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RESEARCH ARTICLE

Cerebrospinal α-Synuclein Oligomers Reflect Disease Motor Severity in DeNoPa Longitudinal Cohort

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ABSTRACT: Background: Tangible efforts have been made to identify biomarkers for Parkinson's disease (PD) diagnosis and progression, with α-synuclein (α-syn) related biomarkers being at the forefront.
Objectives: The objectives of this study were to explore whether cerebrospinal fluid (CSF) levels of total, oligomeric, phosphorylated Ser 129 α-synuclein, along with total tau, phosphorylated tau 181, and β-amyloid 1–42 are (1) informative as diagnostic markers for PD, (2) changed over disease progression, and/or (3) correlated with motor and cognitive indices of disease progression in the longitudinal De Novo Parkinson cohort.
Methods: A total of 94 de novo PD patients and 52 controls at baseline and 24- and 48-month follow-up were included, all of whom had longitudinal lumbar punctures and clinical assessments for both cognitive and motor functions. Using our in-house enzymelinked immunosorbent assays and commercially available assays, different forms of α-synuclein, tau, and β-amyloid 1–42 were quantified in CSF samples from the De Novo Parkinson cohort.
Results: Baseline CSF total α-synuclein was significantly lower in early de novo PD compared with healthy controls, whereas the ratio of oligomeric/total and phosphorylated/total were significantly higher in the PD group. CSF oligomeric-α-synuclein longitudinally increased over the 4-year follow-up in the PD group and correlated with PD motor progression. Patients at advanced stages of PD presented with elevated CSF oligomeric-α-synuclein levels compared with healthy controls.
Conclusions: Longitudinal transitions of CSF biomarkers over disease progression might not occur linearly and are susceptible to disease state. CSF oligomeric-α-synuclein levels appear to increase with diseases severity and reflect PD motor rather than cognitive trajectories. © 2021 The Authors. *Movement Disorders* published by Wiley Periodicals LLC on behalf of International Parkinson and Movement Disorder Society

Key Words: Parkinson's disease; α-synuclein; oligomers; biomarkers; DeNoPa; longitudinal cohort; disease progression

Parkinson's disease (PD) is a complex, progressive neurodegenerative disorder that is linked to α-synuclein (α-syn) misfolding and aggregation, leading to neuronal damage. Clinical diagnosis of PD is made according to UK Brain Bank Society criteria based on motor symptoms and a positive effect of dopamine replacement therapy.[1,2]

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The complex nature of the disease, the heterogeneity of the clinical phenotypes, and the significant clinical and pathological overlap with other neurodegenerative diseases all hinder the clinical diagnostic accuracy of PD, especially in the early stage of the disease.[3-5] Clinical trials for PD therapeutics are hampered by the heterogeneity of the patient population and lack of well-established end points and/or biomarkers to assess disease progression and response to therapy. To address these needs, several promising studies have been conducted to identify PD-specific biomarkers comprising risk factors and diagnostic and progression biomarkers.

The most relevant forms of α-syn to disease-underlying mechanisms have long been a source of debate and speculation for researchers. Several research groups including ours explored the diagnostic and progression biomarker potential of total- (t-), oligomeric (o-), and phosphorylated Ser 129 (pS129-) α-syn forms either individually or in combination with other biomarkers using different quantification methods.[4,5] Levels of t-α-syn in cerebrospinal fluid (CSF) are mostly lowered in PD and other synucleinopathies.[6-10] CSF o-α-syn levels are significantly elevated in PD, dementia with Lewy bodies, and individuals at high risk of developing PD.[9-11] CSF o-α-syn (but not t-α-syn) levels correlate with PD motor scales.[9,12] Reports about the levels of pS129-α-syn in CSF are more contradictory, with CSF pS129-α-syn either higher in PD or indifferent compared with control groups.[9,10,13] Longitudinal changes in CSF α-syn forms have also been examined by several groups in PD cohorts for up to 2 years of follow-up.[12,14-17] Characterizing the longitudinal dynamics of CSF α-syn forms over PD progression compared with healthy aging is crucial to advance our understanding of how α-syn reflects disease progression and could also provide reference data for improving the design and management of current and upcoming disease-modifying clinical trials for PD.

