α-Synuclein Strain Dynamics Correlate with Cognitive Shifts in Parkinson's Disease

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Abstract

α-Synuclein (α-syn) strains can serve as discriminators between Parkinson's disease (PD) from other α-synucleinopathies. The relationship between α-syn strain dynamics and clinical performance as patients transition from normal cognition (NC) to cognitive impairment (CI) is not known. Here, we show that the biophysical properties and neurotoxicity of α-syn strains change as PD cognitive status transitions from NC to mild cognitive impairment (PD-MCI) and dementia (PD-D). Both cross-sectional and longitudinal analyses reveal distinct α-syn strains in PD patients correlating to their level of cognitive impairment. This study presents evidence that individuals with PD have different α-syn strains that change in accordance with their cognitive status and highlights the potential of α-syn strain dynamics to guide future diagnosis, management, and stratification of PD patients.

One Sentence Summary: Distinct features of α-syn strains change with cognitive decline in Parkinson's disease.

Keywords: α-synuclein, strain, SAA, cognitive impairment

Introduction:

Misfolded α-synuclein (α-syn) is a key pathological feature underlying the motor and cognitive changes in individuals with Parkinson's disease (PD). Seed amplification assays (SAAs) of α-syn can serve as potential diagnostic biomarkers for PD, including the discrimination of PD from multiple system atrophy (MSA)[1-3]. Progression biomarkers directly related to PD-pathophysiology are needed to improve patient care, facilitate clinical trial cohort selection, and aid in the development of new therapeutics. Disease progression in PD includes greater motor impairment over time, often coupled with the worsening of non-motor symptoms, including cognitive change. Patients initially demonstrate limited or no cognitive changes early in the disease (PD-NC) and later develop cognitive impairment (PD-CI), including PD-MCI (mild CI) and then PD-D (dementia). The rate and severity of this cognitive change are highly variable between patients, which has been attributed, in part, to the presence of other proteinopathies at autopsy[4] and the extent of α-syn cortical burden[5].

We theorized that if α-syn strains underlie the heterogeneity of α-synucleinopathies, it is possible that α-syn strains change with the different cognitive stages of PD and that α-syn strain properties differ amongst individuals and within an individual over time. To investigate this possibility, we collected cerebrospinal fluid (CSF) samples from clinically well-characterized patients and characterized the biophysical, biochemical, and cellular properties of the amplified α-syn aggregates in relation to the patient's clinical characteristics. Notably we found that α-syn exhibits different aggregation and biophysical properties among PD patients as they transition from normal cognition (NC) to cognitive impairment (CI). These findings indicate that cross-sectional and longitudinal characterization of α-syn strain properties may be of value in the management of PD patients as they transition from NC to CI.

Results:

α-Syn strains change in relation to cognitive status

Protein misfolding cyclic amplification (PMCA) was used as the SAA from cerebrospinal fluid (CSF) obtained in a cross-sectional manner from PD patients and controls from Johns Hopkins (**Fig. 1a**). A series of biophysical and cellular studies including Thioflavin T (ThT) assessments (**Fig. 1a**) were performed to characterize the features of aggregated α-syn. The cognitive status of our cohort was stratified according to Montreal Cognitive Assessment (MoCA) (**Extended data table 1**).

ThT features distinguishes α-syn strains among the cognitive groups

The ThT results showed that the maximal fluorescence intensity (mfi), Lag time (t_{lag}) and time at which aggregation is 50% complete (t_{50}) were different according to cognitive groups. ThT-mfi was significantly higher in PD-D, then decreased in a stepwise fashion with significant differences between PD-D and PD-MCI, PD-MCI and PD-NC, and PD-NC and HC (**Fig. 1b**). The ThT t_{lag} also differed among the cognitive groups. The PD-D group had a smaller ThT-t_{lag} compared to the PD-MCI and PD-NC groups (**Fig. 1c**). The PD-MCI group ThT-t_{lag} was less than the PD-NC group (**Fig. 1c**). There is no ThT-t_{lag} for the HC group due to the minimal fluorescence intensity (**Fig. 1b,c**). The ThT-t_{50} of PD-D was significantly lower than ThT-t_{50} of the PD-NC group (**Fig. 1d**).

Dynamic light scattering (DLS) properties of α-syn strains

DLS was utilized to assess the size profiles (i.e. peak number, intensity, size) of amplified α-syn strains (**Fig. 2a**). The typical DLS spectra of both the PD-MCI and PD-D groups showed significantly greater homogeneity with one peak compared to the PD-NC and HC groups, which exhibited two peaks (**Fig. 2b,c, and Extended Data figure 2a,b,c,d**). Peak size was not significantly different between PDD and PD-MCI, but decreased in a stepwise fashion with significant differences between PD-D or PD-MCI versus PD-NC and HC (**Fig. 2d, e**). Peak intensity was also not different between PD-D and PD-MCI, but increased in a stepwise fashion with significant differences between PD-D or PD-MCI versus PD-NC and HC (**Fig. 2f,**

g). %polydispersity (%PD) of peak 2 was not different between PD-D and PD-MCI, but significant differences were observed between PD-MCI versus PD-NC versus HC (**Fig. 2h, i**).

α-Syn strains are differentially neurotoxic

The amplified α-syn strains were applied to primary mouse cortical neurons and neurotoxicity was assessed by quantifying NeuN immunostaining (**Fig. 2j**). The amplified α-syn strains from the PD-NC group exhibited more neurotoxicity than the HC group (**Fig. 2j,k**). The PD-MCI group showed significantly increased neurotoxicity compared to the PD-NC group, and the PD-D group exhibited the highest neurotoxicity (**Fig. 2k**).

α-Syn strains exhibit different Proteinase K (PK) digestion patterns

PK digestion was performed on the amplified α-syn strains over time and followed with a dot-blot assay for α-syn immunoreactivity (**Fig. 2l,m**) and silver staining (**Fig. 2n,o**) as previously described[6,7]. In the dot-blot assay, the PD-D strain showed the strongest resistance to PK digestion as evidenced by the remaining α-syn signal (**Fig. 2l,m and Extended Data figure 2e,f**); the PD-MCI strain exhibited less resistance than PD-D strain, but more resistance than PD-NC strain (**Fig. 2l,m and Extended Data figure 2e,f**); the PD-NC strain exhibited mild resistance to PK digestion; the HC strain demonstrated minimal resistance to PK digestion (**Fig. 2l,m and Extended Data figure 2e,f**). To assess the digested band patterns in response to PK digestion via silver staining, the digested bands between the 5th and 7th bands were quantified (**Fig. 2n,o**). The results showed significant differences between the PD-D and PD-MCI strains (**Fig. 2n,o**), and between the PD-MCI and PD-NC strains. However, there was no difference between the PD-NC and HC strains (**Fig. 2o**).

