Proteinopathy and longitudinal changes in functional connectivity networks in Parkinson disease

Meghan C. Campbell, PhD, Joshua J. Jackson, PhD, Jonathan M. Koller, BS, Abraham Z. Snyder, MD, PhD,
Paul T. Kotzbauer, MD, PhD, and Joel S. Perlmutter, MD

Correspondence
Dr. Campbell
meghanc@wustl.edu

Abstract

Objective
To evaluate resting-state functional connectivity as a potential prognostic biomarker of Parkinson disease (PD) progression. The study examined longitudinal changes in cortical resting-state functional connectivity networks in participants with PD compared to controls as well as in relation to baseline protein measures and longitudinal clinical progression.

Methods
Individuals with PD without dementia (n = 64) and control participants (n = 27) completed longitudinal resting-state MRI scans and clinical assessments including full neuropsychological testing after overnight withdrawal of PD medications ("off"). A total of 55 participants with PD and 20 control participants also completed baseline β-amyloid PET scans and lumbar punctures for CSF protein levels of α-synuclein, β-amyloid, and tau. Longitudinal analyses were conducted with multilevel growth curve modeling, a type of mixed-effects model.

Results
Functional connectivity within the sensorimotor network and the interaction between the dorsal attention network with the frontoparietal control network decreased significantly over time in participants with PD compared to controls. Baseline CSF α-synuclein protein levels predicted decline in the sensorimotor network. The longitudinal decline in the dorsal attention–frontoparietal internetwork strength correlated with the decline in cognitive function.
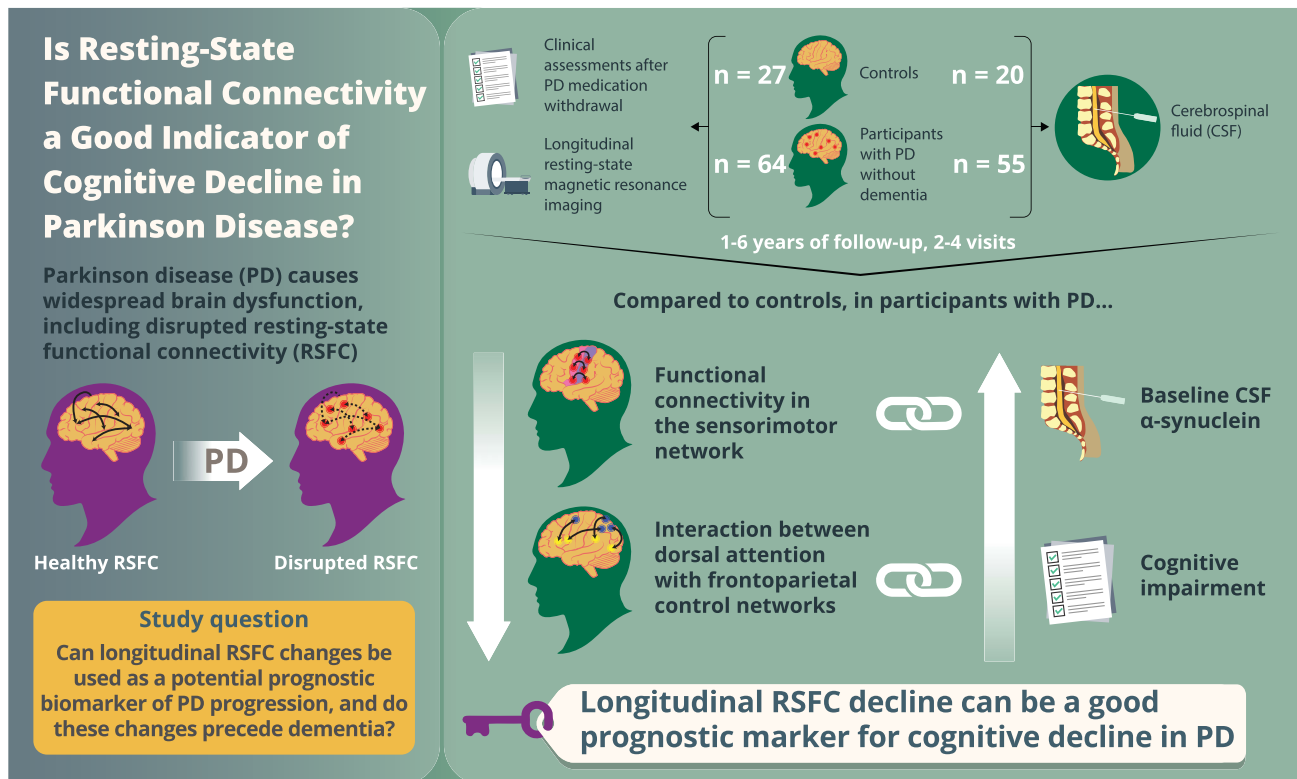
Conclusions
These results indicate that α-synuclein levels may influence longitudinal declines in motor-related functional connectivity networks. Further, the interaction between cortical association networks declines over time in PD prior to dementia onset and may serve as a prognostic marker for the development of dementia.

From the Departments of Neurology (M.C.C., A.Z.S., P.T.K., J.S.P.), Radiology (M.C.C., A.Z.S., J.S.P.), Psychiatry (J.M.K.), and Neuroscience (J.S.P.), Program in Occupational Therapy (J.S.P.), and Program in Physical Therapy (J.S.P.), Washington University School of Medicine; and Department of Psychological and Brain Sciences (J.J.J.), Washington University in St. Louis, MO.

Glossary

Is Resting-State Functional Connectivity a Good Indicator of Cognitive Decline in Parkinson Disease?