The De Novo Parkinson (DeNoPa) cohort represents a longitudinal, single-center, prospective observational study of drug-naive PD patients and age-, sex-, and education-matched neurologically healthy controls (HCs). The study aims to discover diagnostic, progression, and prognostic biomarkers for PD, scored at baseline with biannual follow-up.[18,19]

In the current study, we explored whether a composite panel of CSF t-, o-, pS129-α-syn, total tau (t-tau), phosphorylated tau 181 (p-tau), and β-amyloid 1–42 (Aβ42) would be instructive as diagnostic and/or progression biomarkers of PD versus HCs. We also explored which biomarkers at baseline of different modalities would correlate with motor and cognitive indices of disease progression over the 24- and 48-month follow-up (FU). Given that PD is a complex syndrome with clinical heterogeneity, we also investigated if α-syn levels differed among the different PD clinical phenotypes.

Methods

CSF Sample Collection

Lumbar punctures were performed between 8 AM and 9 AM under fasting conditions, and CSF samples were collected in polypropylene tubes and processed according to standard operating procedures and as described.[18,20] Samples were then aliquoted and stored at −80°C within 20 to 30 minutes after sample collection until further analysis. CSF samples with hemoglobin (Hb) levels higher than 200 ng/mL were excluded from analysis, as traces of blood may influence CSF α-syn level[19] (for details of subjects' study flow, please see Fig. 1).

Study Population

Detailed inclusion and exclusion criteria of the DeNoPa cohort, demographics, and study design have been described in previous publications.[18,21] Briefly, newly diagnosed de novo PD patients aged between 40 and 85 years old were recruited. Patients had to fulfill de novo PD criteria with levodopa exposure no longer than 2 weeks and not within 4 weeks prior to study entry. The clinical diagnosis was established according to UK Brain Bank Society criteria,[1] with the investigators blinded to the outcome of nonmotor symptom assessments. To ensure diagnostic accuracy at this early stage, the patients were reexamined after 24 and 48 months by 2 independent teams of neurologists.[22] Healthy controls were matched for age, sex, and education. Family history of idiopathic PD was ruled out for all healthy controls. All subjects were recruited between September 2008 and January 2012 at the Paracelsus-Elena Klinik in Kassel, Germany. Longitudinal data for both patients and controls including motor and cognitive assessments were collected at baseline, at 24-month follow-up (24FU), and at 48-month follow-up (48FU). In the current study, we report data from available CSF samples of 94 PD patients and 52 healthy controls at baseline.

Clinical Assessment

Patients were classified into different subgroups based on Hoehn and Yahr (H&Y) stage at baseline. The H&Y stage I group was defined as patients with H&Y score ≤ 1.5, whereas the H&Y stage II group was defined as patients with H&Y score ≥ 2.

Measurements of CSF Biomarkers

We measured different CSF biomarkers that have been previously discussed as potential diagnostic markers of PD diagnosis and/or progression, including t-α-syn, o-α-syn, pS129-α-syn, t-tau, p-tau, and Aβ42. Please see supporting data for experimental details on the protocols used. For all assays, samples were run in duplicates and measured in a blinded fashion, and both

FIG. 1. Flowchart of enrolled and followed subjects with Parkinson's disease and healthy controls. Flowchart presenting the number of cases at each point, exclusion criteria, and number of matched cases. [Color figure can be viewed at wileyonlinelibrary.com]

groups were randomized over the plates. The baseline and follow-up samples from the same individual were run in the same plate next to each other. A series of internal controls (n = 5) were also run to normalize for inter-run variation. Specified calibrators were used to generate an 8-point standard curve to which a 4-parameter logistic curve of all plates was fitted and used to quantify unknown concentrations using GraphPad Prism software.

Statistical Analysis

Statistical analyses were performed using IBM SPSS software (version 24.0; Chicago, IL), GraphPad Prism (version 8.3.0), and R software (version 3.4.4) where appropriate. For plotting the scatterplots, receiver operator characteristic curves, and correlation analysis, GraphPad Prism was used. For DeNoPa data extracting, analyzing, and matching the statistic, R software (version 3.4.4) was used. For plotting Figure 2, function "geom_smooth" from the R "ggplot" package was used. All data sets were tested for normality and the presence of outliers. As data were considered inappropriate for parametric analyses, Spearman rank order correlation coefficients were used to examine correlations within the study groups. Continuous variables were described using median and interquartile range. Categorical variables were presented as count or percentages. The Mann–Whitney *U* test was used for comparisons between PD and HC diagnostic groups across visits. Differences between baseline and follow-up were assessed using the Wilcoxon signed rank test for paired comparisons. Changes in biomarkers and clinical parameters were calculated as the differences between observations at 24FU and baseline and between 48FU and baseline. In all the analyses, $P < 0.05$ was set as the level of statistical significance. As some patients received dopamine replacement therapy (ie, levodopa) at the 24- and 48-month FUs, we explored whether medication had any confounding impact. The levodopa-equivalent dose was calculated according to Tomlinson conversion formuls.[23]