α-Syn strains change in relation to cognitive status in the same individual

To determine whether α-syn strains change within the same individuals, the CSF samples from the same individuals obtained at the initial and last visits (either a 3-, 4-, or 5-year follow-up time) of JHU cohort were used to amplify α-syn strains followed by characterization and correlation studies (**Fig. 3 and 4**). ThT-mfi did not change if the cognitive status remained the same between the initial and last visits (**Fig. 1a**). That is, patients who remained HC, PD-NC, PD-MCI, or PD-D between their initial and last visits had a stable ThT-mfi that matched their cognitive strata.

ThT parameters change with cognitive decline

In contrast, the ThT-mfi significantly increased in the individuals whose cognition declined by the last visit compared to the initial visit: PD-NC→PD-MCI, and PD-MCI→PD-D (**Fig. 3a**). Because the ThT results are correlated with the longitudinal cognitive decline, the time resolution was extended to determine whether the ThT profile could predict cognitive decline. In the ThT studies of α-syn strains amplified from these yearly collected longitudinal CSF samples, the results showed the ThT-mfi remained the same in those individuals without cognitive change (**Fig. 3b,c,f**), consistent with the ThT results of the first-last visit (**Fig. 3a**). In those individuals whose cognitive status changed, including PD-NC→PD-MCI, and PD-MCI→PD-D, the results showed that the ThT-mfi increased when cognition progressed from PD-NC to PD-MCI, and PD-MCI to PD-D (**Fig. 3d,e**). Similarly, ThT-t_{lag} (**Extended Data figure 3a,c,d**) and ThT-t_{50} (**Extended Data figure 4a,c,d**) were significantly reduced in individuals whose cognition changed, PD-NC→PD-MCI, and PD-MCI→PD-D. The groups without cognitive change PD-NC→PD-NC, and PD-D→PD-D did not show any change in ThT-t_{lag} (**Extended Data figure 3a,b,e**) or ThT-t_{50} (**Extended Data figure 4a,b,e**). These two ThT features only changed once cognition declined; it did not predate the cognitive decline.

α-Syn strains become more neurotoxic with cognitive decline, but not disease duration

A significant increase in neurotoxicity was observed in patients transitioning to different cognitive states, specifically the PD-NC→PD-MCI, and PD-MCI→PD-D groups (**Fig. 4a**). In contrast, groups without

cognitive change (HC→HC, PD-NC→PD-NC, and PD-D→PD-D), showed no significant changes in neurotoxicity during the follow-up time (**Fig. 4a**). The neurotoxicity of these α-syn strains amplified from yearly-collected CSF was also assessed. Notably, in the groups of HC→HC, PD-NC→PD-NC, and PD-D→PD-D, the results demonstrated consistent neurotoxicity levels annually (**Fig. 4b,c,f**). During the progression from the PD-NC to PD-MCI, and PD-MCI to PD-D, the enhanced neurotoxicity occurred at the time of the diagnosis of the cognitive change, but the neurotoxicity did not predate the cognitive decline (**Fig. 4d,e**). Taken together, these results indicate that the neurotoxicity of amplified α-syn strains increases when PD patients undergo cognitive decline but remains the same when the cognitive status is stable.

Discussion:

The major finding of this paper is the observation that α-syn strain properties change in association with cognitive decline in PD. Both cross-sectional and longitudinal studies showed a strong correlation between α-syn strain features and cognitive status. α-syn strains exhibited differences in the three cognitive stages (PD-NC, PD-MCI, and PD-D).

Our results suggest that characterization of α-syn strain features and dynamics has the promise of greatly enhancing the diagnostic power of characterizing α-syn strains. This enhancement could potentially position them as valuable clinical biomarkers in the management of PD cognitive status transitions from NC to MCI and dementia.

Methods:

Patient enrollment and biosample collection

Individuals with PD and those without motoric evidence of parkinsonism (healthy controls) were enrolled at JHU as part of our participation in the NINDS Parkinson's Disease Biomarker Program (PDBP)[8]. Inclusion criteria for participants included meeting UK Brain Bank criteria for idiopathic PD, modified to allow for individuals with a family history of PD, and receiving levodopa therapy for their PD. Inclusion criteria for healthy controls included Montreal Cognitive Assessment score indicating no cognitive impairment (MoCA > 25) and the absence of any first-degree relative with parkinsonism. All participants had to agree to and be eligible for an annual lumbar puncture. All individuals underwent the PDBP standard set of motor, psychiatric, and cognitive assessments every 6 months for the first 5 years of the investigation as well as a one-time 12-month follow-up visit at the end of the investigation, and an annual lumbar puncture.

Clinical and Cognitive Assessments and Diagnosis Determination

Individuals were divided into HC and PD based on motoric symptoms and then divided into normal cognition, mild cognitive impairment (cognitive impairment no dementia (CIND)), and dementia according to MoCA scores. PD-NC: MoCA score of 26 or above, PD-MCI: MoCA score between 21 and 25, PD-D: MoCA score of 20 or below.

Expression and purification of α-synuclein protein:

Recombinant human α-syn protein was prepared according to the previous method[9]. pRK172-α-syn plasmid was transduced in BL21 (DE3) cells and cultured at 37°C in lysogeny broth overnight. The E.coli pellets were resuspended with osmotic shock buffer (3.63 g Tris-base, 400 g sucrose, and 0.744 g EDTA were dissolved in 1 L DDW, pH 7.2) by agitation. The mixture was then centrifuged at 10,000 g for 30 min to remove the supernatant, and DDW containing proteinase inhibitor and 80 μL saturated $MgCl_2$ were added to resuspend the pallets. The supernatant was collected (10,000 g, 30 min centrifugation) and filtered through a 0.45 μm filter, followed by the dialysis with low salt buffer (20 mM Tris-base, 50 mM NaCl in DDW; pH 8.0) overnight at 4°C. α-Syn protein was purified with fast protein liquid chromatography (FPLC). The endotoxin was removed using Thermo scientific pierce high capacity endotoxin removal spin

column. The purity was evaluated with Coomassie brilliant blue staining and immunoblot. The concentration was measured with a BCA assay.

Amplification of pathogenic α-syn in patient CSF with SAA:
The amplification of α-syn strains from patient-derived CSF samples was performed using the SAA method by referring to previous work[1,10,11] with some modifications. The SAA equipment containing the microplate horn (#431MPX), a sound enclosure (#432MP), and a thermoelectric chiller (#4900) was purchased from Qsonica. Briefly, recombinant α-syn was centrifuged at 100,000 g for 30 min at 4°C to remove any preformed aggregates before use. Then, α-syn was diluted with SAA buffer (1% Triton X-100 in PBS), and 100 μL was transferred into PCR tubes containing a suitable amount of silicon beads (diameter 1.0 mm, purchased from BioSpec products), and 10 μL CSF samples were added as seeds in triplicate. The final concentration of α-syn was 0.3 mg/mL. After mixing, the samples were subjected to sonication (Amplitude: 5; 40 sec sonication and 29 min 20 sec incubated at 37°C). In total there were 40 amplification circles for 1-day reaction and 280 amplification circles for 7-day reaction. 5 μL samples were collected every day and amplification was monitored by measuring Thioflavin T (ThT) (Sigma-Aldrich, cat No. T3516) fluorescence using a Fluorescence Spectrophotometer (Varioskan LUX plate reader, Thermo Fisher Scientific) with fixed excitation and emission wavelength at 450 nm and 485 nm respectively. After 7 days, the mixture was transferred into the centrifugal filter (Millipore, MCW: 3000) containing 15 mL PBS, and centrifuged at 4000 g for 30 min at 4°C to remove Triton X-100. The washing with 15 mL PBS and centrifugation was repeated 8 times and the final SAA products were collected. SAA products were spun for 30 min at 20,000 g, and the amount of monomeric α-syn in the supernatant was assessed by BCA assay and the pelleted assemblies were resuspended in PBS buffer.