Formation of midbrain Lewy bodies, containing aggregated α-synuclein, results in dopaminergic cell loss and motor deficits in Parkinson disease (PD), whereas cortical Lewy bodies likely contribute to dementia.[1,2] β-amyloid (Aβ) accumulation,[3,4] as well as serotonergic, norepinephrine, and cholinergic system deficits,[5–7] also may occur with PD. This heterogeneous neuropathophysiology causes widespread brain dysfunction and a variety of clinical manifestations, including dementia in up to 80% of PD cases.[8] Prognostic biomarkers reflecting the underlying pathophysiology and clinical progression are greatly needed.

Resting-state functional connectivity (RSFC) MRI may provide the link between neuropathophysiology and behavioral dysfunction. Both whole-brain and sensorimotor network (SMN) RSFC relate to CSF α-synuclein,[9,10] providing direct evidence that PD neuropathology disrupts RSFC. Moreover, altered striatal and cortical motor RSFC correlates with motor severity[11,12] and disruption of dorsal attention and frontoparietal networks relates to cognitive dysfunction in PD.[13] However, only one study currently reports longitudinal whole-brain RSFC declines in PD associated with cognitive decline and dementia.[14] Thus, longitudinal changes in specific RSFC networks, the role of proteinopathy in longitudinal RSFC changes, and whether RSFC changes precede dementia onset remain to be determined.

Therefore, we investigated longitudinal RSFC network changes in participants with PD without dementia and control participants, in relation to baseline protein levels and longitudinal clinical progression. We hypothesize that RSFC network integrity decreases longitudinally in participants with PD prior to dementia onset and that α-synuclein and Aβ predict RSFC decline. To test these hypotheses, participants completed a baseline lumbar puncture and Aβ PET scan, longitudinal RSFC, and clinical assessments.

Methods

Standard protocol approvals, registrations, and patent consents

The Washington University in St. Louis Human Research Protection Office and Radioactive Drug Research Committee approved this study. All participants provided written informed consent.

Participants

As part of an ongoing longitudinal study, 77 participants with PD and 37 control participants completed longitudinal RSFC scans and longitudinal clinical assessments between January 2010 and July 2016. Portions of the cross-sectional data have been reported[9,15–17]; the present study includes additional participants (original cross-sectional RSFC analyses included 43 participants with PD and 22 controls[9]) and new longitudinal data. Follow-up periods ranged from 1 to 6 years, with 2–4 visits depending on time since enrollment. Idiopathic PD diagnosis was based on modified UK PD Society Brain Bank criteria.[18] Dementia was assessed with the Clinical Dementia Rating (CDR),[19] a global score of 0 = normal, 0.5 = cognitive decline, and ≥1 = dementia, with higher total scores (sum of boxes) indicating increased impairment. Six participants with PD were excluded owing to dementia (CDR ≥ 1) at baseline. Nine participants with PD converted to dementia (CDR = 1) during the follow-up period. Analyses were conducted both with and without these participants to test for RSFC changes prior to dementia onset. Exclusion criteria included neurologic diagnosis other than PD, head injury with loss of consciousness >5 minutes or neurologic sequelae, severe psychiatric disorders (e.g., schizophrenia), treatment with dopaminergic blocking or depleting drugs, or inability to complete MRI. Age-matched controls were recruited through participants with PD (e.g., spouses/partners). Additional criteria for controls included normal neurologic examination, intact cognition (CDR = 0), no first-degree family history of PD, and no evidence of preclinical Alzheimer disease[20] based on Aβ PET (Pittsburgh compound B), as described.[15] Six controls were excluded (1 with CDR = 0.5; 5 with elevated cortical Aβ PET). All remaining controls had normal cognition (CDR = 0 and Mini-Mental State Examination ≥ 24) at baseline and follow-up visits. Thus, 71 participants with PD and 31 control participants were analyzed further.

Protein measures

Due to refusal or ineligibility (e.g., on blood thinners), only a subset of participants (55 PD and 20 controls) completed a lumbar puncture for CSF α-synuclein, Aβ, and tau protein levels. CSF protein levels were quantified using commercially available sandwich ELISAs kits (Covance [Princeton, NJ] for α-synuclein; Innotest from Fujirebio [Malvern, PA] for Aβ and tau), as described.[16] All participants completed baseline Aβ PET (Pittsburgh compound B), with the mean cortical binding potential (MCBP) as the primary measure of Aβ PET.[15]

Motor and cognitive assessments

Longitudinal clinical assessments included motor examinations, full neuropsychological testing, and CDR evaluations. Motor severity was assessed using the Unified Parkinson's Disease Rating Scale motor evaluation (UPDRS-III total score)[21] "off" PD medications. Participants also completed neuropsychological testing, assessing attention (Digit Span[22]; Digit Symbol[22]), memory (Logical Memory[23]; California Verbal Learning Test–II short form[24]), language (Boston Naming Test[25]), visuospatial (Judgement of Line Orientation[26]; Hooper Visual Organization Test[27]) and executive function (Trail-Making Test[28]; Stroop Inhibition[29]; Verbal Fluency Switching[29]), as described[15,17] while "off" medication.[30] Age-adjusted scaled scores were converted to z scores and averaged across cognitive domains to create a global cognition metric (average cognitive z score). Participants completed motor and cognitive assessments at each study visit; change in motor and cognitive function was computed as the change in UPDRS-III total score, CDR sum of boxes, and average cognitive z score from the first to the last time point coinciding with RSFC scans.