Results

Characterization of New Oligomer-Specific ELISA

We developed and validated a new oligomeric-specific enzyme-linked immunosorbent assay (ELISA) for measuring α-syn soluble aggregates (ie, oligomers) in human CSF. To secure a consistent manner of assay performance among future studies, a renewable source of antibodies employed in the assay format is vital. Toward that goal, in the current ELISA, both capture (2A1) and detection (3G7) antibodies employed in the assay format were mouse monoclonal antibodies. The detection antibody was used in its biotinylated form, allowing the use of streptavidin–horseradish peroxidase (HRP) conjugate rather than a secondary antibody-HRP conjugate, further eliminating the use of any polyclonal antibodies (commercial polyclonal antibodies have high batch-to-batch variation). The capture antibody, 2A1, is a conformation-specific antibody that specifically recognizes α-syn aggregates, both full-length (1–140 aa), and C-terminally truncated (1–135, 1–130,

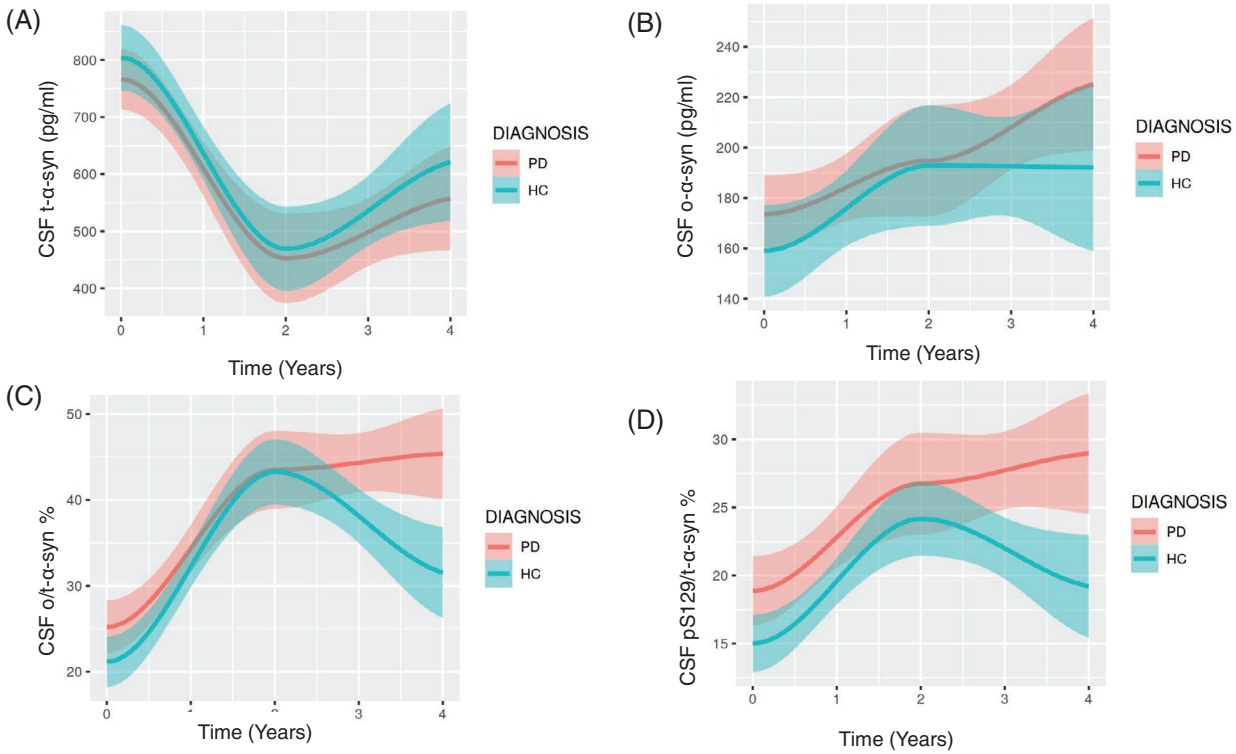

FIG. 2. Longitudinal changes of CSF α-syn biomarkers over time in PD and HC groups. CSF (**A**) total (t-), (**B**) oligomeric (o-), (**C**) oligomeric/total ratio (o-/t- %), and (**D**) phosphorylated S129/total ratio (pS129-t- %) α-syn levels at each visit in Parkinson's disease (PD) and healthy controls (HCs) in the DeNoPa cohort. The solid line gives a smoothed interpolation of the measured data points via locally weighted scatterplot smoothing (LOESS) as implemented in the R package ggplot. The colored ribbon represents the standard errors (standard deviation divided by the square root of the number of measurements) of the measured points and a LOESS fit through them. [Color figure can be viewed at wileyonlinelibrary.com]

