia. We hypothesised that high oligomeric- and low total-α-synuclein CSF levels are associated with worsening motor and cognitive function and increasing cortical atrophy across the premotor–motor–dementia PD continuum. To this end we determined CSF oligomeric- and total-α-synuclein and their motor, neuropsychological, and quantitative MRI correlates in a cohort of subjects with idiopathic REM-sleep behaviour disorder (iRBD) and with PD without and with dementia, intended to represent the premotor–motor–dementia PD continuum.

Methods

Design, participants and clinical protocol

Cross-sectional study of a convenience cohort of 77 subjects from the research programs of the Movement Disorders Unit (controls and PD patients) and the Sleep Disorders Unit (iRBD subjects) of our institution, with these programs sharing the clinical and CSF protocol but differing in other aspects. Controls ($n = 13$) were individuals undergoing intradural anaesthesia for knee surgery who, as per thorough clinical history and examination, did not have any neurological or psychiatric condition. iRBD patients ($n = 23$) were prevalent cases (iRBD mean duration = 10.65 years) diagnosed by clinical history and video-polysomnography, in the absence of cognitive complaints or other neuro-psychiatric features as detailed elsewhere [22]. PD participants ($n = 41$) had a definite clinical diagnosis according to the UKPDSBB criteria [23]. Of them, 21 had no dementia (PDND) and 20 fulfilled the PD-dementia (PDD) diagnostic criteria [24]. Severe depression and MRI abnormalities other than mild white matter hyperintensities were exclusion criteria. Demographic and clinical information, including the motor part of the Unified PD Rating Scale (UPDRS-III) [25], Hoehn & Yahr staging [19], and mini mental state examination (MMSE) as a global cognitive indicator [26], were obtained for all participants. All, but one, PD patients have been reported elsewhere [27]. The study received approval from the Ethics Committee. All participants provided written informed consent after full explanation of all procedures.

CSF collection, pre-processing and storage

All participants underwent lumbar puncture in the L3–L4 space using a 22G needle after overnight fasting and before the morning anti-Parkinsonian medication in the case of PD patients. During extraction, CSF visually contaminated with blood was rejected. CSF was immediately centrifuged for 10 min at 4,000*g* and 4 °C, and subsequently stored at −80 °C in 500 µl polypropylene aliquots until analyses.

CSF analyses

CSF oligomeric-α-synuclein was determined using a modification of the ELISA technique used by others [8,

14], based on the principle of using the same antibody for coating and detection. Shortly, 96-well ELISA plates were coated overnight at 4 °C with anti-α-synuclein antibody (1 μg/ml; mouse 211-antibody, Santa Cruz) in 200 mM NaHCO3, pH 9.6. After 3 × 1 min washes in PBS–Tween and blocking for 2 h in blocking buffer (PBS–Tween–2 % BSA), 50 μl/well of CSF or standard solution were added and incubated for 2 h at 37 °C. Standard α-synuclein oligomers were prepared by incubation at 37 °C for 4 days of a 25 μM α-synuclein solution. After 3 washes in PBS–Tween, 50 μl/well of biotinylated anti-α-synuclein antibody (1:50) was added and incubated for 2 h at 37 °C. Biotinylation of 211-antibody was performed with a kit following the manufacturer's instructions (EZ-Link Sulfo-NHS-LC-Biotinylation kit, #21435, Thermo Scientific). After 3 washes in PBS–Tween, wells were incubated for 1 h at 37 °C in ExtrAvidin–Alkaline Phosphatase (E2636, Sigma) 1:2,000, washed again 3 × 1 min and incubated for 30 min at 37 °C in p-nitrophenyl phosphate solution (N7653, Sigma). Absorbance was read immediately at 405 nm and results were expressed as absorbance values as in previous reports [8, 14].

CSF total-α-synuclein was determined using a commercial ELISA-kit (KHB0061, Invitrogen, Camarillo, CA, USA) with minor modifications. Briefly, 75 μl of each standard solution, CSF and blanks were dispensed in duplicate into ELISA plates. Plates were incubated for 3 h at 37 °C. Hu α-synuclein detection antibody solution (50 μl/well) was added (except for blank-wells). Plates were incubated overnight at 4 °C. Wells were washed 4 × 1 min with 200 μl of wash solution. 100 μl of goat anti-rabbit IgG HRP (1:100) was dispensed per well and incubated at room temperature for 30 min. Solution was decanted and, after four washes, plates were developed in the dark with 100 μl of TMB for 25 min at room temperature. Then, stop solution (100 μl of 0.6N H_2SO_4) was added and plates gently mixed. Absorbance was read at 450 nm and results were expressed in ng/ml.

All samples were run at once in two ELISA plates for each CSF α-synuclein form, with three samples of each group run in both plates to check for inter-plate variability. CSF levels of τ and Aβ [1–42] were determined with commercial ELISA kits (Innogenetics, Ghent, Belgium).

Neuropsychological assessment

iRBD and PD patients underwent neuropsychological assessment within 8 weeks of lumbar puncture. Pre-morbid level was established with years of education and the WAIS-III vocabulary subtest. iRBD patients underwent the Buschke memory test, WAIS-III similarities, FAS phonetic-fluency, and the 60-item Boston naming test (BNT-60). PD patients underwent the Rey auditory verbal learning test (RAVLT), phonetic fluency, the working-memory index (WMI) and the 15-item BNT (BNT-15). The iRBD and PD neuropsychological protocols shared semantic fluency and the visual object space (VOSP) object and space variables. PD patients were examined in "on" condition. All these tests were administered according to conventional procedures [28], with abnormal scores being defined as performance >1.5 SD below normative data or scale score ≤6 [29–32]. CSF comparisons dichotomising neuropsychological tests performance as normal vs. abnormal were carried out in PDND + PDD, but not in iRBD + PDND + PDD (due to protocol differences), neither in each group separately (due to small figures of iRBD and PDND subjects with impaired tests and to the fact that, as expected, most of PDD participants were impaired in most tests). CSF-neuropsychological correlations were carried out in each group separately and in PDND + PDD, but also in iRBD + PDND + PDD in the case of semantic fluency and VOSP variables.