Resting-state functional connectivity

MRI scans were completed "off" PD medications (3T Siemens [Munich, Germany] Trio; 12-channel head coil). Structural scans included T1-weighted magnetization-prepared rapid gradient echo (repetition time [TR] 2,400 ms, inversion time [TI] 1,000 ms, echo time [TE] 16 ms, flip angle 8°, 0.9 mm^3) and T2-weighted MRI (TE 455 ms, TR 3,200 ms, 1 mm^3). Participants completed up to 3 RSFC runs, depending on time and participant tolerance (200 volumes/run, TE 27 ms, TR 2,200 ms, field of view 256 mm, flip angle 90°, 4 mm^3), with eyes closed but awake (confirmed verbally). RSFC runs with observable, sustained movement (e.g., tremor) were excluded.

Standard fMRI preprocessing methods,[9,11,31] using in-house processing scripts, include compensation for systematic, slice-dependent time shifts, elimination of odd–even slice intensity differences due to interleaved acquisition, rigid-body correction for head motion within and across runs, and normalization of signal intensity to a mode value of 1,000. Functional data were coregistered across visits, with functional data from follow-up scans registered to functional data from the first RSFC time point, to ensure that observed longitudinal changes were not attributable to differences in atlas registration across time points. Atlas transformation occurred by composition of affine transforms derived by a sequence of coregistrations of the fMRI volumes, structural scans, and an atlas representative template created from equal numbers of participants with PD and control participants to minimize registration bias of MRI scans.[9,11] Head motion correction was applied in a single resampling that generated volumetric time series in 3 mm^3 atlas space. Standard RSFC preprocessing analyses[11,31] included spatial smoothing (6 mm full width half maximum) and temporal low-pass filtering (<0.1 Hz). Nuisance regressors included 6 rigid body measures derived from head motion correction, white matter and CSF signals, and mean

whole-brain signal, to reduce physiologic noise[32] and motion artifacts.[33,34]

RSFC quality assurance

Motion artifacts and movement confounds were minimized through strict quality assurance measures and motion scrubbing.[34,35] Motion criteria were head motion <1.0 mm root mean squared per run; voxel-wise temporal SD averaged over the brain (after full preprocessing) ≤0.5%; and frame-to-frame signal intensity changes (DVARS[35]) ≤0.3%. Each run required 50 useable volumes and each participant needed ≥150 useable volumes for a minimum of 5.5 minutes of RSFC data. Four control participants and 7 participants with PD were excluded due to excessive motion.

Network-level RSFC

Network-level RSFC analyses focused on 5 previously defined cortical networks: default mode network (DMN), dorsal attention network (DAN), frontoparietal control network (FPCN), salience network (SAL), and SMN.[9,36,37] Networks were chosen based on their relevance for the neuropsychological (DMN, DAN, FPCN, SAL) and sensorimotor (SMN) aspects of PD and previously demonstrated association with CSF proteins.[9] Each network was defined by a set of expanded seed regions (figure 1), using the same approach as established with our previous cross-sectional analyses[9] and previously established network definitions.[9,36,37] Specifically, the DMN included posterior cingulate/precuneus, medial prefrontal cortex (PFC), left and right lateral parietal cortex, and left and right inferior temporal regions; DAN included left and right frontal eye fields, left and right middle temporal area, and both left and right anterior and posterior intraparietal sulcus; FPCN (previously labeled as the control network in our original cross-sectional analyses[9]) included dorsal medial PFC, left and right anterior PFC, and left and right superior parietal cortex; SAL included dorsal anterior cingulate cortex, left and right anterior PFC, left and right insula, and left and right lateral parietal cortex; SMN included left and right motor cortex, supplemental motor area, and left and right primary visual and auditory cortex.

Figure 1 Resting-state functional connectivity (RSFC) network regions
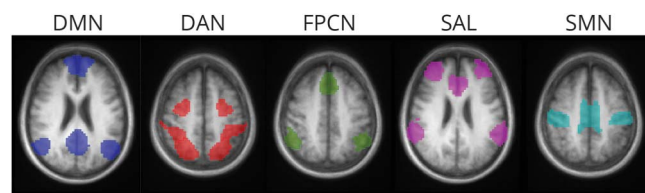
The expanded seed regions for each RSFC network are displayed as a representative slice for each network. Colors indicate the respective RSFC networks as follows: blue = default mode network (DMN); red = dorsal attention network (DAN); green = frontoparietal control network (FPCN); purple = salience network (SAL); teal = sensorimotor network (SMN). See table e-1 in Campbell et al.[9] for a list of seed region coordinates.

To generate regions of interest, a seed-based approach was first applied using 5-mm radius spheres centered on Talairach atlas coordinates as previously defined for the key nodes of each network listed above.[15,32,33] Individual-level Fisher z transformed correlation maps for each seed were averaged across groups. Expanded seed regions were defined as voxels satisfying $|z| > 3$ in the group correlation maps. Using expanded seed regions avoids potential small atlas registration errors and minor individual deviations in peak RSFC locations, similar to a parcel-based approach.[38] Composite scores, representing intranetwork (within) and internetwork (between) RSFC, were computed as the mean of Fisher z transformed correlations between seed region pairs for each network.[9,37] The intranetwork and internetwork composite scores for each participant, at each time point, were analyzed as measures of network-level RSFC.