1–123, 1–122 aa) forms (Fig. S1; for details about the protein preparation, please see Supporting Data S1). The detection antibody, 3G7, is a mouse monoclonal antibody that recognizes a wide range of α-syn forms, including C-terminal truncated forms (Fig. S2). Examining the ELISA specificity, serial dilutions of α-syn monomers and oligomers were tested, and the assay appeared to be oligomer-specific (Fig. S3A). The standard curve was established using full-length α-syn oligomers displaying a typical sigmoid curve (details about oligomeric-ELISA calibrator preparation were previously published[9]). The assay also showed a large linear dynamic range, with fold changes ranging from ~0.020 to 10 ng/mL of lower/upper limits of quantification, respectively. Intra assay variation was calculated based on 3 standard curves and 5 CSF samples, all in duplicate, which were measured on the same day but on separate plates. Interassay variation data were calculated based on 3 pairs of standard curves and 5 pairs of CSF samples performed on 3 nonconsecutive days. Both intra- and interassay variations were below 15% for all evaluated measurements (Fig. S3B). A spike recovery test was conducted to assess potential impact of CSF matrix on oligomers quantification. In 5 independent CSF samples, different concentrations of α-syn oligomers ranging from low to high (40, 80, 160, and 320 pg/mL) were spiked and extracted from the biological matrix. All calculated recovery rates were within the accepted range (80%–120%)[24]; see Figure S3C. As highlighted earlier, the current 2A1 oligomeric ELISA is different than the previously published one in terms of the range of α-syn aggregates that it measures. Although Syn-O2 ELISA would measure aggregates of full-length α-syn only, 2A1 ELISA measures aggregates of C-terminally truncated α-syn as well. To understand how the measurements of our current ELISA compares with our previous one, we have analyzed the same samples set using Syn-O2 ELISA. Correlation analysis revealed a strong correlation between both assays in both diagnostic groups at all times (Figure S4). However, differences in group comparisons and correlation with clinical parameters were more pronounced using 2A1 ELISA than Syn-O2 ELISA (data not shown).

Demographic and Clinical Data

We analyzed a subgroup of 146 subjects (94 PD and 52 HC) with complete data and CSF available from the DeNoPa cohort. Demographic, clinical, and biomarker data for the whole cohort are shown in Table 1. From there it could be seen that the PD and HC cohorts were sex- and age-matched. In our sample of DeNoPa cohort

TABLE 1 *Demographic, clinical and biomarker data by diagnostic group*

Groups Times*	HC			PD		
	BL (n = 52)	24FU (n = 29)	48FU (n = 15)	BL (n = 94)	24FU (n = 48)	48FU (n = 32)
Hb < 200 ng/mL	48	29	14	86	48	28
Age (y)	67 (61–70)	69 (63–72)	67(63–74)	64 (56–71)	68 (60–73)	69 (63–78)
Sex (male/female), n (%)	36/12 (65%)	16/13 (55%)	9/6 (60%)	56/30 (70%)	29/19 (60%)	14/14 (50%)
UPDRS total	2(1–5)	6 (2–14)	8(6–10)	33 (23–46)	40 (27–58)	48 (33–64)
UPDRS motor	0(0–0)	1 (0–3)	1 (0–2)	21 (12–28)	22 (14–33)	27 (19–39)
H&Y score	0(0–0)	0 (0–0)	0 (0–0)	2(1–2)	2 (1–3)	2(1–3)
MMSE score	29(28–29)	28 (28–29)	30 (29–30)	29 (28–30)	28 (28–29)	28(26–29)
T-α-syn (pg/mL)	826 (713–933)	399 (379–552)	577 (481–732)	763 (634–895)[a,c]	406 (310–547)	405 (363–498)
O-α-syn (pg/mL)	152 (120–187)	190 (164–224)	157 (138–266)	172 (139–192)	186 (134–250)	200 (113–297)
pS129-α-syn (pg/mL)	112 (89–129)	101 (75–131)	98 (89–140)	116 (89–160)	105 (78–126)	128 (92–174)
O-/t-α-syn (%)	20 (14–24)	44 (36–48)	29 (21–36)	23 (18–29)[b,c]	42 (36–52)	41 (27–70)[a,c]
pS129-/t-α-syn %	13 (10–17)	19 (16–31)	20 (12–27)	17 (11–22)★★[b]	27 (16–32)	29 (22–42)★★[b]
tTau (pg/mL)	241 (193–328)	246 (211–322)	NA	205 (163–283)	251 (176–355)	NA
pTau (pg/mL)	44 (35–59)	43 (34–55)	NA	39 (31–51)	44 (30–58)	NA
Aβ-42 (pg/mL)	905 (733–1035)	929 (725–1001)	NA	872 (712–992)	883 (751–1067)	NA

