

RESEARCH ARTICLE

Identification of Parkinson's Disease Subtypes from Resting-State Electroencephalography

CME

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ABSTRACT: Background: Parkinson's disease (PD) patients present with a heterogeneous clinical phenotype, including motor, cognitive, sleep, and affective disruptions. However, this heterogeneity is often either ignored or assessed using only clinical assessments.

Objectives: We aimed to identify different PD sub-phenotypes in a longitudinal follow-up analysis and their electrophysiological profile based on resting-state electroencephalography (RS-EEG) and to assess their clinical significance over the course of the disease.

Methods: Using electrophysiological features obtained from RS-EEG recordings and data-driven methods (similarity network fusion and source-space spectral analysis), we have performed a clustering analysis to identify disease sub-phenotypes and we examined whether their different patterns of disruption are predictive of disease outcome.

Results: We showed that PD patients ($n = 44$) can be sub-grouped into three phenotypes with distinct electrophysiological profiles. These clusters are characterized by different levels of disruptions in the somatomotor

network (Δ and β band), the frontotemporal network ($\alpha 2$ band) and the default mode network ($\alpha 1$ band), which consistently correlate with clinical profiles and disease courses. These clusters are classified into either moderate (only-motor) or mild-to-severe (diffuse) disease. We showed that EEG features can predict cognitive evolution of PD patients from baseline, when the cognitive clinical scores were overlapped.

Conclusions: The identification of novel PD subtypes based on electrical brain activity signatures may provide a more accurate prognosis in individual patients in clinical practice and help to stratify subgroups in clinical trials. Innovative profiling in PD can also support new therapeutic strategies that are brain-based and designed to modulate brain activity disruption. © 2023 The Authors. *Movement Disorders* published by Wiley Periodicals LLC on behalf of International Parkinson and Movement Disorder Society.

Key Words: electroencephalography; disease phenotyping; clustering; Parkinson's disease; resting-state

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Introduction

Parkinson's disease (PD) is a neurodegenerative disorder characterized by significant clinical (presentation and prognosis) and pathological heterogeneity.¹⁻⁴ This heterogeneity is delineated by a constellation of motor and non-motor symptoms (sleep disturbance, cognitive decline, emotional and vegetative disorders, etc) and associated with multiple genetic, environmental, and comorbidity factors. It has, therefore, largely endorsed the emerging concept of approaching PD as the final common pathway of multiple disease mechanisms rather than a single entity.⁵⁻⁷ Considering PD as a single disorder has been indeed ineffective when attempting disease modification.⁶ To take this heterogeneity in PD into account, efforts are currently focused on what is known as “precision (or stratified) medicine”, that requires a prior identification of distinct prodromal PD subtypes⁷⁻¹⁰ probably based on differing primary disease processes.

Most previous PD subtyping studies are based only on clinical/behavioral assessments.¹¹⁻¹⁴ Despite revealing clinically relevant PD subtypes, uncovering the extent of the underlying physiopathology remained very limited. In contrast, genome-wide association studies, cerebrospinal fluid analysis, and neuroimaging studies have led to better understanding of the biological underpinnings of the disease by associating common genetic variants, proteins, or neurophysiological processes with different forms of PD in terms of clinical features,¹⁵⁻¹⁷ treatment's responsiveness,^{18,19} and rates of progression.^{20,21} Indeed, integrating features from such data modalities in the clustering approaches would result in better identification of PD subtypes that share latent pathological mechanisms.²²⁻²⁵

Moreover, stratifying PD patients according to their individual disease trajectories is fundamental to improve biomarkers development strategies.⁹ This cannot be done without an adequate methodological framework that includes sufficient longitudinal assessments. In recent years, electroencephalography (EEG) has shown its worthiness as a promising low-cost technique for biomarkers discoveries providing non-invasive access to the electrophysiological activity of the whole brain at the millisecond.^{26,27} Several studies used EEG features and clustering techniques to identify subphenotypes of different brain disorders.²⁸⁻³⁰ In parallel, the advent of longitudinal studies assessing the evolution of clinical and biological features of different PD subtypes has afforded reliable tracking of the clinical trajectory and disease progression by attributing a well-defined biological/clinical profile for each subphenotype.^{13,22-25,31} Yet, the use of EEG in a longitudinal framework to establish PD phenotyping based on neurophysiological brain activity is still missing.

In this study, we used resting-state high-density (HD)-EEG recordings of 44 PD patients from their 5-year follow-up examinations to perform a clustering

analysis based on an unsupervised learning technique called similarity network fusion (SNF).³² We aimed to identify different PD subgroups with distinct electrophysiological profiles and appraise their clinical significance and neurophysiological progression longitudinally. We also investigated the ability of the relevant electrophysiological features to distinguish between PD subtypes from early disease stages at baseline (BL) and associate them with the global cognitive state of patients. Finally, we validated the categorical subtyping by applying a dimensionality reduction analysis on the input features of the clustering analysis and associating the low-dimensional representations with the significant EEG measures.

Materials and Methods

Participants

Patients with idiopathic PD were prospectively recruited from the Movement Disorders Clinic of University Hospital of Basel (City of Basel, Switzerland) as part of a 5-year longitudinal cohort described in our previous study.³³ HD-EEG examinations, along with neurological and cognitive assessments, were performed for the included patients at BL and at follow-up visits after a mean interval of 3 years (3Y) and 5 years (5Y). All patients gave written informed consent to the research protocol, which was approved by the local ethics committees (Ethikkommission beider Basel, Basel; Switzerland; EK 74/09). Of the 77 PD patients recruited for the main cohort BL, only 45 who participated in the 5Y examinations were selected for this study. One patient was subsequently excluded because of significant artifacts in their corresponding EEG recordings. Therefore, the final sample size for the clustering analysis at 5Y is $n = 44$ PD patients. Among these patients, nine did not complete the 3Y follow-up examinations and were excluded because of missing EEG recordings, and two patients had significant artifacts in their corresponding EEG recordings at BL and were also excluded from the study. As a result, the sample size was subsequently reduced to 35 PD patients at 3Y and 42 PD patients at BL. Patients were evaluated while taking their regular dopaminergic medication. The demographic and clinical characteristics (Supplementary Table S1, S2) of this longitudinal study cohort are presented in the Supplementary Data S1, materials and methods section.