Statistical analyses

A series of growth models, a type of longitudinal mixed-effects analysis, were conducted using multilevel modeling to test between-group differences in longitudinal network-level RSFC. This statistical approach allows for variability in the number and duration of longitudinal data points and permits maximal utilization of data analysis without restricting the analyses to a common set of time points as with other approaches (e.g., repeated-measures analyses of variance). Growth was defined as a linear trend with random slopes, modeled as a random effect. Standardized age, education, sex, and disease duration were included as covariates. Time was modeled as months since first RSFC time point and reported as annualized estimates (e.g., change per year) for convenience of interpretation. When examining group effects, controls were set as the reference group (i.e., 0). Model estimates represent a change in the outcome (e.g., SMN) with a 1-unit change of the variable (e.g., 1 year, control to PD group). Interaction terms involving time test the differences in trajectories between groups (e.g., control vs PD) or levels of continuous predictors (e.g., 1 SD above and below the mean). Time interactions were examined for the PD group, protein levels, and changes in motor and cognitive function. To limit the number of statistical comparisons, analyses involving protein levels and behavior were restricted to RSFC networks with significant time by group interactions. Data were analyzed using R[39] with growth models fit using the lme4 package.[40] All tests were 2-tailed and $p ≤ 0.05$ was considered statistically significant.

Data availability

Data presented in this report will be made available to research investigators upon request to the corresponding author.

Results

Participants

Sixty-four participants with PD without dementia and 27 controls had useable RSFC data for ≥2 time points (21 PD, 10 controls with ≥3; 6 PD with 4). At the first RSFC time point,

the PD group was older ($F_{1,87} = 4.57$, $p = 0.04$), more educated (nonparametric Mann-Whitney U test due to non-normally distributed values; $U = 556.5$, $z = -2.72$, $p = 0.007$), and had more men ($\chi^2 = 11.39$, $df = 1$, $p = 0.001$) (see table 1 for demographic and clinical information). Therefore, age, education, and sex were included as covariates in group comparisons of RSFC data.

Cross-sectional analyses

CSF α-synuclein and Aβ were significantly lower in the PD group than in controls (nonparametric Mann-Whitney U test; α-synuclein: $U = 315$, $z = -3.05$, $p = 0.002$; Aβ: $U = 343$, $z = -2.72$, $p = 0.006$), but there were no group differences in total tau ($p = 0.31$) or Aβ PET (MCBP; $p = 0.12$) (table 1). The PD group also demonstrated significantly lower (weaker) SMN RSFC (analysis of covariance [ANCOVA] with age, sex, education covariates; $F_{1,88} = 6.93$, $p = 0.01$). In addition, the PD group demonstrated significantly reduced FPCN-SMN internetwork RSFC (ANCOVA; $F_{1,88} = 4.03$, $p = 0.05$). None of the other networks or internetwork interactions significantly differed between groups ($ps \geq 0.07$) at baseline. Finally, lower CSF α-synuclein correlated with reduced SMN strength in PD ($n = 55$, $r = 0.35$, $p = 0.009$). Thus, baseline cross-sectional results are consistent with our previous findings.[9,15,16]

Longitudinal network-level RSFC

Multilevel growth models, with age, sex, education, and disease duration covariates, compared longitudinal network-level RSFC changes between groups (e.g., time by group). There was a significant group by time effect for within-network SMN integrity, with the PD group declining slightly in contrast to the slight increase for controls (annualized estimates = −0.11, $t = -2.64$, $p = 0.008$; table 2, figure 2A). Splitting the PD group based on cognitive status at baseline (PD CDR = 0, n = 36; PD CDR = 0.5, n = 28; control CDR = 0, n = 27) revealed that both the cognitively normal (annualized estimates = −0.099, SE = 0.047, $t = -2.14$, $p = 0.035$) and cognitively impaired groups (annualized estimates = −0.137, SE = 0.065, $t = -2.11$, $p = 0.022$) showed significant SMN differences compared to controls but not each other (annualized estimates = −0.36, SE = 0.052, $t = 0.69$, $p = 0.49$). Importantly, excluding participants who developed dementia (n = 9) resulted in no substantive changes; the PD group declined more rapidly in SMN compared to controls, prior to dementia onset (annualized estimates = −0.105, SE = 0.043, $t = -2.45$, $p = 0.016$).

There was also a significant group by time interaction for DAN-FPCN trajectories (figure 2B). Participants with PD evidenced greater declines in DAN-FPCN compared to controls (annualized estimates = −0.102, SE = 0.045, $t = -2.29$, $p = 0.02$). Again, both participants with PD with normal cognition (n = 36; annualized estimates = −0.109, SE = 0.054, $t = -2.03$, $p = 0.045$) and those with cognitive decline at baseline showed this effect (n = 28; annualized estimates = −0.099, SE = 0.051, $t = -1.96$, $p = 0.056$) and were not significantly different from each other (annualized estimates = −0.013, SE = 0.064, $t = -0.22$, $p = 0.83$). Further, excluding participants who developed dementia (n = 9) yielded a significant group by time interaction (annualized estimates = −0.100, SE = 0.046, $t = -2.16$, $p = 0.035$),

Table 1 Participant demographics, clinical characteristics, and protein measures at baseline

	Control group (n = 27)	PD group (n = 64)	Group comparison
Sex, M/F	6/21	39/25	$p < 0.001$
Age, y	61.5 (9.4)	65.6 (7.9)	$p = 0.04$
Education, y	13 (10–19)	16 (12–21)	$p = 0.007$
PD duration, y	—	6.7 (4.0)	—
UPDRS-III "off" total	—	22.4 (7.4)	—
MMSE[a]	29.0 (1.0)	28.6 (1.6)	$p = 0.10$
Baseline CDR (0/0.5)	27/0	32/32	$p < 0.01$
Baseline CDR sum of boxes	0.02 (0.10)	0.58 (0.76)	$p < 0.01$
Average cognition Z score	0.78 (0.41)	0.45 (0.62)	$p = 0.004$
Aβ PET MCBP	−0.03 (0.05)	0.02 (0.15)	$p = 0.12$
CSF α-synuclein, pg/mL	2,266.15 (721.40)	176.01 (573.2)	$p = 0.002$
CSF Aβ, pg/mL	947.01 (147.4)	826.2 (196.2)	$p = 0.006$
CSF tau, pg/mL	237.7 (105.6)	217.9 (97.7)	$p = 0.31$

Abbreviations: Aβ = β-amyloid; CDR = Clinical Dementia Rating; MCBP = mean cortical binding potential; MMSE = Mini-Mental State Examination; PD = Parkinson disease; Unified Parkinson's Disease Rating Scale.
Values represent mean (SD), except education, which is non-normally distributed and reported as median (range).
[a] MMSE scores for 58 of the participants with PD.

