Two distinct trajectories of clinical and neurodegeneration events identified in Parkinson's disease

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Abstract

Background

Increasing evidence suggests that Parkinson’s disease (PD) may have distinct spatial and temporal progression patterns. We aimed to disentangle the distinct trajectories of clinical and neurodegeneration events of PD by using a novel data-driven Subtype and Stage Inference (SuStaIn) model.

Methods

We enrolled 201 PD patients and 118 healthy controls with comprehensive olfactory, autonomic function, cognitive, sleep, emotional tests, and multimodality MRI scanning (T1-weighted imaging, diffusion tensor imaging, and neuromelanin-sensitive imaging). The volume and mean diffusivity of cortical/subcortical regions, the signal of substantia nigra (SN) and locus coeruleus (LC) were then obtained. Above features were input into SuStaIn model to identify PD subtypes with distinct progress trajectories. An independent dataset with 151 PD patients and 57 healthy controls was used to validate our findings. Finally, the differences in clinical, imaging, biochemical profiles, levodopa treatment response, and longitudinal prognosis between PD subtypes were assessed.

Results

We identified two distinct PD subtypes: subtype 1 with rapid eye movement sleep behavior disorder (RBD), autonomic dysfunction, SN and LC degeneration as early manifestations and cognitive impairment, limbic degeneration as advanced manifestation; while subtype 2 with cognitive impairment, SN and limbic degeneration as early manifestation, and RBD, LC degeneration as advanced manifestations. The two subtypes were successfully validated in the independent dataset. In addition, we found that subtype 1 showed poorer levodopa response and longitudinal prognosis when compared with subtype 2.

Conclusion

We demonstrated two PD subtypes with distinct clinical and neurodegeneration trajectories, which are consistent with the recently proposed “body-first” and “brain-first” hypothesis. Meanwhile, the distinct treatment response and prognosis between two subtypes provided new insights into the underlying disease biology and may be useful for personalized treatment for individuals with different subtypes of PD.

Introduction

Parkinson’s disease (PD) is the second most common neurodegenerative disease, characterized by loss of dopaminergic neurons within the substantia nigra (SN), and progressive and irreversible aggregation of misfolded α-synuclein in multi brain regions\(^1\). PD varies dramatically in its manifestations, therapeutic effect, and long-term prognosis, suggesting the existence of PD subtypes\(^2\). Identification of PD subtypes
is crucial for better understanding disease mechanisms, predicting disease prognosis, and ultimately delivering personalized medicine\(^3\).

Great efforts have been made to identify PD subtypes, from the initial classification based on a single motor symptom, to classification based on multidomain phenotypes including motor and nonmotor symptoms, neuroimage data, and biochemical markers\(^3\)–\(^8\). On account of the inconsistent reliability and difficulty in characterizing disease heterogeneity, the empirical classification methods fade away, and have been replaced by data-driven methods without a priori hypotheses\(^9\)–\(^14\). These data-driven studies provide invaluable information toward the understanding of complex mechanisms but have limitations. Typical data-driven subtyping methods are limited by the confound of disease stage, as the samples included in studies are usually at different points in the disease course\(^12,15\). Restricted by feasibility issues such as sample size, it is difficult for research to stratify patients by disease duration or stage. Therefore, patients may be misclassified by data-driven subtyping methods if individuals at different durations or stages are included. In addition, typical clustering analysis may not work well when patients have various progression trajectories\(^16\). We speculate that this is also one of the reasons why a lot of these data-driven PD subtype classification systems lack reproducibility\(^17\). Recently, brain-first and body-first subtypes of PD has been proposed, which may suggest two contrasting spreading routes of a-synuclein pathology\(^12,18,19\). In this sense, uncovering both temporal and phenotype heterogeneity when conducting the data-driven clustering analysis is quite meaningful.

An emerging model called the Subtype and Stage Inference (SuStaIn) model has been successfully used to uncover the heterogeneity and temporal complexity of neurodegenerative diseases including Alzheimer's disease, multiple sclerosis, and frontotemporal dementia\(^15,16,20\)–\(^23\). SuStaIn model is a machine-learning method that disentangles temporal and phenotypic heterogeneity to identify subtypes with distinct progression trajectories from readily-accessible cross-sectional patients. Then, this model assigns each patient into the most appropriate subtype and calculates each patient's stage in this subtype. In this model, progression trajectory refers to the sequence of abnormalities in clinical or imaging features, and stage refers to the cumulative degree of abnormalities in clinical or imaging features. For a certain type of patients, if all have atrophy in subcortical nuclei, but only some of these patients have atrophy in neocortex, we would infer with high confidence that subcortical atrophy comes before neocortical atrophy in this type of patients\(^24\). And the patient with neocortical atrophy would have a high/heavy disease stage when compared with the patient with pure subcortical atrophy.