Data Acquisition, Preprocessing, and Source Reconstruction

The experimental paradigm consisted of 12 minutes of continuous resting state with closed eyes. Patients were seated in a comfortable chair and asked to relax while staying awake with minimum body movements. HD-EEG data were collected using a 256-channel

system (Netstation 300, EGI, Eugene, OR) and sampled at 1000 Hz. Segmentation and preprocessing procedures are detailed in the Supplementary Data S1, methods section. Further, to estimate brain activity at the cortical level rather than at the scalp level, we applied the first step of the EEG source connectivity method.³⁴ This involved reconstructing the dynamics of the brain at the cortical-source level from the artifact-free data segments by solving the inverse problem. The procedure included co-registering EEG channels with a magnetic resonance imaging (MRI) template (ICBM152), building a realistic head model (using the OpenMEEG toolbox),³⁵ and applying the weighted minimum norm estimate method.³⁶ The outputs were the time-varying signals of the 210 cortical regions of interest (ROIs) from the Brainnetome atlas.³⁷

Power Spectral and Functional Connectivity Features

The 210 source-reconstructed signals were used to estimate the power spectrum at the cortical level using the Welch method.³⁸ Relative band power features were then estimated in five different EEG frequency bands: Δ (1–4 Hz), θ (4–8 Hz), α_1 (8–10 Hz), α_2 (10–13 Hz), and β (13–30 Hz) and a total of $[210 \times 5]$ power spectral features were obtained for each patient for further analysis. Regarding functional connectivity features, in each frequency band, statistical couplings were estimated between the 210 regional time series using the “Amplitude Envelope Correlation (AEC)” method³⁹ with leakage correction to reduce the volume conduction effect and a total of $[219 \ 45 \times 5]$ unique connectivity features were attributed to each patient for further analysis. More details about

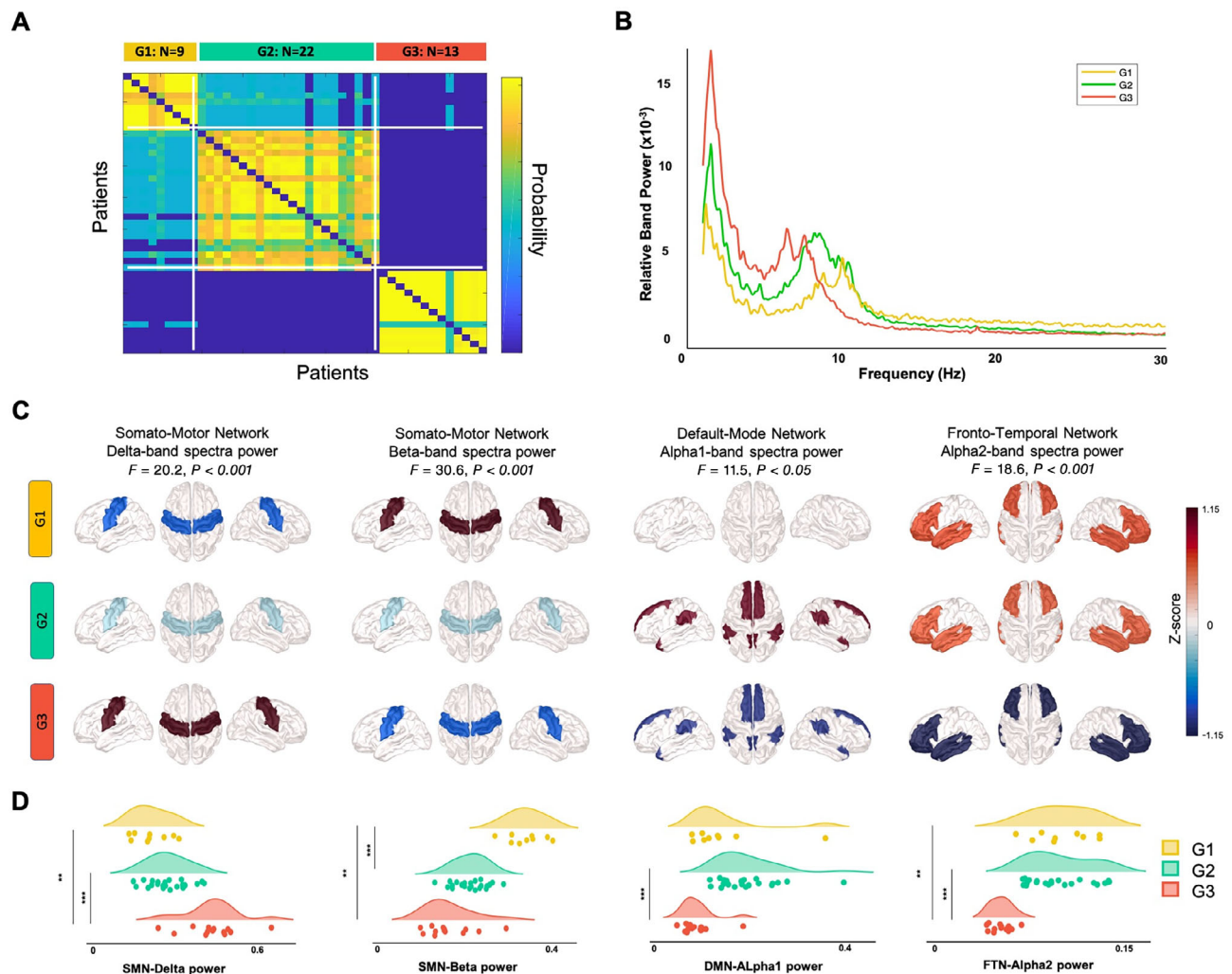


FIG. 1. Parkinson's disease (PD) subtypes and their electrophysiological profiles. **(A)** Patient co-assignment matrix representing the three identified subgroups and indicating the probability of two patients being assigned to the same cluster across clustering solutions. **(B)** Changes in the relative power spectra for the three PD subtypes. **(C)** Distinct neurophysiological profiles characterizing PD patients in each subgroup across frequency bands. For representation purposes, the average power in each brain network was converted to z-scores. **(D)** Distribution of the quantifiable power measures between the three PD subgroups: average power of somatomotor network (SMN) in Δ and β , default mode network (DMN) in α_1 , fronto-temporal network (FTN) in α_2 . Significant differences between subgroups are marked as $**P < 0.01$ and $***P < 0.001$. P -values were corrected for multiple comparisons using Bonferroni. [Color figure can be viewed at wileyonlinelibrary.com]

these approaches are provided in the Supplementary Data S1, methods section.