ThT fluorescence assay:
The SAA sample (5 μL) was taken out and added into a 55 μL ThT solution (20 μM). Samples were subsequently plated in triplicate on 384 well black/clear bottom plates (Sigma-Aldrich, cat no. P6491), and the fluorescence was measured at 450/485 nm excitation/emission with a microplate reader (Varioskan LUX plate reader, Thermo Fisher Scientific). Following data acquisition, the kinetic curves were fitted to Equation. 1 to calculate the half-time (t_{50}) value and lag time (t_{lag}) for each curve.

$$y = A2 + (A1-A2)/(1 + \exp((x-x_0)/dx)) \qquad \text{Equation. 1}$$

Where A1 represents initial fluorescence, A2 is the final fluorescence value, x_0 is the half-time (t_{50}) value, and dx represents the time constant.

$$t_{lag} = x_0 - 2dx \qquad \text{Equation. 2}$$

Proteinase K digestion, dot blot, and silver staining:
The SAA samples (7 μg) were mixed with proteinase K (PK) and incubated at 37°C at different time points (0, 5, 15, and 30 min). For the dot blot assay, the PK-digested SAA samples were loaded onto the nitrocellulose membrane (Bio-Rad, cat no. 1620112) and blocked by 5% bovine serum albumin (BSA) (Sigma-Aldrich, cat no. A7906) in TBST for 1 hr at room temperature (RT). The membrane was then transferred into the mouse anti-α-syn mAb (1:2000 dilution, BD Biosciences, cat no. 610787) in TBST with 5% BSA overnight at 4°C. Following with TBST wash (3 times x 5 min), the membrane was incubated with anti-mouse IgG-HRP (1:5000 dilution, GE Healthcare, cat no. NA931) for 1 hr at RT. After TBST wash, the signal was developed with SuperSignal West Pico Plus chemiluminescent substrate (Thermo Fisher Scientific, cat no. 34096). For the silver staining, PK-digested SAA samples were loaded on the SDS-PAGE (15%) gels. Silver staining was performed using Pierce Silver Stain Kit (Thermo Fisher Scientific, cat no. 24612). All the images were acquired and processed with Amersham Image 600 (GE Healthcare Life Sciences).

Dynamic light scattering (DLS):
The SAA sample (10 μg) was mixed with filtered phosphate buffer (990 μL). Measurements were performed using a Zetasizer Nano-ZS (Malvern Instruments, Malvern, UK) equipped with a He-Ne laser.

Each sample was measured in 1-cm path-length polystyrene semi-micro disposable cuvettes (Fisher Emergo, Landsmeer, The Netherlands). The cell holder was maintained at 25 °C. For each sample, 10 runs were performed, with three repetitions.

***Primary cortical neuron culture, neurotoxicity of SAA products**:*
Mouse primary cortical neurons were cultured from embryonic 15.5-day pups of CD-1 pregnant mice (Charles River). 48 well plates were coated via Poly-L-ornithine solution (0.2 mg/mL) for 1 hr at 37°C and washed 3 times with sterile dd-water. Primary neurons at 7 days in vitro (DIV) were treated with SAA samples with a final concentration of 10 µg/well. Neurotoxicity was assessed at 21 and 28 DIV individually. The primary cortical neurons were washed with PBS, fixed in 4% paraformaldehyde (PFA), followed by blocking in 3% goat serum containing PBST (0.1% Tween-20) for 1 hr. Anti-NeuN (1:250, MAB377, Sigma-Aldrich) were incubated overnight at 4°C, followed by Alexa-fluor 488 secondary antibodies (1:2000, Thermo Fisher Scientific) and Hoechst (1:5000, Thermo Fisher Scientific) at RT for 1 hr. The images were obtained on a fluorescence microscope (Zeiss). The number of NeuN was quantified using ImageJ software (National Institute of Health, Bethesda, MD).

Statistical analysis
Statistical analysis was performed using the statistical software Stata (version 18). The baseline demographic and clinical characteristics of the participants are presented as mean +/- standard deviation (SD) or number (%). The characteristics were compared using the student's *t*-test or chi squared as appropriate. Logistic regression analyses were performed with the binary cognition variable as the outcome variable and the biomarker of interest as the independent variables, adjusting for covariates, such as age and gender. Receiver operating characteristic curves were calculated to visualize and compare the predictivity of the biomarkers. The Cox proportional hazard model was used to determine whether baseline biomarker data is associated with the progression to cognitive impairment. Two-sided p-values <0.05 were considered significant.

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Author contributions:
KG performed all the basic science experiments and data analysis. EQX, NW, XDZ, JD, JY, AW, YJC, RK, HQL, LLN, RC, and SZ contributed to biophysical, biochemical, cellular experiments and data interpretation. LSR provided key patients' samples and clinical information. LTJ, YJL, and MYY helped with the concept generation, manuscript writing, and experiment design. XM and LSR designed the project direction and research strategy. XM, LSR, TMD, and VLD wrote and revised the paper. All authors reviewed, edited, and approved the paper.

Data and materials availability:
Further information and requests for resources and reagents should be directed to and will be fulfilled by Xiaobo Mao (xmao4@jhmi.edu). Information related to biospecimens will be provided by Liana Rosenthal (Liana.Rosenthal@jhmi.edu). There are no restrictions on any data or materials presented in this paper. All data are available in the main text or the Extended Data.