Here, we aimed to identify PD subtypes with distinct trajectories based on this data-driven method with multidimensional data, including comprehensive clinical and magnetic resonance imaging (MRI) features (derived from T1-weighted imaging, diffusion tensor imaging, and neuromelanin-sensitive imaging). In previous studies, these MRI measurement of macro- and microstructural changes have been related to PD pathology\(^25\)–\(^28\). Meanwhile, we fitted the analogous multidimensional data from Parkinson's Progression Markers Initiative (PPMI) dataset into SuStaIn model to verify the repeatability of PD subtypes. Finally, we
explored the differences in clinical characteristics, structural and functional imaging features, treatment response, and longitudinal prognosis between PD subtypes.

Materials And Methods

Participants

The discovery dataset is from the Second Affiliated Hospital of Zhejiang University School of Medicine. Informed written consent in accordance with the Declaration of Helsinki was obtained from all participants and all protocols were approved by the local ethical review board. The discovery dataset included 232 PD patients and 132 healthy controls. PD was diagnosed by experienced neurologists according to the United Kingdom Parkinson's disease Society Brain Bank criteria. Participant who had missing values in clinical assessments or MRI scans that used in SuStaIn model was excluded. In addition, all images were visually checked, and images with intracranial mass, cerebrovascular disorders, and obvious artifacts were excluded. Finally, 201 PD patients and 118 healthy controls were included.

Standard Protocol Approvals, Registrations, and Patient Consents

The protocol, patient information, consent form, and other relevant study documentation were approved by the ethics committee of the Second Affiliated Hospital of Zhejiang University School of Medicine before study initiation. The studies were performed in accordance with the Declaration of Helsinki and consistent with Good Clinical Practice. Before enrollment, all patients provided written informed consent.

Clinical and neuropsychological assessments

The Unified Parkinson's Disease Rating Scale (UPDRS) and Hoehn and Yahr (HY) scales were conducted in PD patients. The modified Brief Smell Identification Test (BSIT) for Chinese, Scales for Outcomes in Parkinson's Disease-Autonomic (SCOPA-AUT), Rapid Eye Movement (REM) Sleep Behavior Disorder Questionnaire-Chinese University of Hong Kong version (RBDQ-HK), Epworth Sleepiness Scale (ESS), Pittsburgh Sleep Quality Index scale (PSQI), Hamilton Depression scale (HAMD), Hamilton Anxiety scale (HAMA), and Montreal Cognitive Assessment (MoCA) scales were conducted in all participants. Cognitive performance in different domains were assessed using the following neuropsychological battery: (1) Symbol Digit Modality Test (SDMT, attention); (2) Trail Making Test (TMT, attention); (3) Digit span test (DST, executive function); (4) Semantic fluency Test - Animal (SF, executive function); (5) 30-item Boston Naming Test (BNT, language function); (6) Auditory Verbal Learning Test (AVLT, memory). For PD patients, all of these assessments were conducted during the OFF state (at least 12 hours after withholding PD medications).
In addition, 97 patients conducted levodopa challenge test according to personal willingness. The UPDRS-III score was assessed during OFF state and repeated one hour after administration of 200 mg levodopa and 50 mg benserazide (ON state, drug concentrations at peak)\(^{29}\). The levodopa response was represented as the difference between UPDRS-III\(_{\text{OFF}}\) and UPDRS-III\(_{\text{ON}}\) (UPDRS-III\(_{\text{OFF}}\) − UPDRS-III\(_{\text{ON}}\)), and the change rate of UPDRS-III score \([(\text{UPDRS-III}\(_{\text{OFF}}\) − \text{UPDRS-III}\(_{\text{ON}}\)) / \text{UPDRS-III}\(_{\text{OFF}}\)]\).

### Magnetic resonance imaging data acquisition and analysis

#### Image acquisition

All participants were scanned on a GE Discovery MR750 3.0 T MRI scanner. Earplugs and foam pads were used to reduce noise and head motion. High-resolution 3D T1-weighted structural MRI, diffusion tensor imaging (DTI), neuromelanin sensitive imaging (NM-MRI), arterial spin labeling (ASL) imaging, and Blood Oxygen Level-Dependent resting-state functional MRI (rs-fMRI) were acquired. Detailed scanning parameters were described in supplementary materials.

#### T1-weighted image processing

T1-weighted image was processed using FreeSurfer (v6, http://surfer.nmr.mgh.harvard.edu). The “recon-all -all” procedure was used to perform its full reconstruction pipeline, which provides cortical and subcortical segmentation and volume measurement. The cortical and subcortical volume features were extracted according to the labels of the Desikan-Killiany atlas.