Clustering Analysis and Dimensionality Reduction

To retrieve clusters of patients with distinct neurophysiological profiles, we followed a data-driven unsupervised clustering approach based on the SNF method.³² SNF is a novel approach for combining features from different data sources into a single fused similarity network that captures both mutual and complementary information between features offering insights into the strength of relationship between patients. Here, we used SNF to extract and combine similarity networks corresponding to the power spectral (or/and functional connectivity) features from the different frequency bands. More details about the method and the clustering pipeline can be found in the Supplementary Data S1, method section.

Further, to validate the categorical representation derived from the clustering analysis, we conducted a dimensionality reduction analysis using diffusion map embedding as described in Markello et al.²³ This approach produced a continuous low-dimensional representation that contained the most meaningful properties of the original data (ie, the underlying pathology). See Supplementary Data S1, method section, for more technical details about the process.

Neurophysiological Profiles of the Clusters and Correlation Analysis

To attribute a specific neurophysiological profile for each of the identified clusters, we defined four spectral power/connectivity measures corresponding to the average relative power/connectivity of regions within four

brain networks (somatomotor network [SMN]; default mode network [DMN]; fronto-temporal network [FTN]; and fronto-parietal network [FPN]). Those networks are recognized to be activated at rest⁴⁰ and to be disrupted in PD.^{41,42} The affiliation of brain regions to each of these networks (Supplementary Table S3), along with more in-depth details about the statistical and correlation analysis are provided in the method section of the Supplementary Data S1.

Results

EEG Reveals Three PD Clusters with Distinct Electrophysiological Profiles

The clustering analysis applied to the EEG power spectral features at the five different frequency bands yielded the identification of three PD subgroups: G1 ($n = 9$), G2 ($n = 22$), and G3 ($n = 13$) (Fig. 1A). The clusters were stable under perturbations in the data with an average robustness coefficient of 91% over 100 iterations. The overall changes in their relative spectral power over all brain regions are illustrated in Figure 1B. Higher relative power appears to characterize the neural activity of G3 compared to G1 and G2 at low frequency bands (Δ , θ), in contrast a shift toward slower neural activity for G3 compared to G1 and G2 was observed for both α and β frequency bands.

Concerning the most relevant brain networks in maximally dissociating the identified clusters in terms of relative spectral power, results showed that the SMN was the most significant in both Δ ($F = 20.2$, $P < 0.001$) and β ($F = 30.6$, $P < 0.001$), the DMN in $\alpha 1$ ($F = 11.5$, $P < 0.05$) and the FTN in $\alpha 2$ ($F = 18.6$, $P < 0.001$). No network appeared to significantly distinguish between groups in the θ band. This approach allowed us to attribute a distinctive neurophysiological profile to each

TABLE 1 Demographic and clinical characteristics of the three identified subgroups of PD patients expressed as mean (standard deviation)

		G1 (n = 9)	G2 (n = 22)	G3 (n = 13)	P-value	
Demographic	Age (year)	65.6 (6.4)	72.6 (7.8)	74.4 (6.7)	0.021	G1 < G3
	Sex (M/F)	6/3	15/7	9/4	0.992	—
	Education (year)	14.8 (2.9)	14.9 (3.4)	15.6 (2.8)	0.785	—
Clinical	Disease duration (year)	12.25 (5.8)	9.3 (5.2)	10.9 (3.5)	0.301	—
	Disease side (LPD/RPD/both)	7/2/0	14/8/0	7/4/2	—	—
	UPDRS-II	10.4 (7.1)	8.3 (6.6)	11.6 (6.5)	0.420	—
	UPDRS-III	21.5(12.7)	15.9 (12.8)	25.3 (13)	0.209	—
Medication	LEDD (mg/day)	839 (399)	613 (411)	524 (292)	0.160	—
Global cognition	MoCA	27.7 (1.6)	26.3 (1.9)	21 (8)	0.001	G1, G2 > G3
	MMSE	28.9 (1.7)	28.6 (1.3)	27.2 (2.6)	0.051	—

Abbreviations: LEDD, levodopa equivalent daily dose; LPD, left-affected PD; M/F, male/female; MMSE, Mini Mental State Examinations; MoCA, Montreal Cognitive Assessment; PD, Parkinson's disease; both: bilateral PD; RPD, right-affected PD; UPDRS-II, Unified Parkinson's Disease Rating Scale motor experiences of daily living; UPDRS-III, Unified Parkinson's Disease Rating Scale motor examinations.

subgroup (Fig. 1C). For example, G1 is characterized with low SMN power in Δ and high SMN power in β , whereas G2 shows a high power within the DMN in $\alpha 1$. Moreover, high power within the FTN in $\alpha 2$ appeared to represent both G1 and G2. Finally, high Δ power for the SMN in contrast with low powers for the DMN in $\alpha 1$, the FTN in $\alpha 2$ and the SMN in β were characteristic of the neurophysiological profile of G3.

Furthermore, by associating a relevant brain network for each frequency band, we computed a quantifiable power measure at the patient level to investigate their distributions across subtypes. At 5Y, results revealed

significantly higher values for G3 compared to G1 ($P < 0.01$) and G2 in terms of SMN- Δ power ($P < 0.001$). Conversely, this metric presented significantly lower values for G3 when computed for the DMN in $\alpha 1$ ($G3 < G2$, $P < 0.01$), the FTN in $\alpha 2$ ($G3 < G1$, $P < 0.01$; $G3 < G2$, $P < 0.001$) and the SMN in β ($G3 < G1$, $P < 0.01$). Differences between G1 and G2 were only significant for the SMN- β power ($G1 > G2$, $P < 0.001$) (Fig. 1D). All reported P -values were corrected using Bonferroni for multiple comparisons.