Data are expressed as median (interquartile range [IQR]) or n (%). Cross-sectional differences among the study groups were analyzed at the same point using the Mann–Whitney *t* test for nonparametric distribution, whereas longitudinal changes of continuous variables were assessed by the paired *t* test (Wilcox sign) for each 2 visits.

*Times: baseline (BL), 0 months; 24-month follow-up visit (24FU); 48-month follow-up visit (48FU).

[a]$P < 0.05$.

[b]$P < 0.01$.

[c]The difference is statistically significant between PD and HC groups at baseline.

BL, baseline; HC, healthy controls; H&Y, Hoehn and Yahr scale; MMSE, Mini-Mental State Examination; NA, not available; o-α-syn, oligomeric α-synuclein; pSer129-α-synuclein, phosphorylated α-synuclein protein at serine 129; PD, Parkinson's disease patients; t-α-syn, total α-synuclein; UPDRS-total, Unified Parkinson's Disease Rating Scale UPDRS-III, Unified Parkinson's Disease Rating Scale; 24FU, 24-month follow-up; 48FU, 48-month follow-up, NA: not available.

patients, we did not find any significant impact of dopamine replacement therapy (ie, levodopa) on clinical outcomes. We also explored any potential association between dopamine replacement therapy and CSF levels of α-syn species without finding a significant effect. Hence, we concluded that dopamine replacement therapy cannot be assumed as a potential confounder in the current sample set.

Cross-Sectional Analysis of CSF Biomarkers at Baseline

All Patients

CSF biomarker levels for PD and HC groups across visits are given in Table 1. Annual rate assessment of motor and cognitive progression of PD and HC participants in the DeNoPa cohort revealed progressive deterioration of both motor and cognitive function in PD patients over the 48-month FU.[21] When all participants were included, at baseline, CSF t-α-syn levels were significantly lower in PD (median, 763 pg/mL; IQR, 634–895 pg/mL; n = 86), compared with HC groups (median, 826 pg/mL; IQR, 713–933 pg/mL; n = 48), with large overlap between the diagnostic groups ($P < 0.05$). The ratios of CSF oligomers to total (o-/t-α-syn %) and phosphorylated S129 to total (pS129-/t-α-syn %) were significantly higher in PD (median, 23%; IQR, 18%–29%; and median, 17%; IQR, 11%–22%, respectively), compared with HC (median, 20%; IQR, 14%–24%; and median, 13%, 10%–17%, respectively; $P < 0.01$). For demonstration of CSF α-syn forms between both diagnostic groups across all visits and their diagnostic potential, please see Figures S5 and S6. CSF AD (Alzheimer's disease) classical biomarkers (t-tau, p-tau, and Aβ42) showed no significant differences between the 2 groups.

H&Y Stage I Versus H&Y Stage II

At baseline, PD patients were categorized as H&Y stage I (H&Y ≤ 1.5, n = 22) and H&Y stage II (H&Y ≥ 2, n = 64). When H&Y stage II PD patients were

compared with the HC group at baseline, distinctively, o-α-syn was significantly elevated in H&Y stage II PD (median, 175; IQR, 143–194; n = 64; $P < 0.05$) but not H&Y stage I (median, 161; IQR, 122–190; n = 22) compared with HC (median, 826 pg/mL; IQR, 713–933 pg/mL; n = 48); see Supplementary Figure 7. The ratio of α-syn subforms remained significantly elevated compared with the HC group (data not shown).