#### Diffusion tensor image processing

DTI processing was performed using FMRIB’s Software Library (FSL, http://www.fmrib.ox.ac.uk/fsl). Fractional anisotropy (FA) and mean diffusivity (MD) were calculated. FA quantifies the microstructural integrity of brain white matter but lacks sensitivity for quantifying cortical microstructural changes related to neuropathology\(^{30}\). As for MD, it quantifies the overall diffusion of water in the tissue, which is suitable for evaluating the damage of cortical and subcortical microstructure\(^{31}\). MD increases reflect reduced integrity of brain microstructure. Consequently, we only extracted the cortical and subcortical MD values for subsequent analysis. To alleviate partial volume effects, we extracted the cortical MD values from the cortical skeleton by using the gray matter-based spatial statistics\(^{32}\). The subcortical MD values were extracted in the native T1-weighted image space (labels were defined using FreeSurfer). Detailed steps were described in supplementary materials (Supplementary Figure 1).

#### Neuromelanin sensitive image processing
Substantia nigra (SN) and locus coeruleus (LC) are vital nodes in the trajectory of PD progression. With the advent of NM-MRI, SN and LC are visible and measurable in vivo (Supplementary Figure 2). Therefore, we used the NM-MRI to measure the degeneration of SN and LC. According to previous method, we calculated the contrast to noise ratio of the SN (CNR\textsubscript{SN}) and LC (CNR\textsubscript{LC}), which represented the integrity of SN and LC\textsuperscript{33}. A lower CNR value means more serious degeneration. Detailed methods were described in supplementary materials.

**Arterial spin labeling image processing**

Brain perfusion alteration is largely reversible which is unsuitable for application in SuStaiIn model. However, considering that changes in perfusion are closely related to clinical symptoms such as cognition, which can deepen our understanding of the characteristics of subtypes, we still assessed the brain perfusion differences between subtypes. The cerebral blood flow (CBF) was calculated by using the GE workstation. The skulls stripping, spatial normalization, and smoothing were conducted in Statistical Parametric Mapping 12 (https://www.fil.ion.ucl.ac.uk/spm/doc/).

**Resting-state functional image processing**

It has been demonstrated that functional synchronization of brain networks have strong correlations with PD manifestations\textsuperscript{34}. Therefore, the network synchronization was assessed, which could help improve our understanding of PD subtypes. Rs-fMRI was processed using Data Processing and Analysis for Resting-State Brain Imaging tools (DPABI, http://rfmri.org/dpabi) based on Statistical Parametric Mapping 12. After processing, the Brainnetome Atlas was used to construct seven classic functional networks, including visual, somatomotor, dorsal attention, ventral attention, limbic, frontoparietal, and default networks\textsuperscript{35}. The synchronization of each network was calculated using an in-house script based on MATLAB. Detailed methods were described in supplementary materials.

**Subtype and Stage Inference model construction**

**Feature selection**

Non-motor variables including BSIT, RBDQ-HK, SCOPA-AUT, HAMD, HAMA, MoCA, SDMT, TMT, SF, AVLT, DST, BNT, FSS, PSQI and ESS scores were included. Motor scores was not included because motor symptoms are only present in patients but not in healthy controls\textsuperscript{24}. For MRI features, the volumes and MD values were integrated into 6 cortical (frontal, temporal, parietal, occipital, cingulate and insula, see supplementary materials) and 7 subcortical (amygdala, hippocampus, nucleus accumbens, caudate, putamen, globus pallidus, and thalamus) regions\textsuperscript{15,21}. The volumes and MD values of the brain stem and cerebellum, the CNR\textsubscript{SN} and CNR\textsubscript{LC} were also included.
The cofounding variables including age, gender, and education were regressed. The influence of total incranial volume on volume features was also regressed. The corrected features were then converted into z-scores relative to the healthy controls (subtracted the mean and divided by the standard deviation of healthy controls) for use as input into SuStaIn model. As volumes and several clinical scores (BSIT, MoCA, SDMT, TMT, SF, AVLT, DST, and BNT) decrease with disease progression, we multiplied their z scores by -1, so that all features with larger z-score mean more serious impairments.

Further, to reduce the data dimension, only features that significantly damaged in PD patients were selected \((p < 0.05)\). Therefore, a total of 33 features \((201 \times 33\) matrix\) were input into SuStaIn model. Additionally, to explore the influence of feature reduction on SuStaIn model results, we constructed another model with only 22 features that showed more significant damage \((p < 0.001)\). Features used in the two SuStaIn models were listed in supplementary materials.

**Modeling**

The pySuStaIn, a Python implementation of SuStaIn algorithm\(^{20}\), was used to identify PD subtypes with distinct progression patterns and grade a stage for each participant. Each feature was assigned three z-scores (1, 2, and 3), which represented three continuous events (stages). In other word, each stage corresponds to a feature reaching a new z-score. The advantage of this method was that it represents the continuous linear accumulation of damage, rather than the instantaneous switch from normal to abnormal. Therefore, the progression pattern of each subtype was characterized by a piecewise linear z score model, which consisted of a series of stages. Individuals were typed according to the likelihood they belonged to each SuStaIn subtype\(^{20}\), and the SuStaIn stage was determined by choosing the most probable stage\(^{20,21}\). The individual who had no biomarker abnormality was defined as stage 0 and had not been assigned to any subtype. The appropriate numbers of subtypes were determined according to prior knowledge. Detailed algorithms and analytical procedures were described in recent studies\(^{15,16,20-22}\).