We, then, investigated the longitudinal evolution of the EEG-based clusters between BL, 3Y, and 5Y. Post

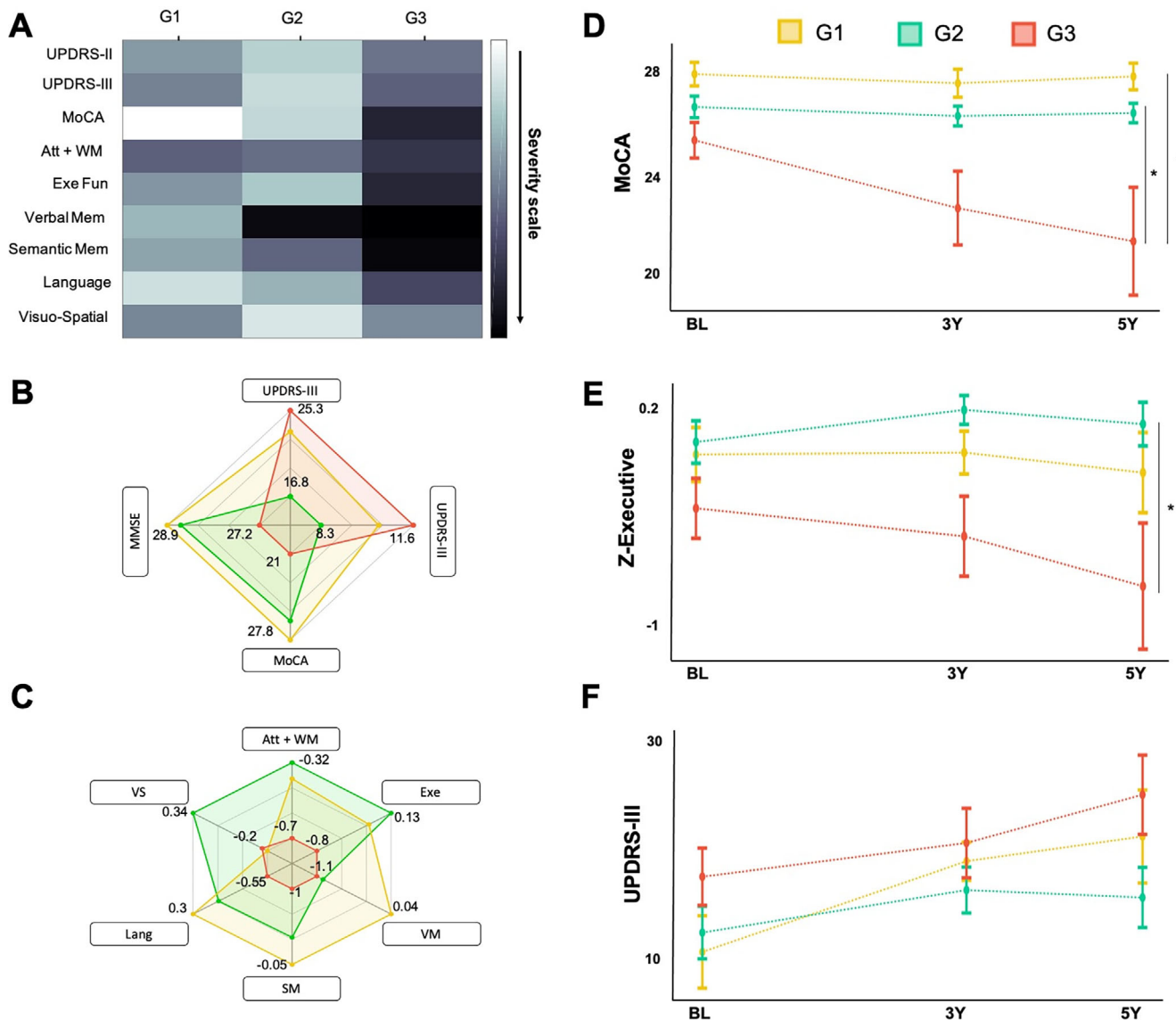


FIG. 2. Parkinson's disease (PD) subtypes and their clinical significance. **(A)** Heatmap illustration of the main global clinical scores (Unified Parkinson's Disease Rating Scale [UPDRS]-II, UPDRS-III, and Montreal Cognitive Assessment score [MoCA]) and the average of the neuropsychological scores per domain (attention + working memory [att + WM], executive function [Exe], verbal memory [VM], semantic memory [SM], language [Lang], and visuo-spatial abilities [VS]) across the three identified PD subtypes. Darker colors represent more severe deficits for the variable. Spider plots of **(B)** the main global clinical scores and **(C)** the average of the neuropsychological z-scores per cognitive domain. Longitudinal progression in time of each of the **(D)** MoCA, **(E)** average z-scores of the tests assessing the executive function and **(F)** UPDRS-III score. Error bars represent the standard error and significant differences between subgroups are marked as $*P < 0.05$. [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com/doi/10.1002/mds.29451)]