Longitudinal Changes in CSF α-Syn Species Over Disease Progression

CSF t-α-syn levels in both PD and HC groups significantly decreased from baseline to the 24-month FU, followed by a small increase from the 24- to 48-month FU (Fig. 2A). In both groups, CSF o-α-syn showed a longitudinal increase over the 24-month FU ($P < 0.05$); however, only in thePD group did the levels continued to increase over the 48 months of disease progression (Fig. 2B). Similar to t-α-syn levels, pS129-α-syn levels slightly dropped from baseline to the24-month FU in both PD and HC groups, but the decrease did not reach statistical significance. Both o-/t-α-syn % and pS129-/t-α-syn ratios generated a significant longitudinal increase from baseline to the 48-month FU in the PD group only (Fig. 2C,D).

Correlation Analysis of α-Syn Species With Clinical Assessments in the PD Group

Data of neither CSF biomarker correlated with clinical assessments in the PD group at baseline or the 24-month FU (data not shown). However, at the 48-month FU, o-α-syn level strongly correlated with H&Y score ($r = 0.65$, $P < 0.001$), MDS-UPDRS III ($r = 0.65$, $P < 0.001$), and MDS-UPDRS total ($r = 0.70$, $P < 0.001$); see Figure 3A–C. All correlation analysis data were stratified by individual (n = 26).

Analyzing the correlation of longitudinal changes in CSF α-syn species and the change in clinical progression variables in PD over the study follow-up duration revealed a positive correlation between the changes in CSF o-α-syn level and the changes in H&Y score from baseline to the 24-month FU ($r = 0.47$, $P < 0.01$, n = 42) and from baseline to the 48-month FU ($r = 0.57$, $P < 0.01$, n = 26); see Figure 4B. When PD cases with H&Y scores stable over visits (change, 0) were excluded from this analysis, the above-mentioned correlations were further strengthened ($r = 0.65$, $P < 0.01$, n = 22; and $r = 0.60$, $P < 0.01$, n = 18, respectively).

Discussion

PD is a clinically heterogeneous disease with a wide range of motor and nonmotor symptoms and large variation in disease-onset and progression patterns among patients. However, there is still unmet need for biomarker(s) that can track disease progression. Longitudinal changes in CSF α-syn forms and other biomarkers in PD have been examined by several research groups in multiple longitudinal cohorts. Among longitudinal studies with up to 2 years' follow-up, 2 studies including ours showed a longitudinal increase in CSF t-α-syn in the early stage of PD,[12,15] 2 reported a decrease,[17,25] whereas 1 showed stable levels.[26] The use of different quantification methodologies and the implementation of different preanalytical procedures and subjects' characteristics may have contributed to these discrepancies.

We investigated CSF α-syn forms in the longitudinal single-center DeNoPa cohort, consisting of 146 CSF samples of de novo PD patients and matched HCs. We also explored the cross-sectional differences between CSF α-syn levels between the PD and HC groups across visits and possible correlations with disease progression clinical variables.

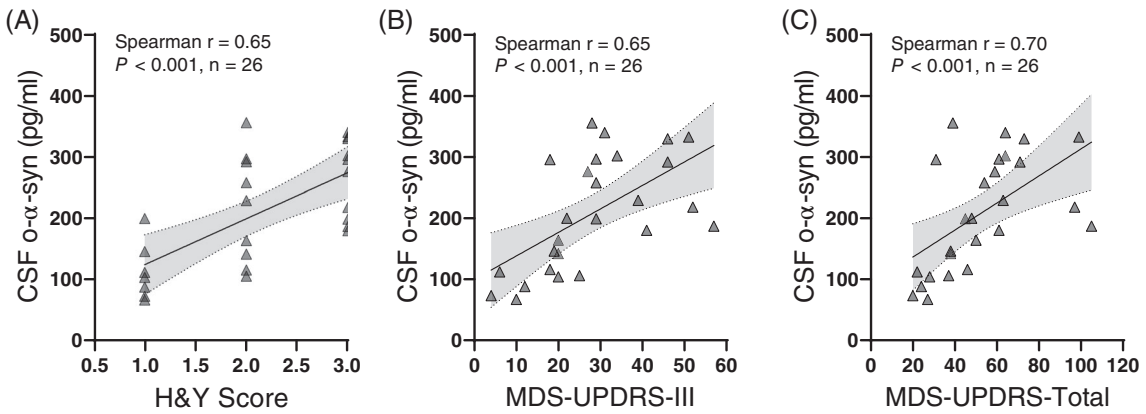

FIG. 3. Spearman correlation analysis of CSF o-α-syn levels for PD patients at 48-month FU with corresponding clinical parameters. Scatterplots showing the correlation between CSF o-α-syn and (**A**) H&Y score ($r = 0.65$, $P < 0.001$), (**B**) MDS-UPDRS-III ($r = 0.65$, $P < 0.001$), and (**C**) MDS-UPDRS-Total ($r = 0.70$, $P < 0.001$). The dotted line highlights the 95% confidence interval for the calculated regression line (solid line).