**Validation dataset**

To validate the reproducibility of PD subtypes, we conducted the SuStaIn model on the PPMI dataset (http://www.ppmi-info.org/)\(^{36}\). Clinical variables similar to the discovery dataset were included. As the PPMI dataset does not collect the NM-MRI data, only T1-weighted and DTI data were included. Finally, 151 PD patients and 57 healthy controls were enrolled after quality control. Detailed clinical assessments and MRI scans were provided in supplementary materials.

The data processing and feature selection steps were the same as the discovery dataset. Finally, 16 clinical and imaging features showed significant damage \((p < 0.05)\) in PD patients were input into SuStaIn model (see supplementary materials).
Statistical analysis

Two-sample t-test was conducted to compare the differences in demographic, disease severity related clinical variables (disease duration, UPDRS-I, UPDRS-II, UPDRS-III, HY stage), and dopaminergic treatment response between PD patients assigned to each of the SuStaIn subtypes. The differences in clinical and imaging variables used in SuStaIn model among PD subtypes and healthy controls were assessed using ANOVA. We also assessed the differences in CBF and network synchronization among PD subtypes and healthy controls in the discovery dataset. Pearson correlation was conducted to assess the relationships between SuStaIn stage and disease severity related clinical variables. In addition, the two-sample t-test was conducted to assess the difference in dopamine transporter binding rate (caudate and putamen), cerebrospinal fluid proteins (amyloid-β42, α-synuclein, and phosphorylated tau protein), and longitudinal prognosis (MDS-UPDRS-I, MDS-UPDRS-II, and MDS-UPDRS-III scores) between PD subtypes in the validation dataset. False discovery rates (FDR) correction was conducted for multiple comparison correction, and a two-tailed \( p \)-value < 0.05 was considered significant. The overall pipeline of the study is summarized in Figure 1.

Results

No significant difference was found in age and education between healthy controls and PD patients. A significant difference was found in gender between the two groups, which is consistent with the epidemiological characteristics of PD\(^1\). PD patients showed significant hyposmia \((p < 0.001)\), autonomic dysfunction \((p < 0.001)\), and RBD symptom \((p < 0.001)\). Detailed demographic and clinical characteristics were shown in Table 1 and Supplementary Table 1.

Table 1

Demographics and clinical characteristics of healthy controls and Parkinson’s disease patients.
<table>
<thead>
<tr>
<th></th>
<th>Healthy controls</th>
<th>Parkinson’s disease</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Discovery dataset</strong></td>
<td>n = 118</td>
<td>n = 201</td>
<td></td>
</tr>
<tr>
<td>Gender (male/female)</td>
<td>56/62</td>
<td>126/75</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age</td>
<td>61.61±7.34</td>
<td>59.73±9.47</td>
<td>0.066</td>
</tr>
<tr>
<td>Education</td>
<td>7.78±4.58</td>
<td>8.29±4.54</td>
<td>0.328</td>
</tr>
<tr>
<td>Disease duration</td>
<td>-</td>
<td>4.34±3.26</td>
<td>-</td>
</tr>
<tr>
<td>UPDRS-I</td>
<td>-</td>
<td>1.6±1.58</td>
<td>-</td>
</tr>
<tr>
<td>UPDRS-II</td>
<td>-</td>
<td>9.09±5.48</td>
<td>-</td>
</tr>
<tr>
<td>UPDRS-III</td>
<td>-</td>
<td>22.64±13.17</td>
<td>-</td>
</tr>
<tr>
<td>HY stage</td>
<td>-</td>
<td>2.05±0.62</td>
<td>-</td>
</tr>
<tr>
<td><strong>Validation dataset</strong></td>
<td>n = 60</td>
<td>n = 151</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td>39/21</td>
<td>98/53</td>
<td>0.561</td>
</tr>
<tr>
<td>Age</td>
<td>59.98±11.4</td>
<td>60.97±9.34</td>
<td>0.519</td>
</tr>
<tr>
<td>Education</td>
<td>16.2±2.92</td>
<td>15.34±2.95</td>
<td>0.056</td>
</tr>
<tr>
<td>Disease duration</td>
<td>-</td>
<td>1.83±1.3</td>
<td>-</td>
</tr>
<tr>
<td>MDS-UPDRS-I</td>
<td>-</td>
<td>5.17±3.75</td>
<td>-</td>
</tr>
<tr>
<td>MDS-UPDRS-II</td>
<td>-</td>
<td>5.58±4.06</td>
<td>-</td>
</tr>
<tr>
<td>MDS-UPDRS-III</td>
<td>-</td>
<td>20.93±9.03</td>
<td>-</td>
</tr>
<tr>
<td>HY stage</td>
<td>-</td>
<td>1.57±0.5</td>
<td>-</td>
</tr>
</tbody>
</table>

UPDRS: Unified Parkinson’s Disease Rating Scale; HY: Hoehn-Yahr; MDS: Movement Disorders Society.