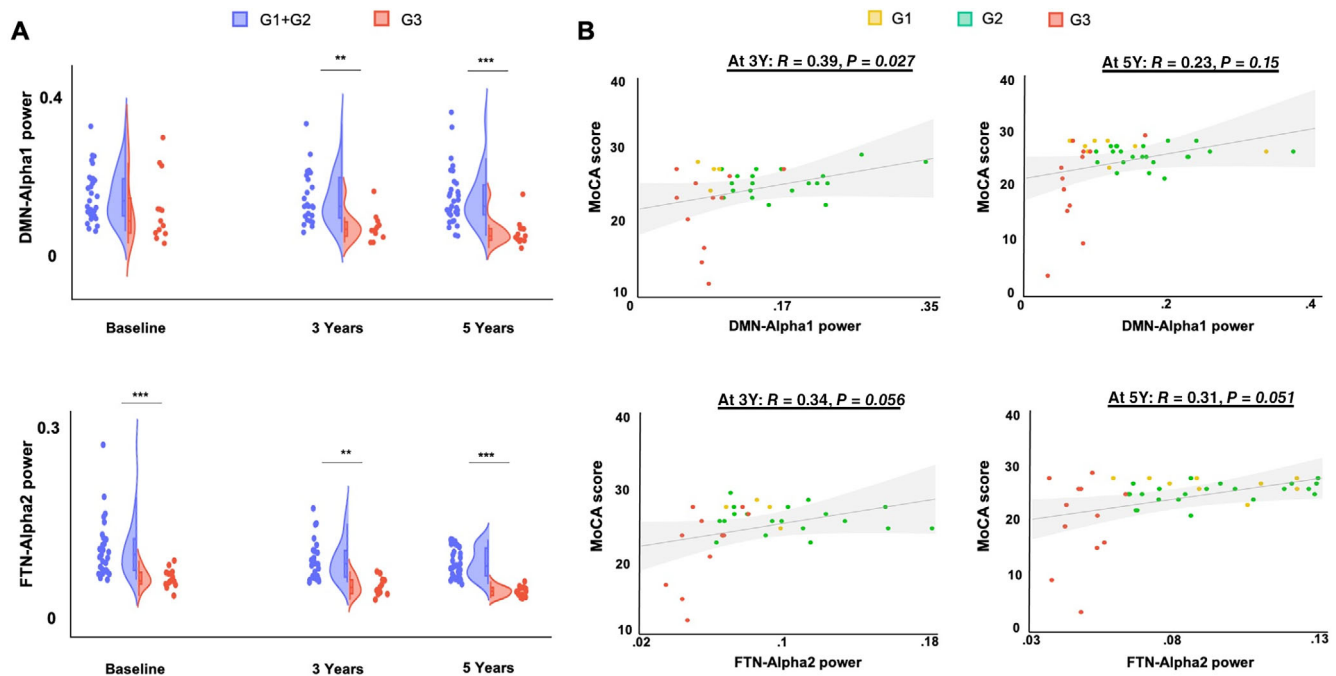


FIG. 3. Longitudinal dissociation between the severe and the moderate groups. (A) Longitudinal progression of the default mode network (DMN) power in $\alpha 1$ and the fronto-temporal network (FTN) power in $\alpha 2$ for the moderate group G1 + G2 against the severe group G3. Significant differences between both groups are marked as ** $P < 0.01$ and *** $P < 0.001$. (B) Correlation between DMN- $\alpha 1$ /FTN- $\alpha 2$ power measures and the global cognitive score Montreal Cognitive Assessment score at 3Y and 5Y. [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com/doi/10.1002/mds.29451)]

hoc analysis revealed that both G1 and G2 showed a relatively constant power spectrum over time compared to G3 where an increase in EEG power between BL and 5Y was clearly observed mainly at lower frequency bands Δ and θ (Supplementary Fig. S1). A broad examination on the longitudinal progression of the significant EEG-based features that characterize the three groups was also assessed for the different frequency bands (Supplementary Fig. S2). We also validated the categorical representation over continuous severity dimension showing that the three groups are dissociable along the first two dimensions of the embedding (Supplementary Fig. S3). Detailed results are provided in the Supplementary Data S1.

Clinical Profiles of EEG Clusters

The demographic and clinical characteristics of the identified clusters are represented in Table 1. Significant inter-group differences were observed only for age ($P = 0.021$) between G1 and G3, with the latter group being older, and for the Montreal Cognitive Assessment score (MoCA) ($P = 0.001$) with G3 having significantly lower scores compared to G1 and G2. To assess the clinical relevance of each subgroup, we conducted a series of one-way ANOVAs on the different neuropsychological tests. Performances of each PD subgroup in those tests are represented in the Supplementary Table S4. Significant inter-groups differences were found

mainly in tests assessing the executive function with G3 having significantly lower scores than G1 and G2. Overall, G3 showed lower scores in all cognitive domains (Fig. 2A,C) with significantly lower global cognitive score MoCA, which express a more severe cognitive state compared to G1 and G2, in contrast it showed higher Unified Parkinson's Disease Rating Scale (UPDRS)-II and UPDRS-III scores, which reflects more severe motor deficits (Fig. 2B). We also investigated for the three subtypes, the longitudinal patterns of progression of the MoCA scores (Fig. 2D), the UPDRS-III scores (Fig. 2F), and the mean Z-score of the tests assessing the executive function (as several tests assessing this domain were discriminable between subgroups at 5Y) (Fig. 2E). Results showed that at BL, these scores overlapped with no significant differences between subgroups, however longitudinally, G3 had the most prominent decline in general cognitive ability and executive function in contrast to relatively unchanged patterns in G1 and G2. When considering the different scores collectively (Fig. 2), G3 can be categorized as a mild-to-severe (diffuse) PD group with progressive decline in cognitive function, whereas both G1 and G2 can be viewed as moderate (motor only) groups, with G1 exhibiting a more rapid motor progression.

Finally, to further investigate the plausibility of the spectral power features in identifying clinically relevant PD subtypes with distinct neurophysiological profiles, we conducted the same previous analysis using source-space

functional connectivity solely and on the combination of both functional connectivity and spectral power features. Detailed results are available in the Supplementary Data S1.

EEG Distinguish Mild-to-Severe PD from Moderate PD Longitudinally

Here, we investigate further the effectiveness of the EEG-based features that characterize these subtypes at 5Y in discriminating between the mild-to-severe PD group and the moderate PD groups from early disease stages at BL, where the clinical scores were not discriminative yet. To do so, we aggregated both G1 and G2 into one moderate only-motor group (G1 + G2) and we compared their corresponding discriminative measures with those of the mild-to-severe group G3 longitudinally. Results showed that the DMN power in $\alpha 1$ and the FTN power in $\alpha 2$ were the most prominent in significantly distinguishing both PD groups longitudinally. In particular, the power of the FTN in $\alpha 2$ was the most relevant in terms of statistical differences between PD subgroups throughout the disease trajectory. The statistical differences were significant from BL even after eliminating the outliers (Cohen's $d = 1.9$, $P < 0.001$) toward the follow-up examinations at 3Y (Cohen's $d = 1.5$, $P < 0.01$), and at 5Y (Cohen's $d = 2.2$, $P < 0.001$) (Fig. 3A). The power of the FTN in $\alpha 2$ could, therefore, be considered predictive of the disease's evolution toward either only-motor or diffuse disease.

We also computed Spearman correlation (with covariate control) between EEG measures and the MoCA scores. Results revealed a significant positive correlation between the DMN- $\alpha 1$ power and the MoCA score at 3Y ($R = 0.39$, $P = 0.027$) and not at 5Y. As for the FTN- $\alpha 2$ power measures, they were positively correlated with the MoCA scores at 3Y ($R = 0.34$, $P = 0.054$) and at 5Y ($R = 0.31$, $P = 0.051$), but without reaching the significance level (Fig. 3B).

Discussion

As the wide spectrum of motor and non-motor symptoms in PD advocates for an undoubtable heterogeneity between patients, there is a need for disease subtyping frameworks to deal with this heterogeneity and empower disease-modification strategies.^{6,7,9} Until recently, defining PD subtypes was based mainly on clinical features, lacking a characterization of the underlying pathobiology of the identified phenotypes.^{2,13} In the present study, we used resting state HD-EEG to reveal subtypes of PD patients using an unsupervised data-driven clustering approach. The analysis led to identifying three different clinically relevant PD subtypes with distinct electrophysiological profiles.