MRI acquisition

iRBD and PD patients underwent 3T brain MRI in the same MRI machine (MAGNETOM Trio scanner, Siemens, Germany) without sedation and within 8 weeks of lumbar puncture. The scanning protocol for iRBD patients included high-resolution 3-dimensional T1-weighted images acquired in the sagittal plane (TR = 2,300 ms, TE = 2.98 ms, TI 900 ms, 240 slices, FOV = 256 mm; matrix size = 256 × 256; 1 mm isotropic voxel). In PD patients the MRI protocol included high-resolution 3-dimensional T1-weighted images in the coronal plane (TR = 2,300 ms, TE = 3.01 ms, TI 900 ms, 160 slices, FOV = 240 mm; matrix size = 240 × 240 × 192, 1 mm voxel).

Cortical-thickness (CTh) analyses

Cortical thickness was assessed using the automated FreeSurfer stream (version 5.3; http://surfer.nmr.harvard.edu) by means of removal of non-brain data, intensity normalisation, grey-matter/white matter boundary tessellation, automated-topology correction and accurate surface deformation to identify tissue borders [33, 34]. CTh was calculated as the distance between the white and grey-matter surfaces at each vertex of the reconstructed cortical mantle. Each subject's results were visually inspected to ensure registration accuracy, skull stripping, segmentation, and cortical-surface reconstruction. Accordingly, MRI was suitable for CTh analyses in 19 iRBD, 16 PDND and 12 PDD patients. Due to different MRI acquisitions, CSF-CTh correlations were run separately in iRBD, PDND and PDD. Briefly, the CSF-CTh correlation was assessed with a

Table 1 Baseline demographic, clinical and CSF biomarkers data across study groups

	Controls ($n = 13$)	iRBD ($n = 23$)	PDND ($n = 21$)	PDD ($n = 20$)	p
Sex (women)	6 (46 %)	7 (30 %)	7 (33 %)	12 (60 %)	0.198
Age (years)	73.00 (63.50–78.00)	69.00 (66.00–76.00)	68.00 (63.50–73.50)	73.50 (66.00–78.00)	0.245
Years of parkinsonism	NA	NA	10.00 (7.00–15.75)	9.00 (7.25–11.50)	0.370
Time to dementia (years)	NA	NA	NA	7.00 (5.25–9.75)	NA
H&Y-off: II/III/IV–V	NA	NA	III (II–III)	IV (III–V)	<0.001*
UPDRS-III	0 (0–0)	1 (0–2)	28 (20–37)	32 (28–45)	<0.001*,#
MMSE	30 (28–30)	29 (27–29)	28 (27–29)	18 (16–22)	<0.001*,†
CSF o-α-Syn (A at 405 nm)	0.182 (0.103–0.346)	0.118 (0.102–0.157)	0.182 (0.115–0.497)	0.341 (0.164–0.661)	0.003*,±
CSF t-α-Syn (ng/ml)	0.395 (0.271–0.629)	0.320 (0.280–0.490)	0.387 (0.216–0.749)	0.453 (0.215–0.679)	0.792
CSF τ (pg/ml)	183.12 (142.25–234.28)	211.00 (169.20–274.20)	216.55 (180.94–286.20)	362.85 (255.77–735.67)	0.001*,¶
CSF $A\beta_{(1–42)}$ (pg/ml)	712.81 (545.09–773.38)	678.50 (517.60–764.100	588.54 (348.63–702.19)	368.61 (278.24–480.17)	0.001*,§

Data presented as *n* (%) or median (IQ range) and compared by means of Chi-square/Fisher's exact tests or Kruskal–Wallis/Mann–Whitney's *U* tests where appropriate

NA not applicable, *CSF t-α-Syn* CSF t-α-synuclein, *CSF o-α-Syn* CSF o-α-synuclein, *A* absorbance

* Significant differences

Significant pair-wise comparisons: # UPDRS-III higher in RBD vs. controls ($p = 0.024$), in PDND vs. controls and vs. RBD (all $p < 0.001$), and in PDD vs. controls, and vs. RBD (all $p < 0.001$), † MMSE lower in RBD vs. controls ($p = 0.039$), in PDND vs. controls ($p = 0.009$), and in PDD vs. controls, RBD and PDD (all $p < 0.001$), ± CSF o-α-Syn higher in PDND vs. RBD ($p = 0.043$), and in PDD vs. controls ($p = 0.043$) and vs. RBD ($p = 0.001$), ¶ CSF τ higher in PDD vs. controls, vs. RBD, and vs. PDND (all $p = 0.002$), § CSF Aβ lower in PDD vs. controls ($p < 0.001$), vs. RBD ($p = 0.005$), and vs. PDND ($p = 0.022$)

vertex-by-vertex general linear model including potentially modifying covariates. Maps were smoothed using a circularly symmetric Gaussian kernel across the surface with a full-width-at-half-maximum of 15 mm. The software Qdec was used and default *abs* option allowed testing positive and negative correlations. Initial vertex-wise threshold was set at $p = 0.05$ (1.3) to find clusters, and Monte Carlo simulation (supported by Qdec software) with 10,000 repeats was tested to avoid false positives (clusters by chance due to multiple comparisons), with $p \leq 0.05$.

Statistical analyses

Qualitative variables are presented as frequencies/percentages and quantitative ones as medians/interquartile ranges (quantitative variables), and were analysed with PASW 18.0 (IBM, New York, USA). Qualitative variables were compared with Chi-square or Fisher's exact tests and quantitative ones with Kruskal–Wallis or Mann–Whitney's *U* tests. Binary logistic regressions, where appropriate, resulted in odds ratios + 95 % confidence intervals [OR (95 % CIs)], reflecting the risk reduction or increase for each unit increase of the tested variable. Correlations were tested with Spearman's rho in each group separately and, where applicable, in the entire cohort, in the purported premotor–motor–dementia continuum (iRBD + PDND + PDD), and in all PD (PDND + PDD) patients (see above). All statistical tests were two sided, with p threshold set at ≤0.05. No adjusts for multiplicity were applied (except for CTh analyses) due to the exploratory study design [35].