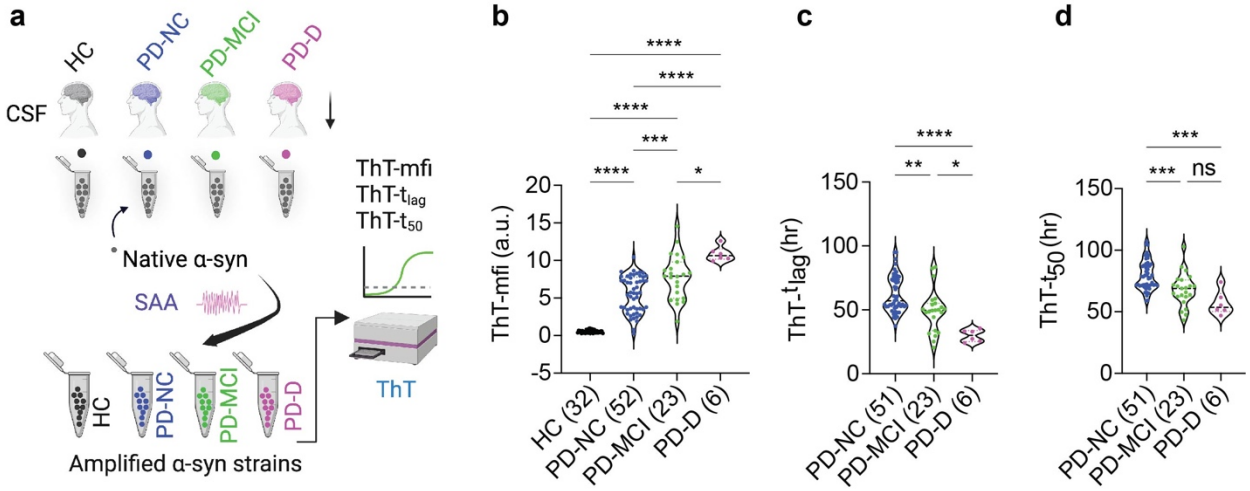

Figure 1. Differentiating amplified α-syn strains via Thioflavin T (ThT) derived from patients with PD-NC, PD-MCI, and PD-D. (**a**) Schematic representation of α-syn amplification and characterization using ThT assay. In these groups: HC (health control), PD-NC (PD with normal cognition), PD-MCI (PD with mild cognitive impairment), PD-D (PD with dementia), and CSF samples were amplified with SAA and the ThT assay was performed. (**b**) ThT-mfi (maximal fluorescence intensity) of CSF-SAA samples. HC (n = 32), PD-NC (n = 52), PD-MCI (n = 23), and PD-D (n = 6). (**c**) ThT-t_{lag} (time at which aggregation started) of CSF-SAA samples. PD-NC (n = 51), PD-MCI (n = 23), and PD-D (n = 6). (**d**) ThT-t_{50} (time at which aggregation is 50% complete) of CSF-SAA samples. PD-NC (n = 51), PD-MCI (n = 23), and PD-D (n = 6). Data are mean ± SEM. The statistical significance was evaluated via one-way ANOVA with Tukey's multiple comparisons test. No significant difference (ns) $P > 0.05$, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$. Every dot indicates an individual biological sample measured in duplicate.

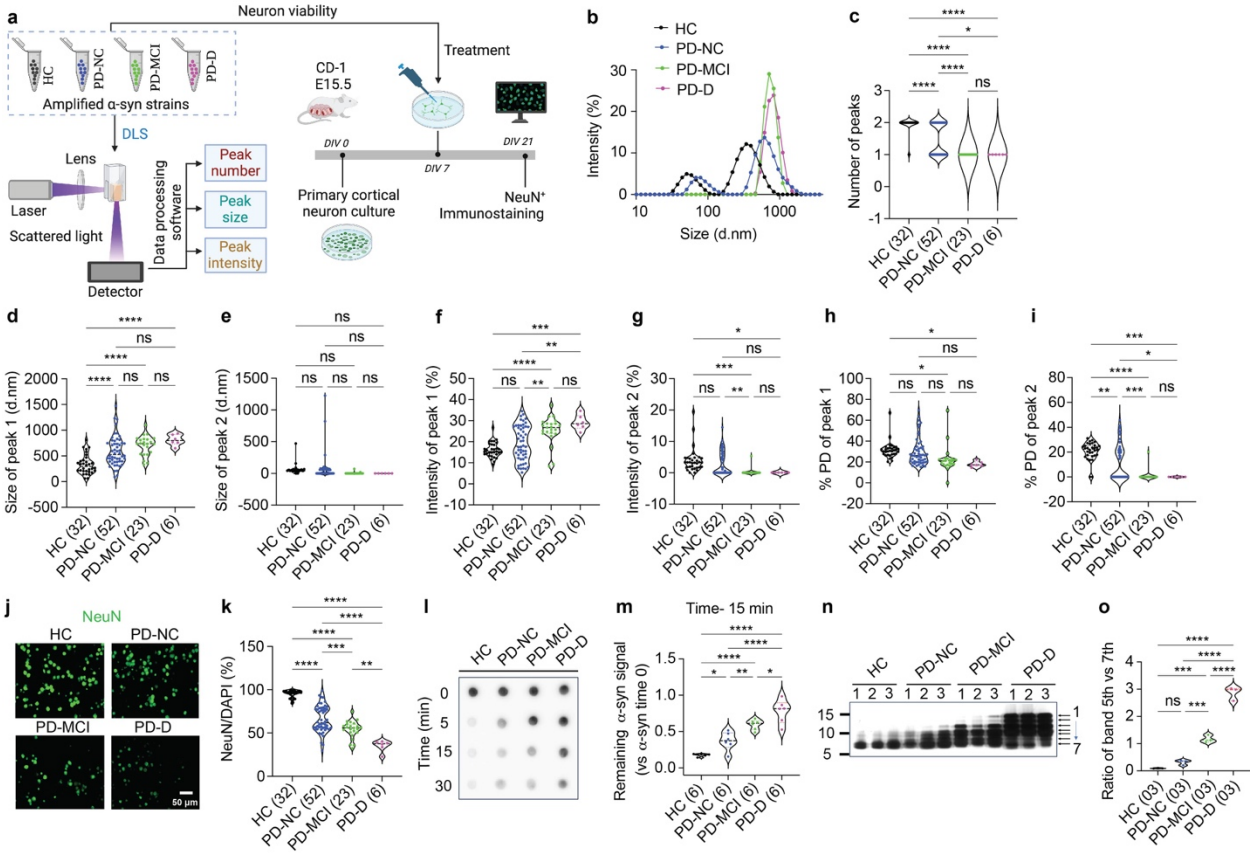

Figure 2. Differentiating α-syn strains using dynamic light scattering (DLS), cell-based, and biochemical assays. (**a**) Schematic representation of the characterization of α-syn strains from HC, PD-NC, PD-MCI, and PD-D. DLS data processing provides peak number, peak size, and peak intensities. Neuronal culture were used to assess neurotoxicity. (**b**) DLS spectra of amplified α-syn strains from HC, PD-NC, PD-MCI, and PD-D. (**c**) The number of DLS peaks for HC (n = 32), PD-NC (n = 52), PD-MCI (n = 23), and PD-D (n = 6). (**d**) Size of peak 1 from DLS for amplified α-syn strains from HC, PD-NC, PD-MCI, and PD-D. (**e**) Size of peak 2 from DLS for amplified α-syn strains from HC, PD-NC, PD-MCI, and PD-D. (**f**) Intensity of peak 1 from DLS for amplified α-syn strains from HC, PD-NC, PD-MCI, and PD-D. (**g**) Intensity of peak 2 from DLS for amplified α-syn strains from HC, PD-NC, PD-MCI, and PD-D. (**h**) %PD of peak 1 from DLS for amplified α-syn strains from HC, PD-NC, PD-MCI, and PD-D. (**i**) %PD of peak 2 from DLS for amplified α-syn strains from HC, PD-NC, PD-MCI, and PD-D. (**j & k**) Neurotoxicity of α-syn strains assessed with the immunostaining and quantification of anti-NeuN (neuronal nuclei marker). Scale bar, 50 µm. (**l**) α-Syn dot-blot immunostaining after proteinase K (PK)-digestion and **(m)** quantification. (**n**) SDS–PAGE followed by silver staining of PK-digested α-syn strains and (**o**) quantification. (**c, d, e, f, g, h, i, k, m,o**) Each dot represents an individual biological sample. The violin plot shows all the points. Data are presented as the mean ± SEM. The statistical significance was evaluated via one-way ANOVA with Tukey's multiple comparisons test. No significant difference (ns) $P > 0.05$, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$.