Resting State EEG-Based PD Clusters

Several studies have used quantitative EEG to characterize PD electrophysiological abnormalities.⁴³⁻⁴⁵ Higher spectral power in the low frequency bands (Δ and θ) and lower power in the high frequency bands (α and β) were associated with cognitive deterioration of PD patients in several electrophysiological cross-sectional^{46,47} and longitudinal studies.⁴⁸⁻⁵⁰ Interestingly, those spectral patterns appeared to characterize our identified subgroup G3 compared to G1 and G2. Moreover, the electrophysiological profile attributed to this cluster is characterized by prominent low powers within the DMN and the FTNs. Intrinsic metabolic,⁵¹ oscillatory brain activity,⁵² and functional connectivity^{17,50,53-55} abnormalities within those networks were strongly associated with PD-related cognitive decline and dementias. This may suggest that patients of G3 are more cognitively impaired than those of G1 and G2, which was confirmed by the high proportion of patients classified clinically with Mild Cognitive Impairment (MCI) within G3 (9/13 = 69%) and by their clinical assessments with stronger deficits in both their motor and non-motor scores.

Concerning the electrophysiological differences between G1 and G2, both groups had relatively similar EEG characteristics in all frequency bands except for β , where G1 showed significantly higher power than G2, mainly within the SMN. Accordingly, the clinical profile of those clusters overlapped in almost all assessments. However, only the motor functionalities of G1 have decayed more rapidly over time than G2, suggesting a motor predominant profile for this cluster. These observations correlated with previous EEG findings that associated the increase in β power with low non-dopaminergic disease severity⁵⁶ and impairments in motor symptoms.⁵⁷

Furthermore, disruptions in functional connectivity have been broadly associated with clinical phenotyping of PD using EEG,^{54,58} magnetoencephalography,^{55,59} or functional MRI (fMRI)³¹ studies. Here, investigating the prospect of HD-EEG functional connectivity features in dissociating multiple phenotypes of PD was also promising and has led to identify three subgroups different from those issued using the spectral power features. Despite presenting inter-group statistical differences, some eccentric values of connectivity for few patients have weakened the relevance of the corresponding electrophysiological profiles of the clusters (Supplementary Fig. S4). This was confirmed later by the lack of pronounced clinical differences between subtypes and the major overlap endorsed by the low-dimensional projections of the input connectivity features. Additionally, integrating both spectral power and functional connectivity features has also generated three clusters, but with more similar affiliations to those yielded when using only the spectral power features (Supplementary Fig. S5). One possible interpretation of these findings could be the

preponderance of information derived from power features over those of connectivity. It is worth mentioning that handling the same analysis by using spectral power features computed at the scalp level rather than at source level yielded to the identification of two subtypes rather than three, the first combining G1 and G2 and the second involving the same patients of G3. Although this approach has separated the moderate group from the mild-to-severe group clinically, features computed at source level were more effective in splitting the moderate group into two subtypes with different clinical and electrophysiological profiles.

Finally, several longitudinal studies on PD subtyping, mainly on the Parkinson Progression Marker Initiative (PPMI) cohort, have used clustering, machine learning, and deep learning techniques. In line with our study, they also identified three PD subgroups with different disease severity and progression rates.²²⁻²⁵ In particular, Fereshtehnejad et al²² and Markello et al²³ have demonstrated that integrating structural data into clustering approaches is very relevant in identifying clinically meaningful clusters attributed with several patterns of disruptions, in terms of brain atrophy and dopamine binding. The HD-EEG adds complementary information to these observations in terms of underlying electrophysiology. That said, HD-EEG provides some advantages as a direct measure of neuronal activity, relatively easy-to-use, portable, widely available in a low-income environment, and has excellent time resolution to analyze fast brain dynamics.

Clinical Prominence of EEG Clusters

Our longitudinal assessment has enabled an accurate clinical/electrophysiological tracking of the disease progression in each subphenotype. The most clinically distinct identified subtype was the mild-to-severe group G3 that showed longitudinal deteriorations in both motor and non-motor scores. Patients of this cluster were older with relatively shorter disease durations than those of the moderate groups, endorsing, therefore, the association of older age at onset with the severity of motor and non-motor features.⁶⁰ In addition, a rapid motor progression has differentiated the moderate subtype G1 from the moderate subtype G2 despite the “on” dopaminergic medication state of the patients and the heterogeneity of their levodopa equivalent daily dose intakes. Indeed, those findings would suggest that the unique neurophysiological patterns of the EEG-based subphenotypes presumably reflect their distinction in terms of non-dopaminergic disease severity, in accordance with a previous quantitative EEG study.⁵⁶

Furthermore, the longitudinal assessment has also shown an extensive overlap between the cognitive scores of the mild-to-severe subgroup and the moderate subgroups at BL. However, their distinct electrophysiological profiles, mainly in the α band, remained separable not

only at the 5Y examinations, but at earlier stages of the disease. The presented work has, therefore, emphasized a potential predictive ability for the resting state EEG in distinguishing between patients according to their future clinical deterioration trajectories. This is in line with several longitudinal subtyping studies that showed that identifying biological-based subphenotypes of PD²²⁻²⁵ or other neurodegenerative disorders^{28,29} provides a link toward causative pathological mechanisms with a confirmed clinical relevance contrary to exclusively clinical-based subtyping studies. In this context, insights provided by the EEG are promising for what is known as “stratified medicine” since prior biologically and clinically relevant subtyping would ultimately yield to improve disease modifying/interventions strategies in well-defined targeted patients.^{6,7,9}

Limitations

Despite the 5-year longitudinal assessments provided for this work, the number of patients who participated in the 5Y examinations and included in the clustering analysis was relatively low because of the complexity in upholding long-term follow-up studies. It is important to admit the limitations of the study and the exploratory nature of the results, particularly when dealing with small sample sizes and a large number of features and statistical tests. In our study, this issue specifically impacted the statistical comparisons for G2, which consisted of only four patients at the 3Y assessment. On another note, although the “on” dopaminergic medication state of the patients appraised the non-dopaminergic severity of the disease in the identified subphenotypes, nevertheless it may also conceal the magnitude of the motor signs in patients, hindering therefore, the clinical interpretation of the inter-group motor dissimilarities. Last, a more-in-depth analysis that explores the consistency of the presented results to be replicated on independent longitudinal cohorts is much needed for further validation. Such endeavors will verify the capacity of spectral power and connectivity features in predicting patient’s stratification without prior knowledge of their disease severity.