Results

Demographic, clinical and neuropsychological data

There were no significant differences in demographic and clinical variables among groups other than the expected worse UPDRS-III in PD and MMSE scores in PDD (Table 1). Formal neuropsychological comparisons were precluded by the intergroup protocol differences, but considering data as descriptive, overall cognitive performance worsened from iRBD to PDND and then PDD (Table 2).

CSF biomarkers findings

For both CSF total- and oligomeric-α-synuclein ELISAs, the R2 of the standard curves were >0.99, and the inter-plate variability was <10 %.

CSF oligomeric-αSYN significantly differed across groups ($p = 0.003$), due to significantly higher levels in PDD patients vs. controls ($p = 0.043$) and vs. iRBD

Table 2 Descriptive neuropsychological data in iRBD, PDND and PDD patients

Cognitive domain	Test	iRBD		Test	PDND		PDD	
		Median (IQR)	*n* (%) impaired		Median (IQR)	*n* (%) impaired	Median (IQR)	*n* (%) impaired
Memory	Buschke-FR	19 (15–28)	3/21 (14 %)	RAVLT-learning	28 (24–34)	9/21 (43 %)	13 (6–18)	20/20 (100 %)
	Buschke-TR	43 (36–46)	1/21 (5 %)	RAVLT-recall	4 (3–6)	7/21 (33 %)	0 (0–0)	19/20 (95 %)
	Buschke-FDR	9 (4–11)	2/21 (9.5 %)	RAVLT-recognition	26 (23–28)	3/20 (14 %)	17 (11–23)	13/20 (65 %)
	Buschke-TDR	15 (12–16)	2/21 (9.5 %)					
	Total	–	2/21 (9.5 %)	Total	–	10/21 (48 %)	–	19/20 (95 %)
Attention/ executive	Similarities (WAIS-III)	11 (8–14)	0/22 (0 %)	WMI	3 (1–4)	10/19 (53 %)	3 (2, 3)	14/19 (74 %)
	FAS	23 (13–34)	2/23 (9 %)	Phonetic fluency	9 (5–14)	12/21 (57 %)	4 (1–5)	19/20 (95 %)
	Semantic fluency	16 (13–20)	1/23 (4 %)	Semantic fluency	12 (8–16)	10/21 (48 %)	4 (3–8)	17/20 (85 %)
Language	BNT-60	49 (44–56)	1/22 (4 %)	BNT-15	13 (12–14)	1/21 (5 %)	10 (8–13)	11/20 (55 %)
Visuo-perceptive	VOSP-O-1	NA	NA	VOSP-O-1	20 (18–20)	0/21 (0 %)	17 (16–18)	1/20 (5 %)
	VOSP-O-2	19 (18–20)	1/21 (5 %)	VOSP-O-2	18 (16–20)	1/21 (5 %)	11 (7–15)	15/20 (75 %)
	VOSP-O-3	16 (15–18)	1/22 (4 %)	VOSP-O-3	15 (12–16)	5/21 (24 %)	9 (4–10)	17/20 (85 %)
	VOSP-O-4	18 (15–22)	3/22 (13 %)	VOSP-O-4	19 (15–20)	4/21 (19 %)	10 (7–16)	13/20 (65 %)
	VOSP-O-5	10 (9–13)	2/22 (9 %)	VOSP-O-5	12 (10–14)	2/21 (9.5 %)	14 (11–17)	13/20 (65 %)
	VOSP-S-1	20 (19–20)	1/22 (4 %)	VOSP-S-1	20 (19, 20)	0/21 (0 %)	12 (0–19)	12/20 (60 %)
	VOSP-S-2	8 (7–10)	1/22 (4 %)	VOSP-S-2	9 (7–10)	2/21 (9.5 %)	2 (0–6)	13/20 (65 %)
	VOSP-S-3	10 (10–10)	0/22 (0 %)	VOSP-S-3	10 (10–10)	0/21 (0 %)	8 (6–9)	12/20 (60 %)
	VOSP-S-4	9 (8–10)	0/22 (0 %)	VOSP-S-4	9 (8–10)	0/21 (0 %)	4 (2–6)	16/20 (80 %)
	Total	–	3/21 (14 %)	Total	–	5/21 (24 %)	–	20/20 (100 %)

Abbreviations of VOSP-O subtests: *O1* form-detection, *O2* incomplete letters, *O3* object-decision, *O4* silhouettes, *O5* progressive-silhouettes. Abbreviations of VOSP-S subtests: *S1* position-discrimination, *S2* number-location, *S3* dot-counting, *S4* cube analysis

patients ($p = 0.001$), and higher levels in PDND vs. iRBD patients ($p = 0.043$) (Table 1; Fig. 1a). CSF oligomeric-α-synuclein showed a modest significant positive correlation with CSF τ in the entire cohort (Suppl. Table 1).

CSF total-α-synuclein did not significantly differ among groups (Table 1; Fig. 1b), but strongly and consistently correlated with CSF τ ($\rho > 0.7$ in all groups separately, in PDND + PDD, in iRBD + PDND + PDD and in the entire cohort; Fig. 2a; Suppl. Table 1), as well as with CSF Aβ, especially in PDND + PDD and in PDND patients separately ($\rho = 0.467$ and 0.719, respectively; Fig. 2b; Suppl. Table 1). In view of the strong total-α-synuclein/τ correlation, cases were stratified for varying CSF τ levels, with exclusion of subjects with CSF $\tau > 250$ pg/ml (chosen after the CSF τ cohort median) resulting in a trend to more PD cases than controls with CSF total-α-synuclein below its median value [1 of 6 controls (17 %) vs. 11 of 17 PD patients (65 %); $p = 0.059$; Suppl. Figure 1]. Such a trend was not seen in iRBD (not shown).

CSF total- and oligomeric-α-synuclein did not significantly correlate to each other in any data-sets, and there were not significant differences in the total-oligomeric ratio (data not shown).

CSF τ levels were similarly low in controls, iRBD, and PDND and significantly higher in PDD, whereas CSF Aβ decreased from controls, iRBD, and PDND to PDD (Table 1).