**Clinical and neurodegeneration trajectories of subtypes**

Amongst the 201 PD patients, only 27 patients (13%) were categorized as stage 0, and not assigned to any subtype. One hundred and seventy-four patients (87%) were assigned to two subtypes: 100 patients (57%) were assigned to subtype 1; 74 patients (43%) were assigned to subtype 2. Each subtype was defined by a different combination of abnormalities in clinical symptoms and brain degeneration (*Figure 2 and Supplementary Figure 3A*).
Subtype 1 had earliest dysfunction in autonomic nerve, olfaction, and REM sleep. These were followed by SN and LC degeneration, which were earliest imaging manifestations. Depression, anxiety, and fatigue were also early appeared manifestations. Impairments in executive function, attention, memory, and global cognition were the most advanced manifestations. Before the occurrence of cognitive impairments, extensive macro- and microstructural damage of cortical and subcortical regions were detected. Remarkably, the degeneration of limbic system such as hippocampus and amygdala were manifestations of advanced stages. Subtype 2 showed the earliest dysfunction in cognition (global cognition and attention) and olfaction. SN degeneration was the earliest imaging manifestation. And the early degeneration of cortex, hippocampus, and amygdala were the characteristics of this subtype. In contrast to subtype 1, RBD symptom and LC degeneration were confined to the end of the stages.

Accordingly, more serious RBD symptom ($p < 0.001$), autonomic dysfunction ($p < 0.001$), and LC degeneration ($p < 0.001$) were found in subtype 1. On the contrary, more significant decline in MoCA ($p < 0.001$), SDMT ($p < 0.001$), and TMT ($p < 0.001$) scores were found in subtype 2. More serious degeneration in extensive brain regions such as hippocampus ($p = 0.001$) were also found in subtype 2. The severity of hyposmia and SN degeneration, which were early deteriorated features in both subgroups, showed no significant difference between the two subtypes. Figure 3A showed the differences in several clinical and imaging features, which are crucial nodes in the trajectory of PD, among healthy controls and two subtypes$^{37}$. See Supplementary Table 2 for detailed statistical comparisons of all clinical and imaging variables included in the SuStaIn model.

We further explored the possibility of dividing PD patients into three subtypes. We found that two of them showed high consistency which is characterized by autonomic nerve dysfunction, hyposmia, RBD symptom and SN degeneration as the earliest manifestations (Supplementary Figure 4). Therefore, we adopted the results of dividing PD patients into two subtypes. In addition, we tested the stability of SuStaIn model under a more stringent feature selection threshold (22 features remained). We found that the results of two models (33 features vs 22 features) were highly consistent, and only 15 patients (7.5%) changed their subtype (Supplementary Figure 5).

**Subtypes characterized by distinct disease severity and functional imaging profiles**

Two subtypes had no significant difference in gender, age, education, and disease duration. Subtype 1 had a higher UPDRS-I score ($p = 0.001$). No significant difference was found in UPDRS-II, UPDRS-III, and HY scores between the two subtypes (Supplementary Table 3). Although no difference was found in disease severity between the two subtypes, subtype 1 showed a lower SuStaIn stage when compared with subtype 2 ($p = 0.006$). In other words, subtype 1 showed a heavy disease burden at a relatively early SuStaIn stage.
We compared the difference in levodopa response between two subtypes. Ninety-seven patients (subtype 1 = 53, subtype 2 = 44) who conduct the levodopa challenge test were included. Two subgroups were well matched in age, gender, education, and disease duration. Significantly, we found that subtype 1 showed poor levodopa response when compared with subtype 2 ($p = 0.013$ for the change of UPDRS-III, and $p = 0.033$ for the change rate of UPDRS-III). See Supplementary Table 4 for detailed demographics of the patients who conduct the levodopa challenge test.

Subtype 1 showed significantly decreased CBF in putamen than healthy controls. Subtype 2 had an extensive reduction in CBF of putamen, parietal, frontal, occipital, and temporal lobes when compared with healthy controls (Figure 4A, FDR corrected). No significant difference in CBF was found between the two subtypes. See Supplementary Table 5 for detailed statistical results. Subtype 1 showed decreased synchronization in the dorsal attention network when compared with healthy controls. Subtype 2 showed significantly decreased synchronization in the somatomotor network, ventral attention network, and limbic network than healthy controls (Figure 4B; $p < 0.05$, FDR corrected). No significant difference in synchronization was found between the two subtypes.

In addition, we found significant correlations between SuStaIn stage with UPDRS-I, UPDRS-II, and UPDRS-III scores in both PD subtypes (Figure 5A; $p < 0.05$, FDR corrected). These results suggested that the features used in SuStaIn model were related to PD pathology, and the SuStaIn stages assigned to patients were reliable.