Conclusion

To sum up, we showed for the first time that EEG spectral and connectivity features can be used to cluster PD patients into subphenotypes with distinct clinical/electrophysiological profiles and disease trajectories. This electrophysiological profiling showed potential stratification capabilities at early disease stages before the clear manifestation of the clinical symptoms. Further investigations would enhance biomarkers discovery efforts addressing the heterogeneity of PD and improve the development of neuroprotective therapies. ■

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Conflicts of Interest

The authors report no competing interests.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy and ethical restrictions.

References

- Bloem BR, Okun MS, Klein C. Parkinson's disease. *Lancet* 2021; 397:2284–2303.
- Mestre TA, Fereshtehnejad SM, Berg D, et al. Parkinson's disease subtypes: critical appraisal and recommendations. *J Parkinsons Dis* 2021;11:395–404.
- Surmeier DJ, Obeso JA, Halliday GM. Selective neuronal vulnerability in Parkinson disease. *Nat Rev Neurosci* 2017;18:101–113.
- Horsager J, Andersen KB, Knudsen K, et al. Brain-first versus body-first Parkinson's disease: a multimodal imaging case-control study. *Brain* 2020;143:3077–3088.
- Farrow SL, Cooper AA, O'Sullivan JM. Redefining the hypotheses driving Parkinson's diseases research. *Npj Park. Dis.* 2022;8:1–7.
- Espay AJ, Kalia LV, Gan-Or Z, et al. Disease modification and biomarker development in Parkinson disease: revision or reconstruction? *Neurology* 2020;94:481–494.
- Espay A, Brundin P, Lang A. Precision medicine for disease modification in Parkinson disease. *Nat Rev Neurol* 2017;13(2):119–126.
- Vijaratnam N, Simuni T, Bandmann O, Morris HR, Foltynie T. Progress towards therapies for disease modification in Parkinson's disease. *Lancet Neurol* 2021;20:559–572.
- Espay AJ, Schwarzschild MA, Tanner CM, et al. Biomarker-driven phenotyping in Parkinson disease: a translational missing link in disease-modifying clinical trials. *Mov. Disord. Off. J. Mov. Disord. Soc.* 2017;32:319–324.
- Poewe W, Seppi K, Marini K, Mahlknecht P. New hopes for disease modification in Parkinson's disease. *Neuropharmacology* 2020;171:108085.
- van Rooden SM, Heiser WJ, Kok JN, et al. The identification of Parkinson's disease subtypes using cluster analysis: a systematic review. *Mov Disord* 2010;25:969–978.
- van Rooden SM, Colas F, Martínez-Martín P, et al. Clinical subtypes of Parkinson's disease. *Mov Disord* 2011;26:51–58.
- Lawton M, Ben-Shlomo Y, May MT, et al. Developing and validating Parkinson's disease subtypes and their motor and cognitive progression. *J Neurol Neurosurg Psychiatry* 2018;89:1279–1287.
- Rodríguez-Sánchez F, Rodríguez-Blázquez C, Bielza C, et al. Identifying Parkinson's disease subtypes with motor and non-motor symptoms via model-based multi-partition clustering. *Sci Rep* 2021;11:23645.
- Nalls MA, Blauwendraat C, Vallerga CL, et al. Identification of novel risk loci, causal insights, and heritable risk for Parkinson's disease: a meta-analysis of genome-wide association studies. *Lancet Neurol* 2019;18:1091–1102.
- Wolters AF, van de Weijer SCF, Leentjens AFG, et al. Resting-state fMRI in Parkinson's disease patients with cognitive impairment: a meta-analysis. *Parkinsonism Relat Disord* 2019;62:16–27.
- Majbour NK, Abdi IY, Dakna M, et al. Cerebrospinal α -synuclein oligomers reflect disease motor severity in DeNoPa longitudinal cohort. *Mov Disord Off J Mov Disord Soc* 2021;36:2048–2056.
- Over L, Brüggemann N, Lohmann K. Therapies for genetic forms of Parkinson's disease: systematic literature review. *J Neuromuscul Dis* 2021;8:341–356.
- Mueller K, Jech R, Růžicka F, et al. Brain connectivity changes when comparing effects of subthalamic deep brain stimulation with levodopa treatment in Parkinson's disease. *NeuroImage Clin* 2018; 19:1025–1035.
- Tan MMX, Lawton MA, Jabbari E, et al. Genome-wide association studies of cognitive and motor progression in Parkinson's disease. *Mov Disord* 2021;36:424–433.
- Majbour NK, Vaikath NN, Eusebi P, et al. Longitudinal changes in CSF alpha-synuclein species reflect Parkinson's disease progression. *Mov Disord* 2016;31:1535–1542.
- Fereshtehnejad S-M, Zeighami Y, Dagher A, Postuma RB. Clinical criteria for subtyping Parkinson's disease: biomarkers and longitudinal progression. *Brain J Neurol* 2017;140:1959–1976.
- Markello RD, Shafiei G, Tremblay C, et al. Multimodal phenotypic axes of Parkinson's disease. *Npj Park Dis* 2021;7:1–12.
- Zhang X, Chou J, Liang J, et al. Data-driven subtyping of Parkinson's disease using longitudinal clinical records: a cohort study. *Sci Rep* 2019;9:797.
- Dadu A, Satone V, Kaur R, et al. Identification and prediction of Parkinson's disease subtypes and progression using machine learning in two cohorts. *Npj Park. Dis.* 2022;8:1–12.
- Biasiucci A, Franceschiello B, Murray MM. Electroencephalography. *Curr Biol* 2019;29:R80–R85.
- Müller-Putz GR. Electroencephalography. *Handbook of Clinical Neurology*; 2020;168:249–262.
- Zhang Y, Wu W, Toll RT, et al. Identification of psychiatric disorder subtypes from functional connectivity patterns in resting-state electroencephalography. *Nat Biomed Eng* 2021;5:309–323.
- Dukic S, Mackin R, Costello E, et al. Resting-state EEG reveals four subphenotypes of amyotrophic lateral sclerosis. *Brain* 2022;145(2): 621–631. <https://doi.org/10.1093/brain/awab322>
- Byeon J, Choi TY, Won GH, Lee J, Kim JW. A novel quantitative electroencephalography subtype with high alpha power in ADHD: ADHD or misdiagnosed ADHD? *Plos One* 2020;15:e0242566.
- Filippi M, Basaia S, Sarasso E, et al. Longitudinal brain connectivity changes and clinical evolution in Parkinson's disease. *Mol Psychiatry* 2020;26(9):5429–5440. <https://doi.org/10.1038/s41380-020-0770-0>
- Wang B, Mezlini AM, Demir F, et al. Similarity network fusion for aggregating data types on a genomic scale. *Nat Methods* 2014;11: 333–337.
- Yassine S, Gschwandtner U, Auffret M, et al. Functional brain Dysconnectivity in Parkinson's disease: a 5-year longitudinal study. *Mov. Disord. Off. J. Mov. Disord. Soc.* 2022;37(7):1444–1453. <https://doi.org/10.1002/mds.29026>
- Hassan M, Wendling F. Electroencephalography source connectivity: aiming for high resolution of brain networks in time and space. *IEEE Signal Process Mag* 2018;35:81–96.
- Gramfort A, Papadopoulos T, Olivi E, Clerc M. OpenMEEG: opensource software for quasistatic bioelectromagnetics. *Biomed Eng Online* 2010;9:45.
- Hämäläinen MS, Ilmoniemi RJ. Interpreting magnetic fields of the brain: minimum norm estimates. *Med Biol Eng Comput* 1994;32: 35–42.
- Fan L, Li H, Zhuo J, et al. The human Brainnetome atlas: a new brain atlas based on connectional architecture. *Cereb Cortex NY* 2016;26:3508–3526.
- Welch P. The use of fast Fourier transform for the estimation of power spectra: a method based on time averaging over short, modified periodograms. *IEEE Trans Audio Electroacoustics* 1967;15: 70–73.
- Brookes MJ, Hale JR, Zumer JM, et al. Measuring functional connectivity using MEG: methodology and comparison with fMRI. *Neuroimage* 2011;56:1082–1104.