Correlates of CSF oligomeric α-synuclein

CSF oligomeric-α-synuclein positively correlated with UPDRS-III and negatively with MMSE in the entire cohort and in iRBD + PDND + PDD, in a way that the worse the motor and cognitive scores the higher the CSF oligomeric α-synuclein levels (Fig. 2d, e; Suppl. Table 1). CSF oligomeric-α-synuclein did not significantly differ between PDND + PDD patients with normal vs. impaired neuropsychological tests (Table 3), but showed significant

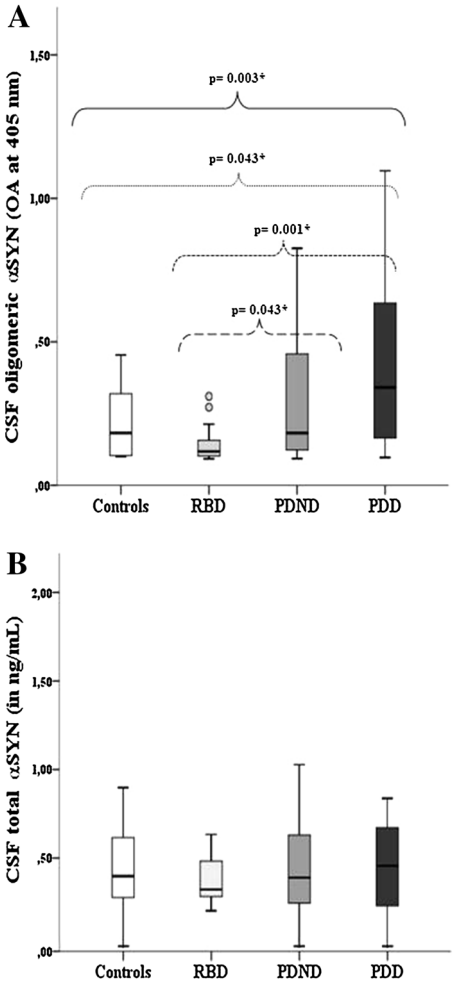

Fig. 1 Box plots of CSF oligomeric-α-synuclein (**a**) and CSF total-α-synuclein (**b**) in controls, iRBD, PDND, PDD. In panel **a**, *solid line* refers to Kruskall-Wallis test among all 4 groups, *dotted line* to Mann–Whitney's *U* test between PDD and controls, the fine-*dashed line* to Mann–Whitney's *U* test between PDD and iRBD and the *thick-dashed line* to Mann–Whitney's *U* test between PDND and iRBD (*asterisk* denotes statistical significance)

negative correlations with semantic fluency and VOSP-O2, S1, S3 and S4 sub-scores in the iRBD + PDND + PDD continuum, and with VOSP-O1 and S3 sub-scores in PDND + PDD (Table 4; Fig. 2f). CSF oligomeric-α-synuclein did not correlate with any CTh measures (data not shown).

Correlates of CSF total α-synuclein

CSF total-α-synuclein positively correlated with age (Fig. 2c; Suppl. Table 1) in the entire cohort, in controls, and, especially, in PDD patients ($\rho = 0.62$; $p = 0.004$). CSF total-α-synuclein was lower in PDND + PDD patients with impaired vs. normal phonetic fluency ($p = 0.026$), but higher in those with impaired vs. normal RAVLT-recognition and BNT-15 ($p = 0.030$ and $p = 0.049$, respectively) (Table 3). However, only the association of low CSF total-α-synuclein with impaired phonetic fluency remained significant when including both CSF total-α-synuclein and CSF τ as covariates in a binary logistic regression with these tests as outcome, while in contrast RAVLT-recognition and BNT-15 were actually associated with high CSF τ (Table 3). CSF total-α-synuclein positively correlated with phonetic fluency and VOSP-O4 scores in PDND patients (Table 4).

CSF total-α-synuclein co-varied for age and for both age and CSF τ showed significant positive correlations with CTh (the lower the CSF total-α-synuclein, the thinner the cortex) mainly of frontal structures in both iRBD and, with greater significance, PDND subjects (Table 5; Fig. 3, top–middle). In PDD patients, CSF total-α-synuclein co-varied for age showed negative correlations with CTh (the higher the CSF total-α-synuclein, the thinner the cortex) mainly of a large right parahippocampal cluster expanding to the isthmus and the precuneus. These correlations did not remain significant when co-varying for both age and CSF τ (Table 5; Fig. 3, bottom), with CSF τ levels negatively correlating with CTh from similar areas (Suppl. Table 2; Suppl. Figure 2).

Discussion

In this cross-sectional study of a cohort intended to represent the premotor–motor–dementia PD continuum, we have found higher levels of CSF oligomeric-α-synuclein in PD mostly in the setting of dementia, albeit without differences in putative premotor (iRBD) cases, and a trend to low CSF total-α-synuclein in PD (but again not iRBD) when controlling for high CSF τ. We have identified clinical, neuropsychological and/or MRI correlates for the studied CSF α-synuclein markers across the premotor–motor–dementia categories. CSF total-α-synuclein correlations with CSF Aβ and CSF τ favour the shared relevance of these proteins in PD, with the correlation with CSF τ potentially accounting for high (instead of low) CSF total-α-synuclein (and its neuropsychological and MRI correlates) in PD patients with cognitive impairment.