**Subtypes validated in an independent dataset**

We validated our findings in the PPMI dataset. Twenty-three patients (15%) were categorized as stage 0, and not assigned to any subtype. The remaining 128 patients were divided into two distinct progression subtypes, which are highly consistent with the discovery dataset (Supplementary Figure 3B).

Subtype 1 ($n = 96, 75%$) had earliest dysfunction in olfaction, autonomic nerve, and REM sleep. These were followed by depression and anxiety. Extensive cognitive decline and brain atrophy were evolved in the advanced stages. As for subtype 2 ($n = 32, 25%$), hippocampus, amygdala, thalamus, temporal atrophy, and cognitive decline were earlier manifestations. Hyposmia, REM symptoms, depression, anxiety, and autonomic dysfunction were limited to the end of the disease stages. As shown in Figure 3B, the subtype 1 showed more serious hyposmia ($p < 0.001$) and autonomic dysfunction ($p < 0.001$). Subtype 1 showed slightly more severe but not significant RBD symptom. On the contrary, significant decreased MoCA score ($p < 0.001$), and atrophy in hippocampus ($p < 0.001$) and amygdala ($p < 0.001$) were shown in subtype 2. Detailed differences of clinical and imaging features were described in Supplementary Table 6 ($p < 0.05$, FDR corrected).

No significant difference was found in gender, age, education, disease duration, dopamine transporter binding rate of caudate and putamen, and amyloid-β42, α-synuclein, and phosphorylated tau protein in cerebrospinal fluid between two subtypes (Supplementary Table 3). Significantly, the subtype 1 had
higher MDS-UPDRS-II scores at baseline and 5 years follow-up (Figure 6, $p = 0.015$, $p = 0.016$, $p = 0.009$, $p = 0.015$, $p = 0.008$, and $p = 0.015$, respectively; FDR corrected). Finally, we found significant correlations between SuStaIn stage and MDS-UPDRS-I, MDS-UPDRS-II, MDS-UPDRS-III scores in the subtype 1 ($p < 0.001$, $p = 0.003$, and $p < 0.001$, respectively) but not in the subtype 2 (Figure 5B, $p < 0.05$, FDR corrected).

**Discussion**

We identified two distinct progression patterns in PD patients: subtype 1 with autonomic dysfunction and RBD symptom as early manifestations and cognitive impairment as advanced manifestation; subtype 2 with cognitive impairment as early manifestation and RBD symptom as advanced manifestation. Meanwhile, subtype 1 was characterized by early degeneration in SN and LC, and eventually spreading to the limbic system such as hippocampus and amygdala across disease stages. While subtype 2 was characterized by early degeneration in SN, hippocampus and amygdala, and finally spreading to LC. Significantly, the validation dataset showed comparable progression patterns with the discovery dataset. Moreover, subtype 1 had poor treatment response and longitudinal prognosis, but less functional alterations in cortex when compared with subtype 2. These findings provide new insights into the heterogeneity of PD from the perspective of disease dynamic evolution while also having potential utility for the stratification of patients in clinical trial and practice.

**Clinical and neurodegeneration trajectories of two subtypes**

According to the braak model, the classic clinical progression trajectory is: the earliest manifestations of PD are autonomic dysfunction, RBD, and hyposmia, which gradually progress to mood disorders, fatigue, movement disorders, and eventually cognitive impairment\(^1\),\(^2\),\(^4\). In this study, we derived a subtype with an identical progress pattern from multidimensional data by using a novel data-driven approach without prior hypotheses. Moreover, we found that the subtype 1 was characterized by early degeneration in SN and LC, which was followed by extensive cortical and basal ganglia degeneration, and eventually spreading to the limbic system such as hippocampus and amygdala across the disease stage. This brain degeneration trajectory was consistent with the Braak staging scheme: the intracerebral formation of abnormal proteinaceous Lewy bodies and Lewy neurites spreading from the medulla oblongata, pontine, midbrain to diffuse cortical regions\(^3\),\(^8\).

However, neuropathological and clinical evidences suggest that the Braak’s hypothesis does not conform to all PD patients. We identified a subtype, which was characterized by an early cognitive impairment, and in the late stage, by dysfunction of REM sleep. And the degeneration in hippocampus, amygdala, and cortex occurred long before LC. It has been recognized that cognitive impairment can occur in the prodromal stage of PD, and it has been adopted as part of the MDS research criteria for prodromal PD\(^3\),\(^9\). Our findings further suggested that the cognitive impairment might not be considered merely a prodromal marker that increases the risk of PD, but rather herald the conversion of a specific PD subtype.
The differences in clinical and imaging profiles between the two subtypes