40. Thomas Yeo BT, Krienen FM, Sepulcre J, et al. The organization of the human cerebral cortex estimated by intrinsic functional connectivity. *J Neurophysiol* 2011;106:1125–1165.
41. Baggio H-C, Sala-Llloch R, Segura B, et al. Functional brain networks and cognitive deficits in Parkinson's disease. *Hum Brain Mapp* 2014;35:4620–4634.
42. Tinaz S. Functional connectome in Parkinson's disease and parkinsonism. *Curr Neurol Neurosci Rep* 2021;21:24.
43. Wang Q, Meng L, Pang J, Zhu X, Ming D. Characterization of EEG data revealing relationships with cognitive and motor symptoms in Parkinson's disease: a systematic review. *Front Aging Neurosci* 2020;12:587396.
44. Geraedts VJ, Boon LI, Marinus J, et al. Clinical correlates of quantitative EEG in Parkinson disease: a systematic review. *Neurology* 2018;91:871–883.
45. Pourzinal D, Yang J, Lawson RA, et al. Systematic review of data-driven cognitive subtypes in Parkinson disease. *Eur J Neurol* 2022; 29(11):3395–3417. <https://doi.org/10.1111/ene.15481>
46. Caviness JN, Utianski RL, Hentz JG, et al. Differential spectral quantitative electroencephalography patterns between control and Parkinson's disease cohorts. *Eur J Neurol* 2016;23:387–392.
47. Bosboom JLW, Stoffers D, Stam CJ, et al. Resting state oscillatory brain dynamics in Parkinson's disease: an MEG study. *Clin Neurophysiol* 2006;117:2521–2531.
48. Caviness JN, Hentz JG, Belden CM, et al. Longitudinal EEG changes correlate with cognitive measure deterioration in Parkinson's disease. *J Parkinsons Dis* 2015;5:117–124.
49. Arnaldi D, De Carli F, Famà F, et al. Prediction of cognitive worsening in de novo Parkinson's disease: clinical use of biomarkers. *Mov Disord* 2017;32:1738–1747.
50. Olde Dubbelink KTE, Stoffers D, Deijen JB, et al. Cognitive decline in Parkinson's disease is associated with slowing of resting-state brain activity: a longitudinal study. *Neurobiol Aging* 2013;34:408–418.
51. Ruppert MC, Greuel A, Freigang J, et al. The default mode network and cognition in Parkinson's disease: a multimodal resting-state network approach. *Hum Brain Mapp* 2021;42:2623–2641.
52. Ponsen MM, Stam CJ, Bosboom JLW, Berendse HW, Hillebrand A. A three dimensional anatomical view of oscillatory resting-state activity and functional connectivity in Parkinson's disease related dementia: an MEG study using atlas-based beamforming. *NeuroImage Clin*. 2013;2:95–102.
53. Hou Y, Yuan X, Wei Q, et al. Primary disruption of the default mode network subsystems in drug-naïve Parkinson's disease with mild cognitive impairments. *Neuroradiology* 2020;62:685–692.
54. Hassan M, Chaton L, Benquet P, et al. Functional connectivity disruptions correlate with cognitive phenotypes in Parkinson's disease. *NeuroImage Clin*. 2017;14:591–601.
55. Olde Dubbelink KTE, Stoffers D, Deijen JB, et al. Resting-state functional connectivity as a marker of disease progression in Parkinson's disease: a longitudinal MEG study. *NeuroImage Clin*. 2013;2: 612–619.
56. Geraedts VJ, Marinus J, Gouw AA, et al. Quantitative EEG reflects non-dopaminergic disease severity in Parkinson's disease. *Clin Neurophysiol* 2018;129:1748–1755.
57. Singh A, Cole RC, Espinoza AI, et al. Frontal theta and beta oscillations during lower-limb movement in Parkinson's disease. *Clin Neurophysiol* 2020;131:694–702.
58. Utianski RL, Caviness JN, van Straaten EC, et al. Graph theory network function in Parkinson's disease assessed with electroencephalography. *Clin Neurophysiol* 2016;127:2228–2236.
59. Dubbelink KTEO, Schoonheim MM, Deijen JB, et al. Functional connectivity and cognitive decline over 3 years in Parkinson disease. *Neurology* 2014;83:2046–2053.
60. Pagano G, Ferrara N, Brooks DJ, Pavese N. Age at onset and Parkinson disease phenotype. *Neurology* 2016;86:1400–1407.

Supporting Data

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