The finding of increased CSF oligomeric-α-synuclein in PD and mostly PDD is in keeping with previous reports [8, 14, 36]. Lack of differences in the oligomeric-total ratio in contrast to two previous reports [8, 18] might be due to lack of differences in CSF total-α-synuclein in our cohort. The large overlap among groups and the lack of association with iRBD cast doubt on CSF oligomeric-α-synuclein as a standalone diagnostic or premotor PD biomarker. Still, high CSF oligomeric-α-synuclein levels, besides being particularly associated with PDD, also showed modest yet significant correlations with motor and both global and

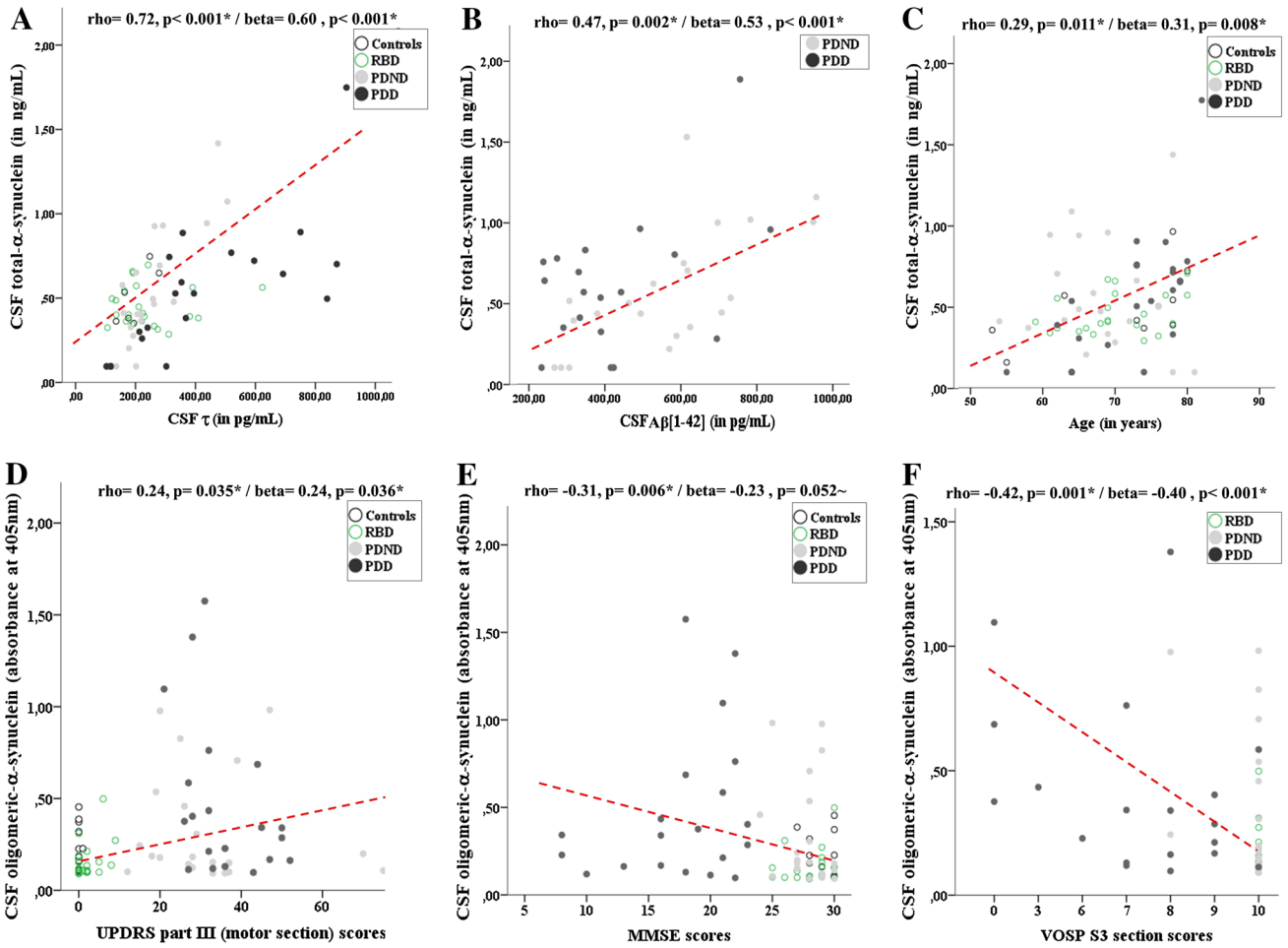

Fig. 2 Scatter plots of significant clinical, CSF or neuropsychological correlations of CSF total-α-synuclein (**a**–**c**) and CSF oligomeric-α-synuclein (**d**–**f**): **a** significant positive correlation between CSF total-α-synuclein and CSF τ levels in the entire cohort; **b** significant positive correlation between CSF total-α-synuclein and CSF Aβ levels in all PD (PDND + PDD) patients; **c** significant positive correlation between CSF total-α-synuclein levels and age at lumbar puncture in the entire cohort; **d** significant positive correlation between CSF oligomeric-α-synuclein levels and UPDRS-III scores in the entire cohort; **e** significant negative correlation between CSF oligomeric-α-synuclein levels and MMSE scores in the entire cohort; **f** significant negative correlation between CSF oligomeric-α-synuclein levels and VOSP-S3 scores in iRBD + PDND + PDD patients

specific (posterior-cortical) cognitive dysfunction across the premotor–motor-dementia continuum, in a way that the higher CSF oligomeric-α-synuclein, the greater UPDRS-III and lower MMSE, semantic fluency and several VOSP-variables scores. Notably, the latter neuropsychological functions with a posterior-cortical basis were predictors of PDD in a longitudinal population-based study [20]. Thus, CSF oligomeric-α-synuclein might be a biomarker of disease progression or severity. The lack of correlations in groups separately might be due to smaller size and predominance of normal scores in iRBD and impaired performance in PDD. The lack of significant CTh correlates of CSF oligomeric-α-synuclein might be due to stringently adjusted CTh analyses and the fact that these were limited to part of the participants and not carried out across the entire continuum after the use of different MRI protocols.