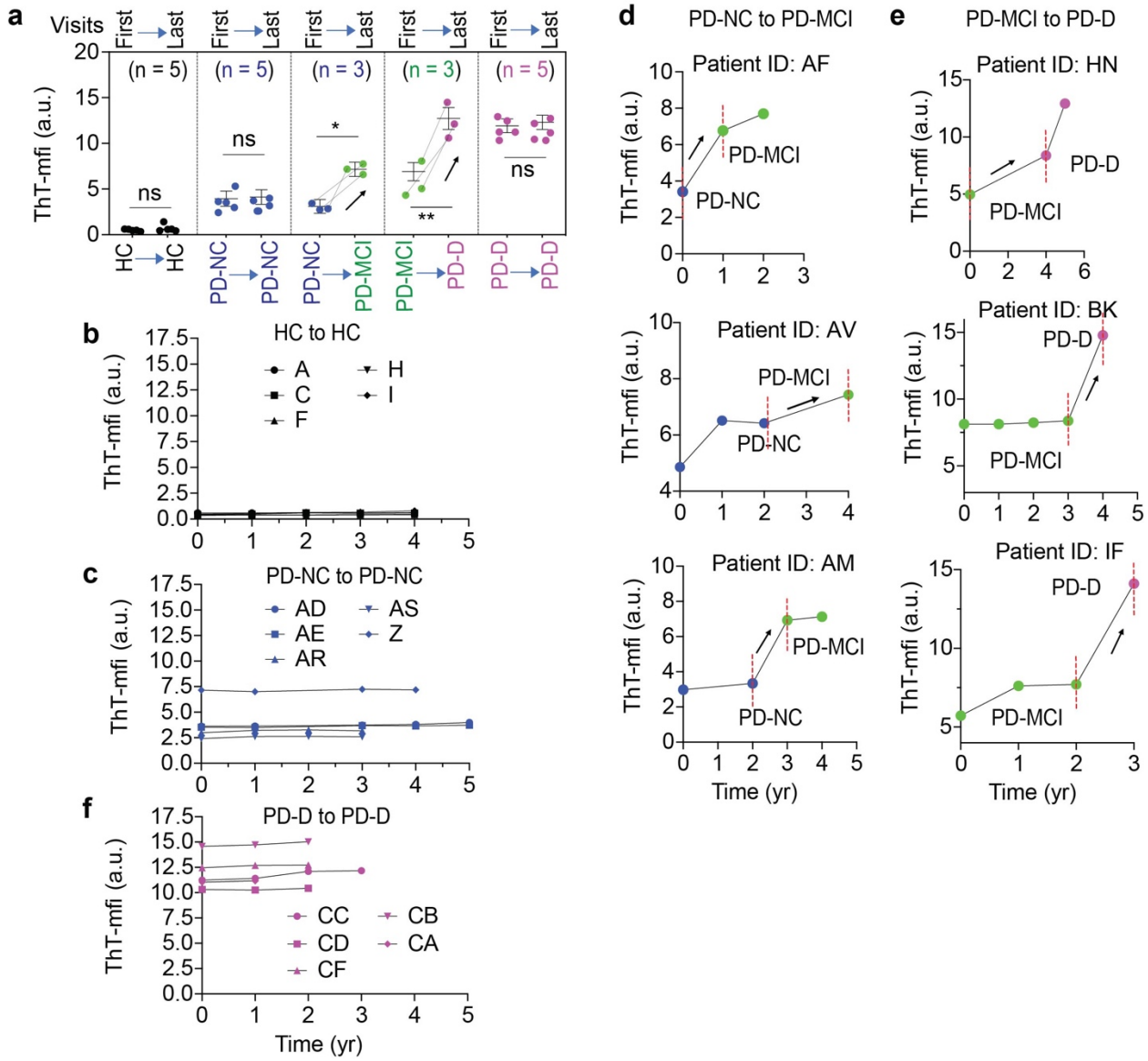

Figure 3. Longitudinal analysis of α-syn strains using ThT-mfi. (**a**) ThT-mfi of amplified α-syn strains from HC, PD-NC, PD-MCI, and PD-D groups between the first- and last-visit of Cohort I. **(b,c,f)** Yearly mapping of ThT-mfi of amplified α-syn strains from individuals with stable cognitive status. **(d,e)** Yearly mapping of ThT-mfi of amplified α-syn strains from individuals with changed cognitive status. The statistical significance was evaluated via one-way ANOVA with Tukey's multiple comparisons test. No significant difference (ns) $P > 0.05$, $*P < 0.05$, $**P < 0.01$.