Table 2 Longitudinal network-level functional connectivity changes in Parkinson disease (PD)

| Predictors	SMN			DAN-FPCN		
	Estimates	95% CI	*p*	Estimates	95% CI	*p*
Intercept	0.120	0.097 to 0.143	<0.001	0.026	0.002 to 0.050	0.032
PD duration	−0.008	−0.020 to 0.004	0.172	0.002	−0.010 to 0.014	0.730
Age	0.013	0.004 to 0.022	0.005	0.000	−0.009 to 0.009	0.972
Sex	−0.001	−0.010 to 0.009	0.867	−0.006	−0.015 to 0.004	0.265
Education	0.003	−0.006 to 0.012	0.497	0.001	−0.008 to 0.010	0.820
Time	0.076	0.012 to 0.141	0.020	0.028	−0.041 to 0.097	0.424
PD	−0.019	−0.048 to 0.010	0.199	0.010	−0.020 to 0.040	0.528
Time × PD	−0.111	−0.194 to −0.029	0.008	−0.101	−0.189 to −0.014	0.023
Observations/no. participants	225/91			225/91		
Marginal R^2/conditional R^2	0.227/0.630			0.035/0.551		

Abbreviations: CI = confidence interval; DAN = dorsal attention network; FPCN = frontoparietal control network; SMN = sensorimotor network.
Model estimates reflect annualized change for each predictor and interaction.

indicating DAN-FPCN internetwork RSFC declines prior to dementia onset.

Proteinopathy and longitudinal RSFC

Growth models testing the interaction between baseline proteins (CSF α-synuclein, Aβ, tau; Aβ PET) and longitudinal RSFC network changes for participants with PD were restricted to only the RSFC networks with significant group by time effects (SMN; DAN-FPCN) to limit the number of statistical tests. No associations were found between proteins and DAN-FPCN (all *p*s > 0.52). As seen in table 3, however, SMN showed a number of associations. Surprisingly, PD with higher cortical Aβ PET binding (e.g., MCBP) had higher SMN RSFC, while there were no significant effects of CSF Aβ. However, as expected, participants with PD with lower CSF α-synuclein, and tau, had lower SMN at

Figure 2 Longitudinal network-level resting-state functional connectivity (RSFC) decline in Parkinson disease (PD)
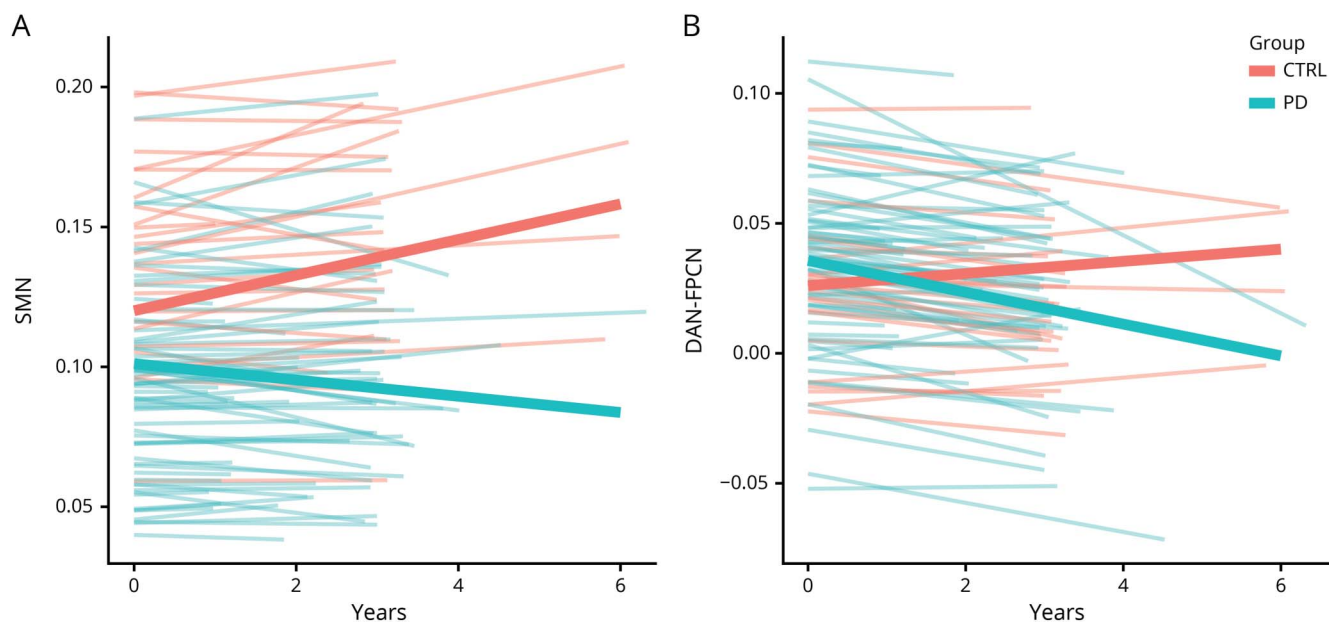

(A) Linear growth curve models reveal a significant group × time interaction for the sensorimotor network (SMN) network, indicating longitudinal decline of SMN RSFC strength for the PD group (blue lines). (B) The interaction, or internetwork RSFC, between dorsal attention network (DAN) and frontoparietal control network (FPCN) declines longitudinally in the PD group (blue lines).