CSF total-α-synuclein has ranged in previous studies from non-significant [13–16] to modest lowering in PD vs. controls [7–12]. Even though the lack of differences in CSF total α-synuclein in our study including for the first time iRBD patients might suggest that CSF total α-synuclein is not a premotor or diagnostic PD biomarker, the presence of significant MRI correlates of this CSF marker even in iRBD patients supports further longitudinal research of the combined use of CSF total α-synuclein and quantitative MRI (CTh) as candidate biomarkers of progression of iRBD to PD. We acknowledge that we have not measured CSF haemoglobin, but the true impact of α-synuclein from red blood cells on CSF total-α-synuclein remains unclear as some studies have found CSF total-α-synuclein differences without adjusting for CSF haemoglobin [8–10]. Besides, the intergroup overlap has been large even in

Table 3 Comparisons of CSF oligomeric- and total-α-synuclein levels in PDND + PDD patients with normal vs. altered neuropsychological tests

	n	CSF oligomeric-α-Syn (A 405 nm)	*p* value	CSF total-α-Syn (ng/ml)	*p* value
Memory domain					
RAVLT-learning					
N	12	0.18 (0.11–0.42)	0.17	0.41 (0.26–0.81)	0.74
A	29	0.29 (0.14–0.72)		0.43 (0.19–0.67)	
RAVLT-recall					
N	15	0.19 (0.14–0.54)	0.66	0.32 (0.11–0.63)	0.30
A	26	0.26 (0.13–0.61)		0.45 (0.24–0.69)	
RAVLT-recognition					
N	24	0.22 (0.14–0.52)	0.96	0.30 (0.03–0.65)	0.030*
A	16	0.22 (0.12–0.54)		0.52 (0.42–0.83)	
Attention/executive domain					
WMI					
N	14	0.21 (0.12–0.72)	0.96	0.47 (0.23–0.68)	0.57
A	24	0.23 (0.14–0.45)		0.40 (0.17–0.68)	
Phonetic fluency					
N	10	0.18 (0.10–0.58)	0.31	0.75 (0.33–0.92)	0.026*
A	31	0.28 (0.15–0.58)		0.39 (0.16–0.59)	
Semantic fluency					
N	14	0.20 (0.14–0.61)	0.89	0.37 (0.21–0.88)	0.44
A	27	0.24 (0.13–0.46)		0.44 (0.18–0.64)	
Language domain					
BNT					
N	29	0.24 (0.13–0.50)	0.74	0.32 (0.13–0.67)	0.049*
A	12	0.22 (0.13–0.66)		0.55 (0.45–0.69)	
Visuo-perceptive domain					
VOSP-o					
N	14	0.19 (0.12–0.48)	0.60	0.52 (0.31–0.88)	0.12
A	26	0.23 (014–0.61)		0.32 (0.18–0.61)	
VOSP-s					
N	22	0.18 (0.10–0.55)	0.087	0.39 (0.16–0.87)	0.98
A	18	0.34 (0.17–0.51)		0.45 (0.24–0.67)	

Data represent median (IQR). Concentrations are expressed in pg/ml. Binary logistic regressions of significant comparisons co-varying for CSF τ: OR (95 % CI) for impaired phonetic fluency, CSF total-α-synuclein = 0.01 (0.00–0.32) ($p = 0.010$*), CSF τ = 1.01 (1.00–1.01) ($p = 0.095$); OR (95 % CI) for impaired RAVLT-recognition, CSF total-α-synuclein = 0.57 (0.03–12.30) ($p = 0.72$), CSF τ = 1.01 (1.001–1.017) ($p = 0.028$*); OR (95 % CI) for impaired BNT, CSF total-α-synuclein = 0.50 (0.03–11.44) ($p = 0.73$), CSF τ = 1.01 (1.001–1.013) ($p = 0.020$*)

N normal, *A* altered

* Significant differences (Mann–Whitney's *U* test)

Table 4 Summary of significant correlations of CSF oligomeric- and total-α-synuclein levels with neuropsychological tests in different subsets of participants

	CSF oligomeric-α-Syn		CSF total-α-Syn	
	ρ	*p*	ρ	*p*
iRBD + PDND + PDD ($n = 64$)				
Semantic fluency	−0.294	0.019*	+0.074	0.566
VOSP-O2	−0.307	0.016*	+0.034	0.796
VOSP-S1	−0.248	0.052	−0.074	0.569
VOSP-S3	−0.419	0.001*	−0.023	0.863
VOSP-S4	−0.300	0.018*	−0.033	0.802
iRBD ($n = 23$)	No significant correlations			
PDND + PDD ($n = 41$)				
VOSP-O1	−0.310	0.052	+0.049	0.766
VOSP-S3	−0.322	0.043*	+0.035	0.833
PDND ($n = 21$)				
Phonetic fluency	+0.049	0.766	+0.478	0.028*
VOSP-O4	+0.035	0.833	+0.468	0.032*
VOSP-S1	+0.049	0.766	+0.113	0.625
PDD ($n = 20$)	No significant correlations			

The *r* and *p* values represent Spearman correlation analyses

* Significant correlations

studies controlling for CSF haemoglobin [7, 11]. Our findings suggest an alternative potential confounding factor in CSF total-α-synuclein studies: CSF τ. The strong positive total-α-synuclein/τ correlation in PD besides being partly a result of low levels of both CSF markers in some cases [11], implies that both markers can also be increased in some PD patients (Fig. 2a). The mechanisms why CSF total-α-synuclein and CSF τ can be either reduced or increased in PD are unknown. Parenchymal sequestration and reduced clearance into CSF might account for their reduction. Conversely, increased leakage to CSF might underlie their increase. Notably, high levels of both CSF total-α-synuclein and CSF τ are being increasingly reported in Alzheimer's disease (AD) [16, 37], and cortical τ neurofibrillary pathology is not rare in PDD and correlates with cortical Lewy pathology [38]. In the light of all this and of the trend to low CSF total-α-synuclein in PD when controlling for high CSF τ, it can be hypothesised that greater cortical pathology (including Alzheimer-type lesions in some instances) in the setting of advanced PD with cognitive impairment or dementia might result in increased leakage of intracellular proteins as α-synuclein and tau to CSF from neurons undergoing degeneration. The lack of a trend to low CSF total-α-synuclein in iRBD when controlling for high CSF τ might be due to more restricted α-synuclein pathology in premotor PD [4].