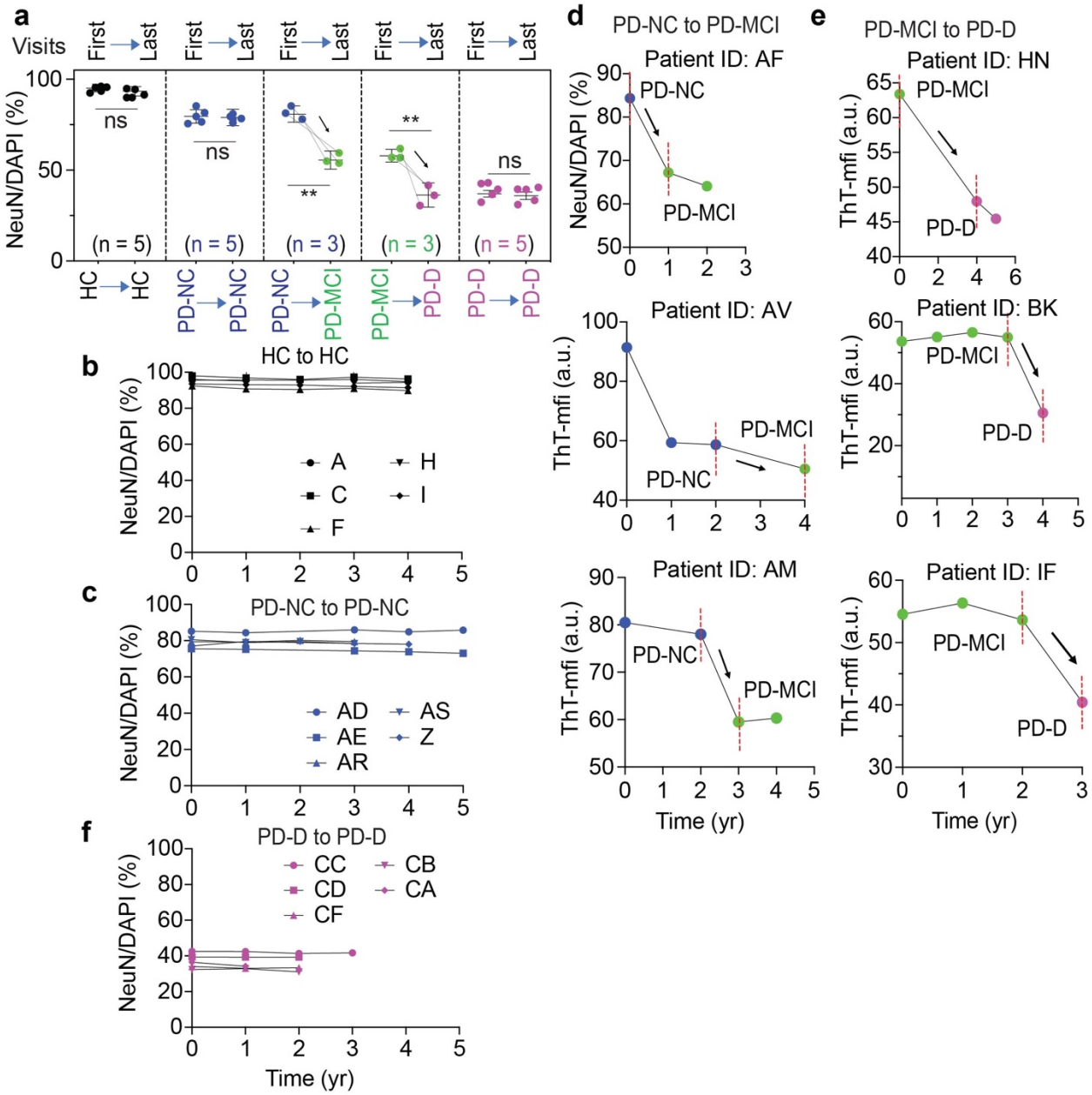

Figure 4. Longitudinal analysis (yearly) for the neurotoxicity of α-syn strains from HC, PD-NC, PD-MCI, and PD-D. Amplified α-syn strains (10 μg/mL) were added to mouse cortical neurons at DIV 7 days. 21 days later neurotoxicity was determined via NeuN counting. (**a**) Neurotoxicity of amplified α-syn strains from longitudinal HC, PD-NC, PD-MCI, and PD-D groups between the first- and last-visit in Cohort I. **(b,c,f)** Yearly mapping of neurotoxicity of amplified α-syn strains from individuals with stable cognitive status. **(d,e)** Yearly mapping of neurotoxicity of amplified α-syn strains from individuals with changed cognitive status. The statistical significance was evaluated via one-way ANOVA with Tukey's multiple comparisons test. No significant difference (ns) $P > 0.05$, $^{**}P < 0.01$.

Supporting information

α-Synuclein Strain Dynamics Correlate with Cognitive Shifts in Parkinson's Disease