Table 3 Proteinopathy and longitudinal sensorimotor network (SMN) decline in Parkinson disease (PD)

Predictors	SMN Estimates	95% CI	p	SMN Estimates	95% CI	p	SMN Estimates	95% CI	p	SMN Estimates	95% CI	p
Intercept	0.100	0.088 to 0.113	<0.001	0.102	0.087 to 0.117	<0.001	0.100	0.086 to 0.114	<0.001	0.101	0.087 to 0.115	<0.001
PD duration	−0.007	−0.018 to 0.005	0.238	−0.005	−0.018 to 0.009	0.488	−0.004	−0.016 to 0.009	0.593	−0.004	−0.017 to 0.008	0.494
Age	0.016	0.005 to 0.027	0.004	0.014	0.001 to 0.027	0.030	0.016	0.003 to 0.028	0.012	0.017	0.004 to 0.030	0.009
Sex	0.001	−0.010 to 0.011	0.921	0.002	−0.010 to 0.014	0.729	0.001	−0.010 to 0.013	0.841	0.001	−0.011 to 0.013	0.867
Education	0.003	−0.007 to 0.013	0.537	0.003	−0.009 to 0.015	0.604	0.004	−0.007 to 0.015	0.525	0.003	−0.008 to 0.014	0.578
Time	−0.033	−0.083 to 0.016	0.182	−0.047	−0.098 to 0.005	0.075	−0.044	−0.094 to 0.006	0.084	−0.046	−0.096 to 0.004	0.072
Aβ PET MCBP	0.085	0.005 to 0.164	0.036									
Time × MCBP	−0.209	−0.535 to 0.116	0.208									
CSF Aβ				−0.002	−0.008 to 0.005	0.653						
Time × CSF Aβ				−0.003	−0.032 to 0.026	0.833						
CSF α-synuclein							0.015	0.003–0.026	0.011			
Time × CSF α-synuclein							−0.049	−0.095 to −0.003	0.038			
CSF tau										0.008	0.001 to 0.015	0.021
Time × CSF tau										−0.024	−0.049 to 0.001	0.063
Observations/no. participants	164/64			140/55			140/55			140/55		
Marginal R²/conditional R²	0.139/0.630			0.085/0.623			0.130/0.625			0.122/0.614		

Abbreviations: Aβ = β-amyloid; CI = confidence interval; MCBP = mean cortical binding potential.
Model estimates reflect annualized change for each predictor and interaction.

Figure 3 Proteinopathy, longitudinal resting-state functional connectivity (RSFC), and cognitive decline in Parkinson disease (PD)
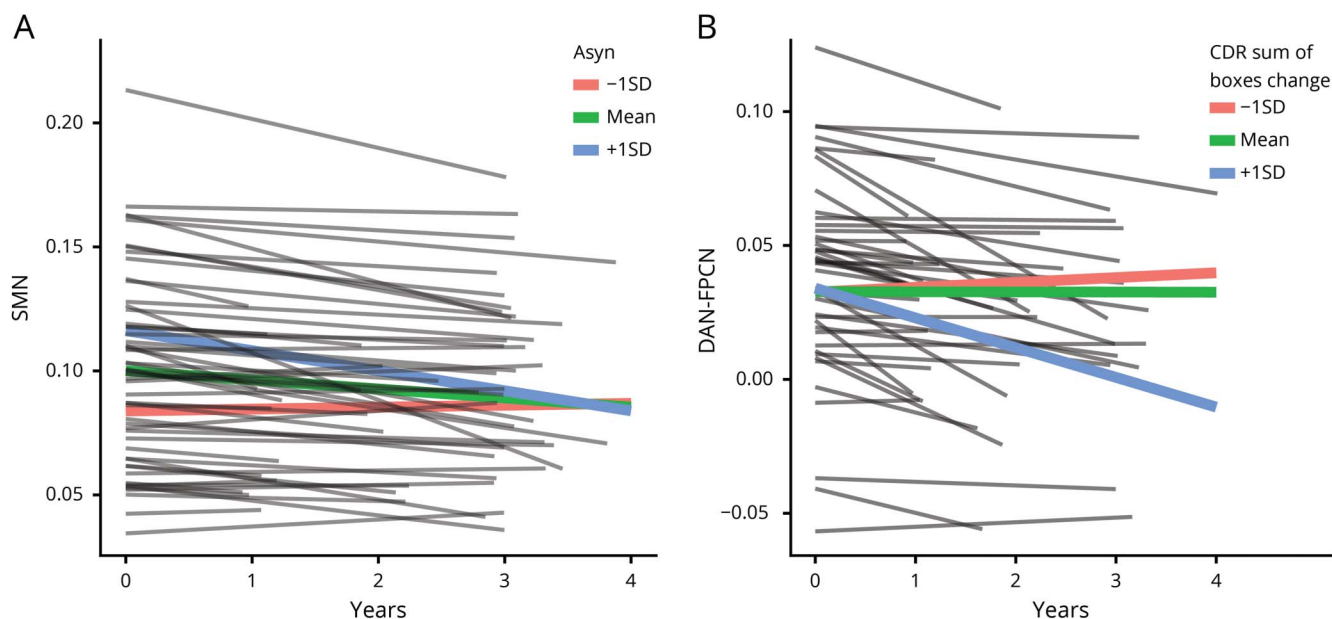
(A) Within the PD group, sensorimotor network (SMN) RSFC strength declines the most for those with higher levels of CSF α-synuclein (i.e., +1 SD above the mean; blue line), while SMN RSFC strength is already reduced and remains relatively stable for those with lower levels (i.e., −1 SD below the mean; red line) of CSF α-synuclein. (B) Dorsal attention network (DAN)-FPCN internetwork strength declined the most among participants with PD with increased cognitive impairment (i.e., +1 SD above the mean; blue line), as measured by increased Clinical Dementia Rating (CDR) sum of boxes scores.