Besides correlating with CSF τ, CSF total-α-synuclein has also shown a significant correlation with CSF Aβ. These correlations could be an in vivo reflection of the close association between all three proteins shown by clinico-pathological studies [39]. Furthermore, Aβ can enhance fibrillisation of α-synuclein [40] and specific α-

Table 5 Correlations of CSF total-α-synuclein levels with cortical thickness in iRBD, PDND and PDD, co-varied for age only, and co-varied for both age and CSF τ levels (see also Fig. 3)

Cortical region	Size mm^2	Tailarach coordinates (x, y, z)	z Max	Corrected Cluster p value
iRBD ($n = 19$)				
Co-varied for age				
Left middle temporal	2,563.14	−41.0, 4.4, −29.5	3.023	0.00040
Left lateral occipital	1,350.99	−24.3, −90.3, 12.1	3.018	0.04140
Left lingual	1,654.51	−13.8, −69.2, −1.2	2.882	0.01220
Right lingual	5,992.25	10.9, −74.2, 2.0	3.595	0.00010
Right post-central	1,675.53	34.9, −26.7, 61.7	2.188	0.01180
Co-varied for age + CSF τ levels				
Left pars opercularis	1,903.89	−44.1, 12.3, 2.6	2.591	0.00520
Right pars triangularis	1,469.58	51.9, 21.6, 8.5	3.410	0.03970
Right superior frontal	2,047.73	8.1, 40.2, 33.5	2.516	0.00420
PDND ($n = 16$)				
Co-varied for age				
Right superior frontal	3,425.02	14.1, 61.0, 7.9	4.330	0.00010
Co-varied for age + CSF τ levels				
Right superior frontal	2,712.45	14.7, 60.6, 7.7	3.945	0.00020
PDD ($n = 12$)				
Co-varied for age				
Left caudal middle frontal	2,492.11	−41.0, 3.8, 44.3	−3.807	0.00050
Right parahippocampal	4,135.62	21.2, −30.1, −10.8	−3.277	0.00010
Right middle temporal	1,530.78	64.2, −34.8, −6.8	−3.059	0.03180
Co-varied for age + CSF τ levels				
None significant	–	–	–	–

Results were obtained using Monte Carlo simulation with 10,000 iterations applied to cortical thickness maps to provide cluster-wise correction for multiple comparisons with p threshold set at <0.05 (1.3)

synuclein strains can induce τ aggregation [41], suggesting a synergism between these proteins. The significant correlation with CSF Aβ in contrast to the preliminary PPMI report in early PD patients [11] might be due to inclusion in our study of more advanced PD patients with dementia and clearly lowered CSF Aβ.

As for the correlates of CSF total-α-synuclein, it has shown modest (entire cohort) to moderate (PDD) positive correlation with age. Thus, caution will probably also be needed when interpreting CSF total-α-synuclein in elder and demented PD patients. In terms of neuropsychological associations, CSF total-α-synuclein was significantly lower in PD patients with impaired phonetic fluency, a typical attentional/frontosubcortical cognitive indicator, in keeping with its mostly frontal CTh correlates, not only in PDND, but also in iRBD. Such associations raise the question whether, despite the lack of cross-sectional significant differences, iRBD and PDND patients with low CSF total-α-synuclein and frontal cortical thinning are at risk of further progression within the PD continuum. Longitudinal studies should address this.

The unexpected neuropsychological and CTh correlates of high (instead of low) CSF total-α-synuclein might also be due to a potential modifying effect of high CSF τ, as these associations did not resist adjust for CSF τ, an AD biomarker. Interestingly these neuropsychological (recognition memory and naming) and MRI (parahippocampal and precuneus cortical thickness) correlates were also AD like. Both in our previous reports with almost the same PD participants and in the current one, high CSF τ has been associated with AD-type neuropsychological and quantitative MRI changes [27, 42]. As speculated above, more aggressive neurodegeneration and sometimes comorbid AD-type τ pathology in PD might contribute to cognitive impairment, greater atrophy, and increased (instead of decreased) CSF total-α-synuclein. It is noteworthy that high (not low) CSF total-α-synuclein has been recently associated with cognitive deterioration in PD using samples from the DATATOP cohort [43]. In this study the authors provided an alternative potential explanation of the presence of high (instead of low) CSF total-α-synuclein in patients with cognitive impairment, suggesting that it might reflect the inability of neurons undergoing more aggressive neurodegeneration of retaining the physiologically active α-synuclein protein intracellularly thus resulting in higher extracellular (i.e., CSF) concentration of this protein [43].

Both CSF α-synuclein markers did not significantly correlate to each other and their correlates have differed, suggesting that they might differently reflect functional and structural changes in the proposed PD continuum. Thus,