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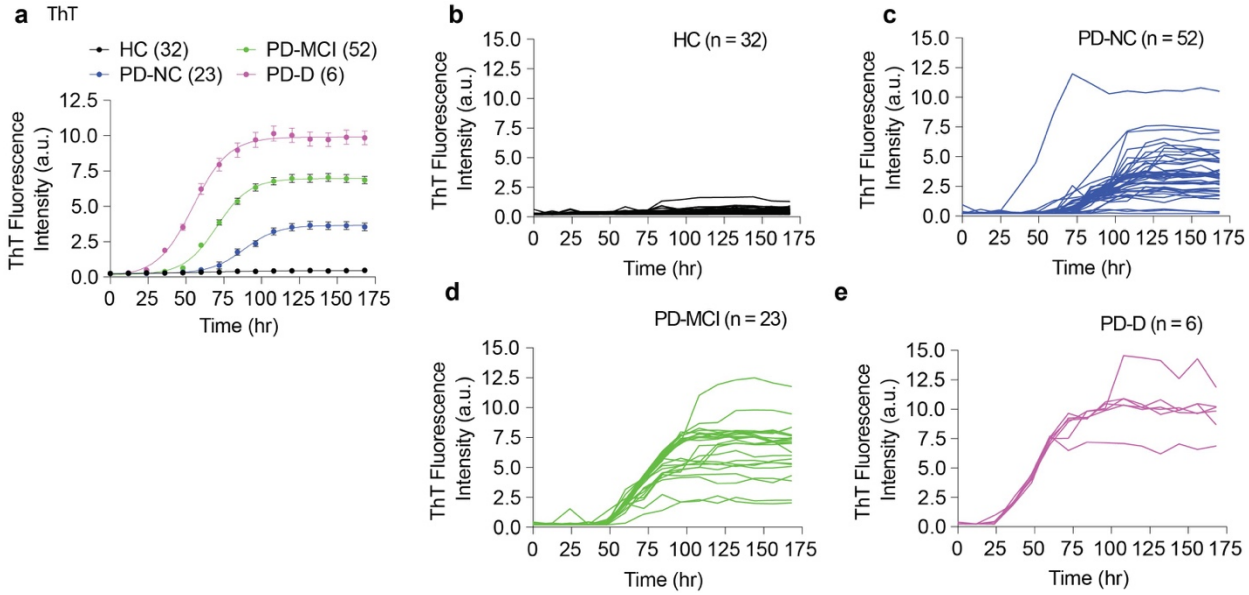
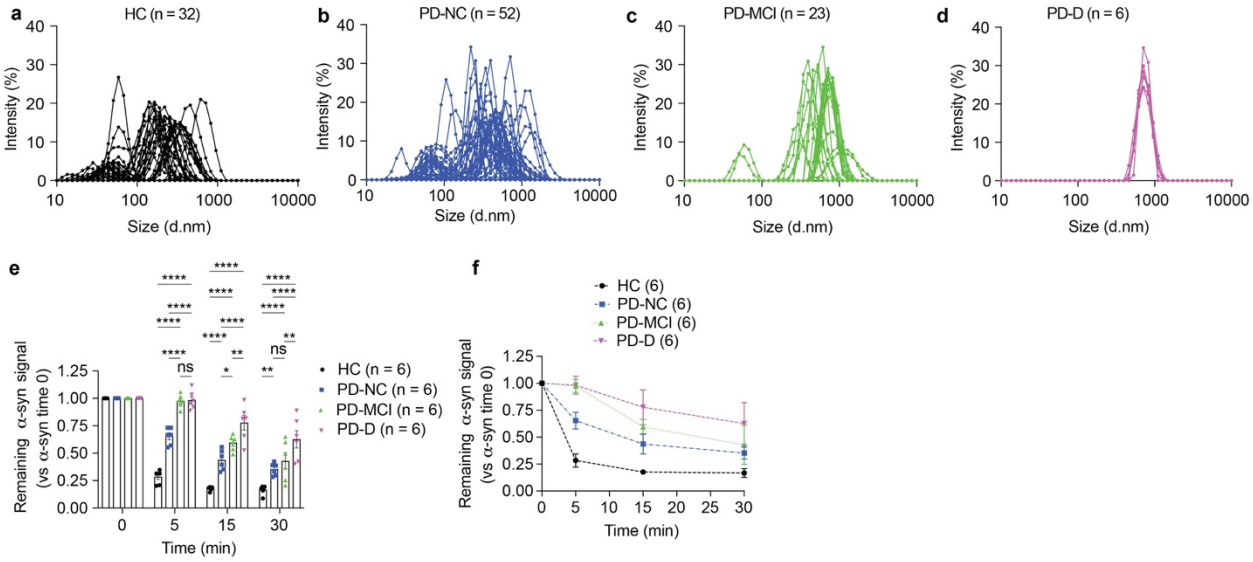
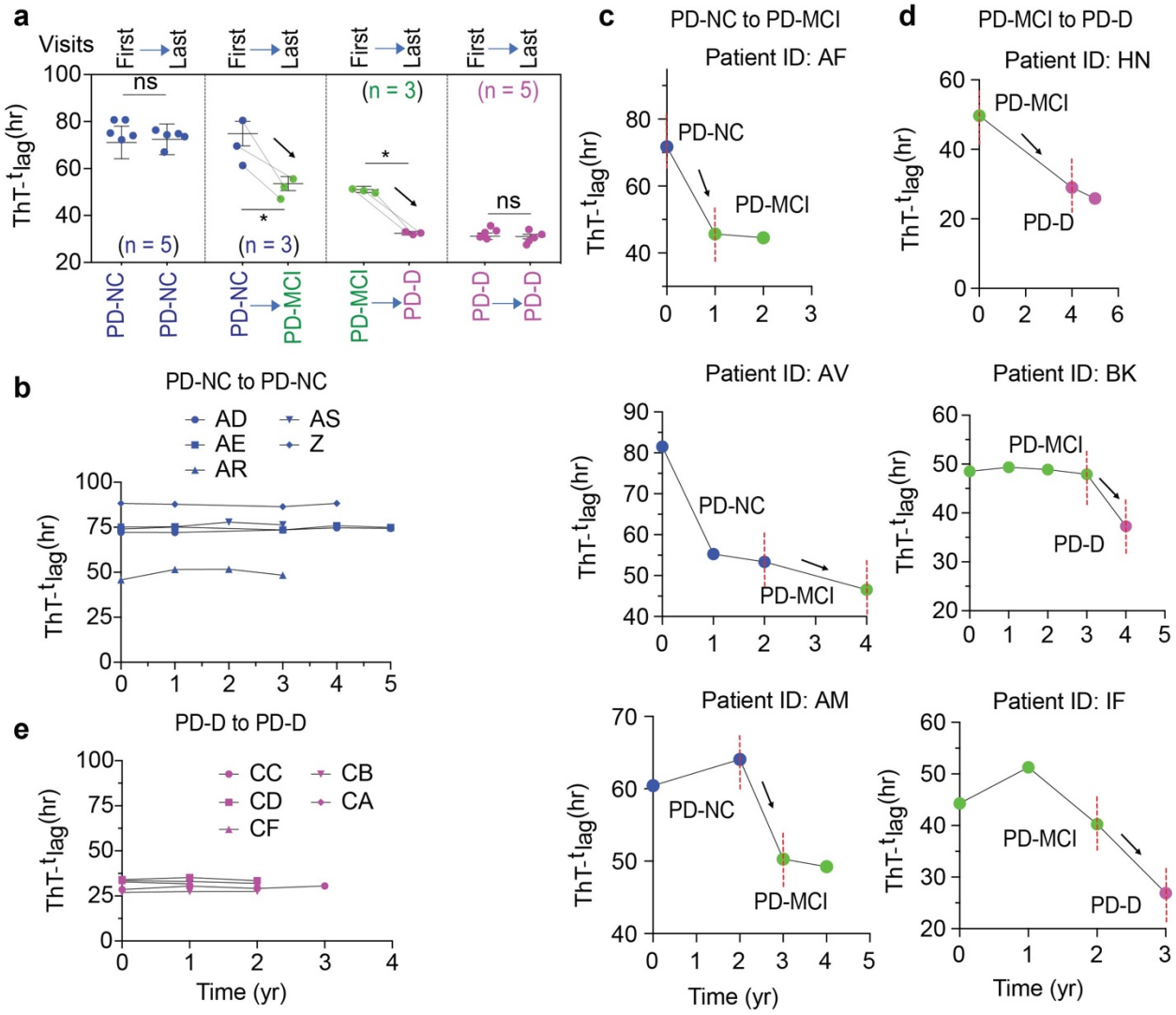