baseline. Interestingly, those with higher baseline CSF α-synuclein declined more rapidly across the study period in SMN strength compared to those with lower levels, who remained relatively stable across time (annualized estimates = −0.049, SE = 0.023, $t = 2.09$, $p = 0.022$; figure 3A). Participants with PD with higher CSF α-synuclein had higher baseline SMN RSFC, but within-network SMN integrity declined over time to match those patients with PD with lower CSF α-synuclein.

PD progression and longitudinal RSFC

Across the study period, motor severity increased (UPDRS-III "off" total mean change = 4.37, SD = 10.62; $t[63] = 4.36$, $p < 0.001$), and global cognition declined (CDR sum of boxes mean change = 0.71, SD = 1.36; $t[60] = 4.07$, $p < 0.001$; average cognitive Z score mean change = −0.12, SD = 0.41; $t[63] = 2.44$, $p = 0.018$). Baseline protein levels were mostly not associated with behavioral changes, except for CDR sum of boxes and CSF Aβ ($r = −0.34$, $p < 0.01$); participants with PD with lower CSF Aβ had worse cognitive function over time. Finally, SMN decline did not relate to increased motor severity (UPDRS-III "off" total, $p = 0.66$) or cognitive impairment (CDR sum of boxes, $p = 0.90$; average cognitive z score, $p = 0.91$). In contrast, DAN-FPCN decline related to cognitive decline (CDR sum of boxes; annualized estimates = −0.005, SE = 0.002, $t = 2.47$, $p = 0.015$; figure 3B). Participants with increased cognitive difficulties showed greater declines in DAN-FPCN functional connectivity.

Discussion

RSFC network integrity declines longitudinally in PD and relates to baseline CSF protein levels. Specifically, SMN intranetwork and DAN-FPCN internetwork RSFC decreased longitudinally in participants with PD without dementia, prior to dementia onset. In fact, baseline CSF α-synuclein levels predicted the longitudinal decline in SMN integrity. Further, longitudinal decline in DAN-FPCN interactions corresponded with increased cognitive impairment. These results suggest that network-level RSFC provides a sensitive measure of PD progression and that altered network-level RSFC precedes cognitive impairment and dementia onset.

Longitudinal SMN decline in PD

Functional connectivity within the SMN declines longitudinally in participants with PD without dementia, consistent with previous reports of cross-sectional SMN deficits[9,17] and the characteristic motor deficits in PD. One other study also reported longitudinal decline in RSFC of motor and occipital regions in PD.[14] Here, we found reduced network-level SMN integrity relates to baseline CSF α-synuclein. Notably, higher baseline CSF α-synuclein predicted greater SMN decline, while PD with lower CSF α-synuclein evidenced reduced, but stable, SMN RSFC. In other words, those with lower CSF α-synuclein already had lower SMN RSFC, while those with relatively higher CSF α-synuclein demonstrated longitudinal decline in SMN integrity.

Although motor function also declined during this time period, the rate of decline did not relate to CSF α-synuclein or reduced SMN RSFC. One possible explanation is that declining SMN integrity relates to specific motor features, such as gait and balance, that are not well-captured by the UPDRS-III ratings. Alternatively, these changes may not temporally co-occur and instead progress at different, perhaps even nonlinear, rates. Longitudinal RSFC changes in additional motor-related regions and networks should also be investigated, especially subcortical and cerebellar regions, which may relate to worsening motor severity.

Longitudinal DAN-FPCN decline in PD

The strength of the interaction between DAN and FPCN also declined longitudinally for participants with PD, prior to dementia onset. Extensive previous research demonstrates the roles of DAN and FPCN in top-down control of goal-directed behaviors.[41,42] Thus, longitudinal decline in DAN-FPCN coupling may reflect decreased cognitive flexibility and reduced top-down cognitive control in PD. In fact, DAN-FPCN decline fits with the classic observation that individuals with PD often rely on externally driven cues and task demands, demonstrating greater difficulty on tasks requiring internally generated thoughts and strategies.[43] Further, recent research suggests that DAN-FPCN internetwork RSFC may regulate visuospatial attention,[44] another area of cognitive dysfunction with PD.[45] Finally, longitudinal DAN-FPCN decline correlated with increased global cognitive impairment in PD, consistent with the notion that reduced DAN-FPCN coupling reflects cognitive dysfunction and precedes dementia onset.

Surprisingly, DAN-FPCN decline did not correlate with baseline protein levels. This may reflect the lack of regional specificity with CSF measures of soluble protein levels as an indirect measure of brain accumulation. Alternatively, these results could suggest that proteinopathy may not be the sole or primary pathology affecting this network interaction. Instead, neurotransmitter deficits, especially dopaminergic and cholinergic systems,[7,30] may play a larger role in disruption of cognitively related associative networks in PD.

Longitudinal RSFC in aging and Alzheimer disease (AD)

The selective pattern of intranetwork and internetwork RSFC decline in PD differs from the pattern observed during healthy aging. For example, healthy older adults demonstrate decreased intranetwork RSFC and increased internetwork RSFC compared to younger adults, primarily affecting associative networks.[46] The increased internetwork RSFC observed with healthy aging, representing network desegregation,[46] is in contrast to the more selective network-level reduction of RSFC in PD[17] as further evidenced by the longitudinal decline in both SMN and DAN-FPCN shown here.