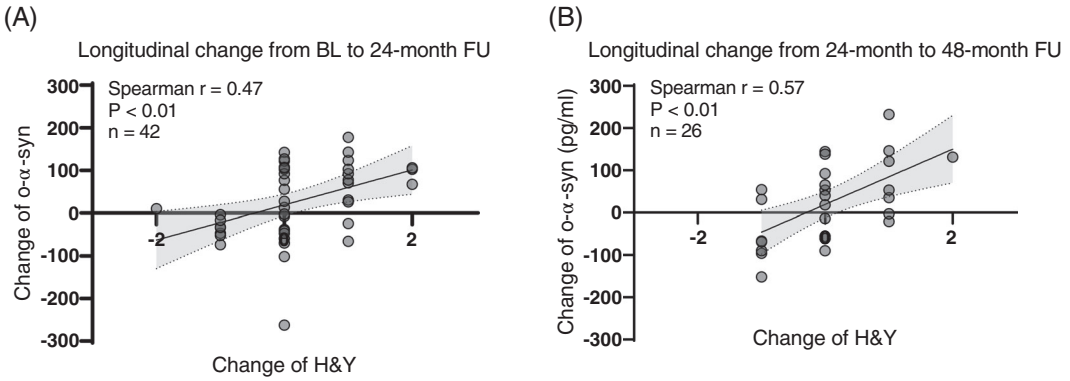

FIG. 4. Scatterplots indicating Spearman correlation analysis between changes in CSF o-α-syn and H&Y score. Spearman correlation between the changes in CSF o-α-syn level and the changes in H&Y score (**A**) from baseline to 24-month FU ($r = 0.47$, $P < 0.001$, n = 42), and (**B**) from baseline to 48-month FU ($r = 0.57$, $P < 0.001$, n = 26). The dotted line highlights the 95% confidence interval for the calculated regression line (solid line).

We found (1) CSF t-α-syn is significantly lower in PD compared with HC, with ratios providing better discrimination at the group level; (2) in PD only, CSF o-α-syn presented with a longitudinal increase over the 4-year follow-up, which correlated with H&Y staging, whereas CSF t-α-syn showed a longitudinal decrease followed by an increase in both PD and HC groups; and (3) clinical subtyping of the PD group showed that PD patients at advanced disease stage (ie, H&Y stage II) presented with elevated CSF o-α-syn levels compared with HCs.

At baseline, CSF t-α-syn levels were significantly lower in PD compared with HC, whereas both o-/t-α-syn % and pS129-/t-α-syn ratios were significantly higher in PD patients compared with HC, providing better group discrimination. A similar observation was also noted at the 48-month FU visit.

Although the reason for the lack of pronounced differences between the groups is not clear, one possible reason could be the complex heterogeneity of the disease among the PD patients.

In contradiction with the current literature reporting different patterns of CSF t-α-syn longitudinal changes between PD and HC,[15,25] in our cohort, CSF t-α-syn appeared to follow U-shaped longitudinal change in both groups. A similar trend was noted in CSF pS129-α-syn levels in the PD group only. On the other hand, CSF o-α-syn continued to increase over the 48-month FU in the PD group only.

Despite the use of similar ELISA assays, different longitudinal changes between the DeNoPa and DATATOP cohorts were noted, as both CSF t- and o-α-syn levels showed a longitudinal increase. The lack of a control group in the DATATOP cohort to compare the dynamic course of α-syn forms between patients and healthy subjects makes it difficult to conclude the reason behind the variability between both studies. Also, the possibility of misdiagnosis, especially in early-stage PD, the genetic underpinnings, and the variability in the velocity of PD clinical progression all challenge the reach for reliable disease progression biomarkers. In DeNoPa cohort, however, misdiagnoses were accounted for through clinical follow-up and reassessment, the gold standard for clinical accuracy.[27]

We also investigated the differences in CSF α-syn forms in different subtypes within the PD group based on H&Y staging. In our cohort, patients at an advanced disease stage (ie, H&Y stage II) presented with elevated CSF o-α-syn levels compared with HCs at baseline, highlighting the potential of CSF o-α-syn to reflect disease severity.