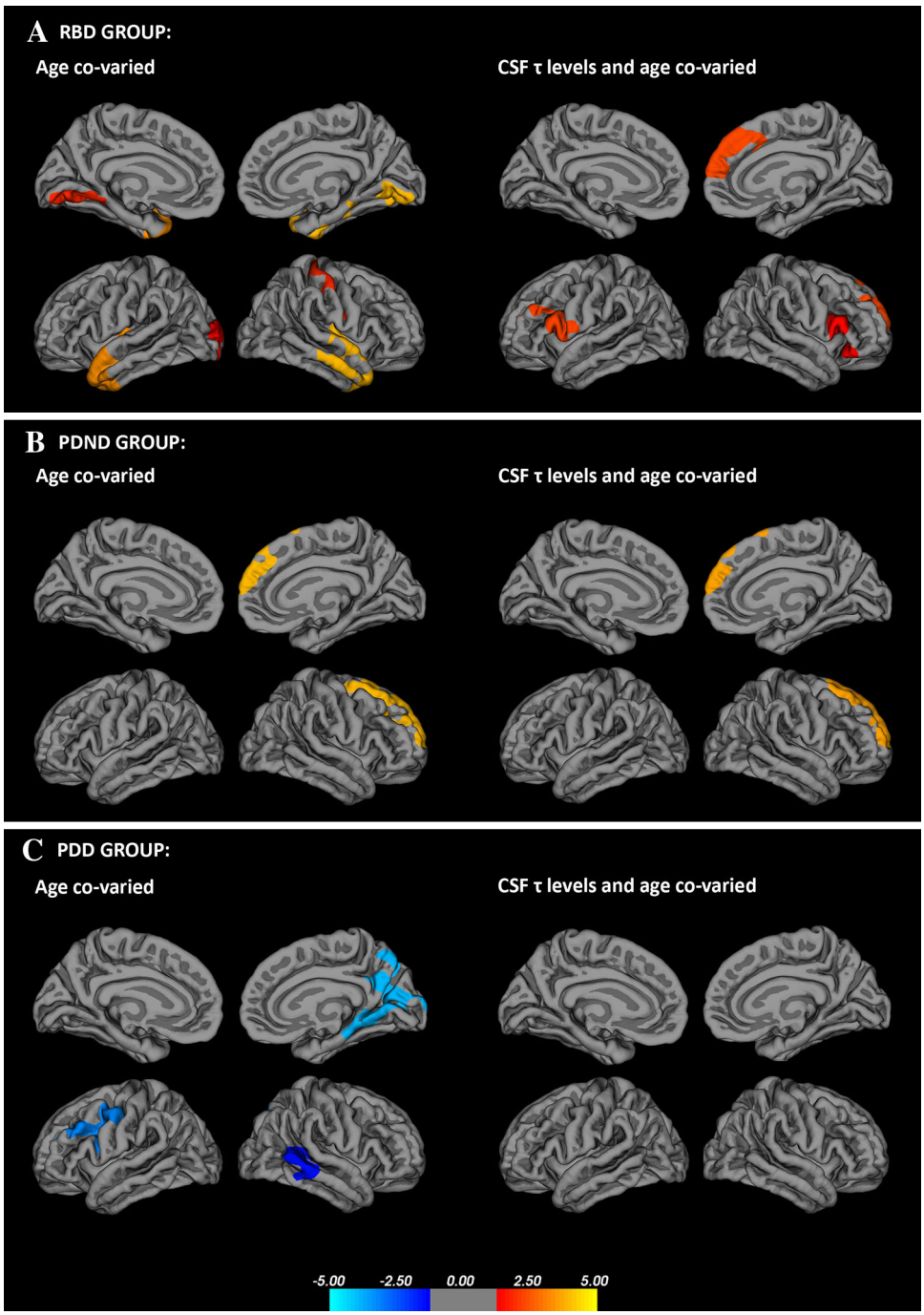

Fig. 3 Significant positive correlations between CSF total-α-synuclein levels and CTh in iRBD (*upper panel*) and PDND (*middle panel*) co-varied for age only (*left*) or for age and CSF τ (*right*), as well as significant negative correlations between CSF total-α-synuclein levels and CTh in PDD patients only when co-varying for age (*bottom left*), not resisting co-variation for both age and CSF τ (*bottom right*). Results were obtained using Monte Carlo simulation with 10,000 iterations applied to CTh maps to provide cluster-wise correction for multiple comparisons (p threshold <0.05; 1.3)

while the neuropsychological and MRI correlates of both low and high CSF total-α-synuclein were noticeably congruent (mostly frontal in the case of low levels and posterior cortical in the case of high levels), the motor and cognitive correlates of CSF oligomeric-α-synuclein were not mirrored by any MRI correlations. Studies of larger cohorts shall better characterise CTh correlates of both CSF oligomeric- and total-α-synuclein.

This study has a number of limitations. The convenience cohort has the hurdle of differences in neuropsychological and MRI protocols that have precluded performing all correlations across all groups. However, it has enabled putting together a cohort that otherwise would not have been easily accessible. The non-availability of neuropsychological assessment and MRI in the small-sized control group does not allow for concluding that the observed

correlations are disease specific and future studies shall definitely include larger control groups and ensure the availability of all biomarker studies in the majority of participants. A limitation of the neuropsychological protocols was the fact that these differed between iRBD and PD patients, along with the lack of tests specifically covering certain functions (i.e., problem-solving and set-shifting abilities), but still most tests used in the present study are part of the recommendations by the international Parkinson's disease and movement disorders society to assess cognition in PD [44]. As stated above, the non-availability of MRI in part of the participants may have accounted for underpowered CTh analyses. This study is cross-sectional and longitudinal surveys combining CSF, MRI and other putative premotor biomarkers (smell testing, transcranial ultrasound, DAT SPECT) will be needed, particularly in the case of iRBD, as it can herald other synucleinopathies, albeit PD is its most frequent outcome [22]. In our cohort disease duration was not significantly longer in PDND vs. PDD groups when it might be expected to be longer in PDD. However, disease progression in terms of both dementia and spread of neuropathological damage to cortical areas does not always correlate with disease duration. Finally, our findings have to be taken with caution due to lack of adjust for CSF haemoglobin and technical differences with other studies. However, the CSF, clinical, neuropsychological and MRI associations in our cohort are plausible and partly in keeping with existing evidence [11, 14, 36, 43].

The present study has also important strengths, as being the first, to the best of our knowledge, assessing both oligomeric and total CSF α-synuclein forms in a cohort attempted to reflect the premotor–motor–dementia PD continuum. The multimodal approach, assessing the clinical, neuropsychological and MRI correlates of both CSF α-synuclein markers, not only in PDND, but also, for the first time, in iRBD and in PDD, is another strength.

In summary, the observed correlates of CSF oligomeric- and total-α-synuclein in a cohort ranging from putative premotor-PD to advanced PD-dementia suggest that these CSF measures might be progression or severity PD biomarkers, differently reflecting functional and/or structural changes across the premotor–motor–dementia PD continuum. Factors such as high CSF τ and increasing age were associated with high CSF total-α-synuclein, thus being potential confounders to consider in future research. The correlations of CSF total-α-synuclein not only with CSF τ but also CSF Aβ are in keeping with the suggestion of synergism between these proteins in PD [38–41]. It remains to be seen whether other CSF α-synuclein-related biomarkers as phosphorylated-α-synuclein [45], DJ-1 [7], glucocerebrosidase [18], or, in the future, molecular imaging of α-synuclein-containing lesions, will eventually overcome the limitations of CSF oligomeric- and total-α-synuclein as PD biomarkers.

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Ethical standard This study was approved by the Institution Ethics Committee and has therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. All participants gave their informed consent prior to their inclusion in the study. There are no details in this manuscript that might disclose the identity of the participants.

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