The longitudinal RSFC declines in SMN and DAN-FPCN for individuals with PD also contrast with the central findings of DMN deficits in AD. Not only does the DMN decline

longitudinally with AD,[47] but interactions between DMN and FPCN also decline.[48] Cross-sectional studies similarly demonstrate DMN deficits with increased cognitive impairment associated with AD.[37] Interestingly, this may be preceded by an initial increase in DMN RSFC with mild cognitive impairment,[49] prior to dementia onset. Additional longitudinal studies with direct comparison between AD and PD are needed to determine disease-specific RSFC markers.

Proteinopathy and RSFC

The relationship between CSF α-synuclein and longitudinal SMN decline follows the known pathology and predominant motor deficits in PD. For example, 2 previous cross-sectional studies report reduced RSFC in relation to CSF α-synuclein levels in PD.[9,10] However, despite numerous studies implicating abnormal Aβ in PD,[3,4,16] Aβ levels were not related to longitudinal RSFC decline in PD. In fact, PD with higher cortical Aβ PET binding had stronger SMN RSFC at baseline, contrary to the expected detrimental effect of abnormal Aβ. Most of the research on Aβ and RSFC, in the context of AD, focuses on the DMN, with the general finding that lower CSF Aβ relates to lower DMN RSFC.[50] Even though CSF Aβ was significantly lower in participants with PD than controls, there were no group differences in DMN RSFC.

The relative lack of an association between Aβ and RSFC in PD raises the question of the functional significance of Aβ for PD. Although one argument might be that soluble Aβ monomers measured with CSF do not adequately reflect neuropathologic Aβ accumulation, the PET measure of fibrillar Aβ also did not correlate with RSFC decline in PD. Instead, CSF Aβ predicted longitudinal cognitive decline in PD. Measures of other Aβ conformations, such as CSF Aβ oligomers or PET tracers for diffuse Aβ plaques, will permit better assessment of the role of Aβ in PD. Thus, it remains unclear if Aβ pathology contributes, either uniquely or in combination with α-synuclein, to PD progression or is secondary to α-synuclein pathology. Additional research on the potential unique and combined effects of Aβ in the context of PD is needed.

Strengths and limitations

The present study provides new insights on RSFC and the relationship between proteinopathy and brain function in PD. This study represents one of the few longitudinal studies of RSFC in PD; many studies, even on aging and AD, use cross-sectional analyses to compare across groups (e.g., young, middle age, older; normal, impaired, dementia).[37,46] Using a relatively large sample, with multiple time points (>2) for better estimation of change over time, we demonstrate network-level RSFC declines in PD. Our focus on both intranetwork and internetwork-level RSFC, rather than region or whole-brain RSFC, represents an important advance on previous research, especially in light of recent evidence of prominent internetwork RSFC reductions in PD.[17] We employed rigorous methodology to control for potential confounds, including removal of motion artifacts, exclusion of potential preclinical AD controls, and age and disease

duration covariates in sophisticated growth curve analyses. Importantly, this study focused on a well-characterized sample of participants with PD without dementia to identify potential antecedent markers of dementia. Finally, using multimodal biomarkers represents a crucial step for understanding the functional consequences of neuropathology and is at the forefront of PD research.

The present study focused on a limited set of cortical networks; larger sample sizes and more advanced statistical approaches would be required to examine longitudinal connectome-level RSFC. Larger sample sizes also would be necessary to detect subtle changes and potential subgroup differences. Although we attempted to restrict the number of analyses, we did not apply a formal multiple comparison correction and results should be interpreted with some caution. We also recognize that PD neuropathology involves more than just proteinopathy and can include neuroinflammation, atrophy, and neurotransmitter deficits. Despite these relative limitations, this study provides important new information regarding longitudinal RSFC changes and the potential role of proteinopathy in brain dysfunction with PD.

Network-level RSFC declines longitudinally in PD and may serve as a biomarker of PD progression. The greatest decline in SMN integrity occurred in participants with PD with less severely depleted CSF α-synuclein, suggesting that SMN integrity tracks severity of α-synuclein neuropathology. Longitudinal CSF measurements will be needed to further clarify this relationship. Further, longitudinal disruption of the interaction between DAN and FPCN associative networks related to cognitive decline, which may yield an antecedent biomarker for development of dementia. Surprisingly, neither Aβ PET nor CSF Aβ levels related to longitudinal RSFC changes in PD, raising further questions regarding the role of Aβ in the context of PD. Overall, this study emphasizes the role of α-synuclein pathology in brain dysfunction and provides initial evidence for network-level RSFC as a prognostic biomarker for PD progression.

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Publication history

Appendix Authors

Name	Location	Role	Contribution
Meghan C. Campbell, PhD	Washington University School of Medicine	Author	Study concept and design, analysis and interpretation of data, drafting/revising the manuscript for content
Joshua J. Jackson, PhD	Washington University in St. Louis	Author	Analysis and interpretation of data, drafting/revising the manuscript for content
Jonathan M. Koller, BSBME, BSEE	Washington University School of Medicine	Author	Analysis and interpretation of data, revising the manuscript for content
Abraham Z. Snyder, MD, PhD	Washington University School of Medicine	Author	Interpretation of data, revising the manuscript for content
Paul T. Kotzbauer, MD, PhD	Washington University School of Medicine	Author	Analysis and interpretation of data, revising the manuscript for content
Joel S. Perlmutter, MD	Washington University School of Medicine	Author	Study concept and design, interpretation of data, revising the manuscript for content

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