The two subtypes showed significant differences between clinical and imaging profiles. Subtype 1 showed more serious RBD symptom, autonomic dysfunction, and LC degeneration. On the contrary, subtype 2 showed more serious cognitive impairment and limbic system degeneration. These characteristics of the two subtypes were aligned with that of body-first and brain-first subtypes\textsuperscript{18,19,37,40}, and the neuroanatomic biotypes identified using a data-driven clustering approach\textsuperscript{14}. They reported two subtypes of PD, one subtype with more severe autonomic dysfunction and RBD symptom compared to another\textsuperscript{14,18,19,37,40}. According to the body-first and brain-first subtypes, alpha-synuclein aggregates originate in the peripheral nervous system or enteric, and gradually invade brainstem and cortex in body-first subtype patients. On the contrary, alpha-synuclein aggregates originate in the limbic system, and gradually invade the brainstem, peripheral nervous system, and enteric in brain-first subtype patients. Consistently, autopsy studies demonstrated two dominant Lewy body pathology distribution patterns in the PD population: a brainstem-predominant pattern (large proportion) and a limbic-predominant pattern (small proportion)\textsuperscript{40-43}. These aggregation and propagation of α-synuclein align well with subtypes identified by this study and SuStaIn staging systems.

Notably, we found that subtype 1 showed a significantly poor response to levodopa replacement therapy than subtype 2, which may suggest the different pathobiological mechanisms. In consistent with this finding, recent animal and human studies have shown that the deficiency of LC-noradrenergic system could complicate PD symptoms and diminishes the therapeutic efficacy of levodopa\textsuperscript{33,44}. We also found that subtype 2 showed significant damage of perfusion and functional synchronization in cortical regions. Cortical hypoperfusion and decreased synchronization were closely associated with cognitive impairment, as has been demonstrated in previous MRI studies\textsuperscript{34,45}.

Additionally, the two subtypes do not differ in the motor symptoms. This was consistent with the viewpoint of the body-first and brain-first hypothesis\textsuperscript{19}. Motor symptoms are therefore may not suitable features to subtype PD. We found that both subtypes showed severe hyposmia and SN degeneration, but only a trend towards a more serious hyposmia was found in subtype 2 and more serious SN degeneration was found in the subtype 2 (the difference between the two subtypes was not statistically significant). Hyposmia is one of the most prevalent symptoms in PD patients, with a prevalence of 50%–90%\textsuperscript{46}. Lewy pathology could directly deposit in olfactory bulb or enter the olfactory bulb through direct connections from LC and SN neurons to the olfactory bulb\textsuperscript{38,40}. Previous studies only found slightly more serious hyposmia in PD patients with RBD symptom\textsuperscript{19,47,48}. We speculated that the susceptibility of the olfactory bulb and the resulting floor effect might also contribute to the significant hyposmia in both subtypes\textsuperscript{38}. Moreover, considering that SN degeneration is the core pathological characteristic of PD, and much more sensitive to alpha-synuclein\textsuperscript{49}, it is therefore reasonable that SN degeneration was defined as an early event in both subtypes.
In addition to classifying patients into different subtypes, we assigned each patient a SuStaIn stage. We found that SuStaIn stage was significantly correlated with UPDRS-I, UPDRS-II, UPDRS-III, HY stage, and disease duration in two subtypes respectively. Considering that these clinical variables are mostly used for evaluating disease severity from different dimensions, these results confirmed the rationality of SuStaIn model used for assigning patients into specific subtypes and stages.

In this study, we identified two PD subtypes with different sequences of clinical and neurodegenerative events using a data-driven approach, which supports the body-first and brain-first hypothesis, and added knowledge about the role of cognitive impairment in PD subtypes. The current discoveries might be meaningful for subtype specific therapies or individualized treatment and eventually prevent PD.

Subtypes validated in an independent dataset

The two progress patterns identified from the validation dataset were consistent with the subtypes in the discovery dataset. It also provided some new knowledge about the two subtypes. Subtype 1 had poorer acitivity of daily life at baseline and 5 years follow-up when compared with subtype 2. This finding was consistent with previous studies that reported a poor prognosis in PD patients with RBD symptoms \(^3^{,40}\). No significant difference was found in DAT imaging between the two PD subtypes. Dopamine deficiency is the core feature of PD, and subjects without dopamine deficiency could not be diagnosed as idiopathic PD. This may explain why DAT binding rate is comparable between the two subtypes. This finding was also consistent with the finding that SN degeneration was comparable between two subtypes in the discovery dataset. In addition, no difference was found in A\(_\beta\)\(_{42}\) and pathological tau protein in CSF, which are crucial biomarkers of Alzheimer's disease. These findings suggested that the severe cognitive impairment in subtype 2 was not caused by Alzheimer's disease related pathology. Taken together, these findings expanded our understanding of PD heterogeneous by showing two subtypes with different temporal and phenotype progression.