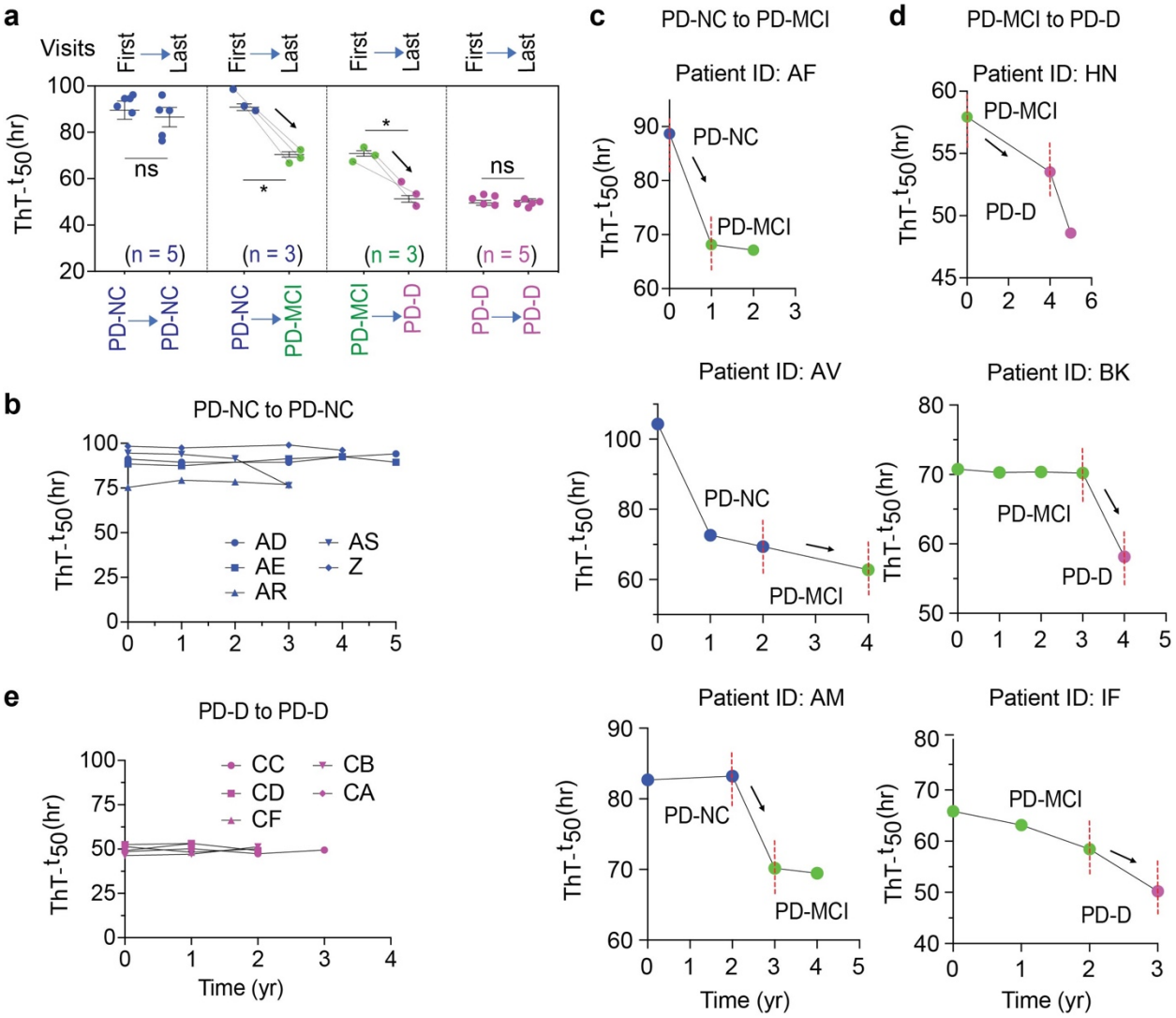
Extended Data figure 1. ThT assay for amplified α-syn strains. (**a**) Averaged ThT fluorescence spectra for amplified α-syn strains from HC, PD-NC, PD-MCI, and PD-D. Data are presented as the mean ± SEM. (**b, c, d,** and **e**) ThT fluorescence spectra for individual samples from HC, PD-NC, PD-MCI, and PD-D respectively.



Extended Data figure 2. DLS and PK-linked dot-blot of amplified α-syn strains. (**a, b, c,** and **d**) DLS spectra for individual samples from HC, PD-NC, PD-MCI, and PD-D respectively. Two peaks were observed in the HC and PD-NC group and one peak in PD-MCI and PD-D. Each peak represents an individual biological sample. **(e,f)** α-syn signal remaining after PK digestion as assessed by dot-blot for α-syn immunoreactivity. Amplified α-syn strains were treated with PK and assessed at different time points (0, 5, 15, and 30 min) after PK administration. Data are presented as the mean ± SEM. The statistical significance was evaluated via one-way ANOVA with Tukey's multiple comparisons test. No significant difference (ns) $P > 0.05$, *$P < 0.05$, **$P < 0.01$, ****$P < 0.0001$.



Extended Data figure 3. Longitudinal analysis (yearly) for α-syn strains using ThT-t_{lag}. (**a**) ThT-t_{lag} of amplified α-syn strains from HC, PD-NC, PD-MCI, and PD-D groups between the first- and last-visit in Cohort I. **(b,e)** Yearly mapping of ThT-t_{lag} of amplified α-syn strains from individuals with stable cognitive status. **(c,d)** Yearly mapping of ThT-t_{lag} of amplified α-syn strains from individuals with changed cognitive status. The statistical significance was evaluated via one-way ANOVA with Tukey's multiple comparisons test. No significant difference (ns) $P > 0.05$, $*P < 0.05$.



Extended Data figure 4. Longitudinal analysis (yearly) for α-syn strains using ThT-t_{50}. (**a**) ThT-t_{50} of amplified α-syn strains from longitudinal HC, PD-NC, PD-MCI, and PD-D groups between the first- and last-visit in Cohort I. **(b,e)** Yearly mapping of ThT-t_{50} of amplified α-syn strains from individuals with stable cognitive status. **(c,d)** Yearly mapping of ThT-t_{50} of amplified α-syn strains from individuals with changed cognitive status. The statistical significance was evaluated via one-way ANOVA with Tukey's multiple comparisons test. No significant difference (ns) $P > 0.05$, $^*P < 0.05$.

Extended Data Table 1. Demographic and clinical characteristics

Factor	PD (n=81)	HC (n=32)	PD-NC (n=52)	PD-MCI (n=23)	PD-D (n=6)	p-value[#]
Age, years (SD)	65.67 (8.03)	67.09 (9.00)	63.63 (6.94)	67.61 (8.28)	17.83 (7.41)	<0.001
Gender, % male	69%	31%	38%	17%	17%	0.14
Race, % Caucasian	96%	88%	96%	100%	83%	0.16
Education, mean years (SD)	16.84 (2.37)	16.78 (2.21)	16.71 (2.40)	17.13 (2.26)	16.83 (2.86)	0.78
Disease duration, mean years (SD)	6.42 (4.17)	n/a	5.98 (3.80)	6.61 (4.58)	9.5 (4.97)	0.14
MoCA Total Score, mean (SD)	25.84 (4.05)	27.81 (1.31)	27.94 (1.40)	23.87 (1.1)	15.17 (6.04)	<0.001
RBD symptoms, % with	56%	12%	62%	35%	83%	0.036
MDS-UPDRS part I Score, mean (SD)	11.05 (5.44)	4.31 (3.45)	9.94 (4.06)	11.74 (5.73)	18 (9.40)	0.001
MDS-UPDRS part II Score, mean (SD)	11.49 (7.44)	0.41 (0.84)	10.04 (5.27)	11.87 (7.94)	22.67 (12.34)	<0.001
MDS-UPDRS part III Score, mean (SD)	31.21 (11.59)	1.34 (1.88)	28.4 (9.01)	32.96 (12.76)	48.83 (11.72)	<0.001
MDS-UPDRS part IV Score, mean (SD)	3.65 (3.41)	0.00 (0.00)	3.65 (3.27)	3.48 (3.45)	4.33 (4.93)	0.86
MDS-UPDRS Total Score, mean (SD)	57.41 (21.94)	6.06 (4.80)	52.04 (13.32)	60.04 (25.07)	93.83 (34.66)	<0.001
UPSIT total score, mean (SD)	19.43 (7.40)	34.91 (4.07)	21.18 (7.14)*	17.52 (6.67)	11.83 (6.62)	0.004

*indicates n=51. [#]*P*-value denotes differences between PD-NC, PD-MCI, and PD-D