Assessing possible correlations between CSF α-syn species and the clinical assessment parameters of PD patients across all visits, significant correlations only emerged at the 48-month FU visit. CSF o-α-syn levels strongly correlated with MDS-UDPRS total and motor, as well as H&Y score ($r > 0.6$). The link between o-α-syn and the disease motor presentation has been previously reported in multiple studies, one of which is the recent phase 1 clinical trial led by AFFiRiS AG,[9,11,12] showing strong ($r > 0.5$) correlation between o-α-syn and MDS-UPDRS III at baseline. Although the correlation was only noted at the 48-month FU in DeNoPa, this could be because of differences in patient characteristics and inclusion/exclusion criteria. It is becoming increasingly evident that CSF o-α-syn is more intimately related to the motor than the cognitive aspects of PD. Previous studies emphasized the association between CSF t-α-syn and cognitive decline.[8-10,17,28] Although such association was absent in the current study, this could be because most patients were cognitively intact at the time of sample collection. Indeed, a Mini–Mental Status Examination (MMSE) score of 24 and above indicates normal cognition,[29] and MMSE scores remained above 26 for PD patients even at the 48-month FU.

Exploring correlations between the longitudinal change of CSF α-syn forms and disease progression, the change in o-α-syn level correlated with the changes in H&Y score from baseline to the 24-month FU and the

48-month FU. H&Y scale is considered the reference standard for impairment measures in PD[30] and significantly correlates with both quality-of-life measures and motor performance.[31,32] A recent report investigating serum neurofilament-light chain (NfL) showed a positive correlation with disease motor scales in PD.[33] This being said, NfL levels are not specific for PD or any other neurodegenerative diseases. Although most studied CSF and blood biomarkers failed to reflect motor progression, CSF o-α-syn, however, is more PD specific[10,34] and could be useful to track disease motor progression. Within PD multistage spectrum, CSF o-α-syn could be particularly important to differentiate early-stage PD from those at advanced diseases stage. This is particularly essential to assist in the selection of the right subgroup of patients for clinical trial inclusion or stratification. It is also worth highlighting that the correlation was only present with the changes in H&Y score but not MDS-UPDRS III, as previously reported in the DATATOP cohort. However, DATATOP patients were assessed using the original UPDRS scale, whereas DeNoPa patients were assessed using MDS-UPDRS scale, which entails a greater number of disease manifestations, including nonmotor symptoms, and thus discriminates mild manifestations of PD better.[35-37] This might explain the missing link coupling CSF biochemical profile of α-syn species with UPDRS subtypes previously noted in the DATATOP cohort.[12] On the other hand, H&Y scale does not comprehensively reflect therapy-related improvements in PD and, unlike MDS-UPDRS III, is less susceptible to ON/OFF disease state, which may jeopardize the reliability of the UPDRS III as a measure of disease severity over time. Such disagreements highlight the challenges to adopt standard clinical tests for detection of cognitive and motor changes at an early disease stage.

The small and unequal group sizes during follow-up might have confounded the statistical analyses. Considering PD starts years prior to manifestation of symptoms, more focus should be shifted to address early changes in the prodromal stage. Another limitation might be the absence of other CSF biomarkers or supporting imaging data to attain further insights about disease state and progression. Other CSF biomarkers are being currently analyzed in DeNoPa to reflect on different underlying disease mechanisms.

In conclusion, our findings highlight the heterogeneity of PD, as longitudinal transitions of CSF biomarkers might not occur linearly but are rather influenced by different biological and pathophysiological mechanisms. Differences in CSF α-syn profiles among different PD subtypes and the reoccurring correlation between CSF o-α-syn and PD motor presentation highlight the significance of oligomers as marker of PD motor progression. Studies at advanced and/or later PD disease stages could also reveal correlations with CSF biomarkers that could be later examined as disease targets. PD subtyping could perhaps benefit from a reverse approach, in which biomarkers rather than clinical profiles are used. Developing disease-modifying therapies would certainly benefit from identifying biologically more homogenous subgroups of PD patients sharing similar molecular and biomarker profiles. The current findings may provide more insights for future studies and clinical trials in respect to group stratification and disease monitoring. ●

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Supporting Data

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