Limitations

We should admit that the current findings were not completely followed the pathological evidence of the body-first and brain-first hypothesis \(^{42}\). The measurement error of clinical and imaging assessments, patient heterogeneity, or different pathological susceptibility of brain regions, might contribute to this inconsistency. Future studies are warranted to investigate this inconsistency. While our study gathered a large sample of PD patients with multidimensional data, the numbers are still small. Incorporate huge amounts of samples could increase the confidence of subtype and stage inference. Moreover, future studies with peripheral system examinations such as \(^{123}\)I-MIBG scintigraphy and intestinal biopsies could improve the understanding of distinct progression subtypes. Finally, considering the complexity of multidimensional data, it is of great significance to develop a subtype and stage inference model more suitable for integrating multidimensional data in the near future \(^{50}\).
Conclusion

In this study, we described two PD subtypes with distinct sequences of clinical and neurodegeneration trajectories. These subtypes exhibit differing clinical and imaging profiles and treatment response. These findings verify the classic opinion on disease progression while revealing new insight into the non-classic trajectory of clinical symptoms and neurodegenerations. This classification might also be useful to stratify patients on entry into clinical trials, and eventually provide more individualized treatment.

Abbreviations

PD: Parkinson's Disease; SN: substantia nigra; LC: locus coeruleus; SuStaIn: Subtype and Stage Inference; MRI: magnetic resonance imaging; PPMI: Parkinson's Progression Markers Initiative; CNR: contrast to noise ratio; UPDRS: Unified Parkinson's Disease Rating Scale; HY: Hoehn-Yahr; MDS: Movement Disorders Society; RBD: rapid eye movement sleep behavior disorder; MD: mean diffusivity; BSIT-Chinese: modified Brief Smell Identification Test for Chinese; RBDQ-HK: Rapid Eye Movement (REM) Sleep Behavior Disorder Questionnaire-Chinese University of Hong Kong version; SCOPA-AUT: Scales for Outcomes in Parkinson's Disease-Autonomic; MoCA: Montreal Cognitive Assessment scale; UPSIT: University of Pennsylvania Smell Identification Test.

Declarations

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Authors contributions


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**Availability of data and materials**

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request. PPMI is a public access dataset and can be obtained by application from https://www.ppmi-info.org.

**Ethics approval**

This study was approved by the Medical Ethics Committee of the Second Affiliated Hospital of Zhejiang University School of Medicine. The author has received consent forms from any participant in a study and has them on file.

The PPMI project was approved by the Institutional Review Board or Independent Ethics Committee of all participating sites in Europe, including Attikon University Hospital (Greece), Hospital Clinic de Barcelona and Hospital Universitario Donostia (Spain), Innsbruck University (Austria), Paracelsus-Elena-Klinik Kassel/University of Marburg (Germany), Imperial College London (UK), Pitié-Salpêtrière Hospital (France), University of Salerno (Italy), and in the USA, including Emory University, Johns Hopkins University, University of Alabama at Birmingham, PD and Movement Disorders Center of Boca Raton, Boston University, Northwestern University, University of Cincinnati, Cleveland Clinic Foundation, Baylor College of Medicine, Institute for Neurodegenerative Disorders, Columbia University Medical Center, Beth Israel Medical Center, University of Pennsylvania, Oregon Health and Science University, University of Rochester, University of California at San Diego, and University of California, San Francisco. Informed consent was provided according to the Declaration of Helsinki.

**Consent for publication**

Not applicable.
Competing interests

The authors declare that they have no conflict of interest.

References


**Figures**

**Figure 1**

**Workflow.** A: Participants enrollment, clinical and imaging data processing; B: Clinical and imaging data normalization; C: Subtype and Stage Inference (SuStaIn) model execution; D: Differences in clinical
variables, images, and treatment response between subtypes; E: Alterations in perfusion and resting-state function between subtypes; F: Correlations between SuStaIn stages and disease severity.

**Figure 2**

*The progression patterns of two Parkinson’s disease subtypes.*

RBD: rapid eye movement sleep behavior disorder; MD: mean diffusivity; SN: substantia nigra; LC: locus coeruleus.

**Figure 3**

*The differences of several crucial features among healthy controls and two Parkinson’s disease subtypes.*

A: Z-score differences in the discovery dataset; B: Z-score differences in the validation dataset. A higher z-score means more serious damage. Z-score was calculated by subtracted the mean and divided by the standard deviation of healthy controls.

BSIT-Chinese: modified Brief Smell Identification Test for Chinese; RBDQ-HK: Rapid Eye Movement (REM) Sleep Behavior Disorder Questionnaire-Chinese University of Hong Kong version; SCOPA-AUT: Scales for Outcomes in Parkinson's Disease-Autonomic; CNR_SN: contrast to noise ratio of the substantia nigra; CNR_LC: contrast to noise ratio of the locus coeruleus; MoCA: Montreal Cognitive Assessment scale; UPSIT: University of Pennsylvania Smell Identification Test.

**Figure 4**

*The differences in cerebral blood flow and network synchronization among healthy controls and two Parkinson’s disease subtypes in the discovery dataset.* A: Differences in cerebral blood flow; B: Differences in network synchronization. FDR corrected, \( p < 0.05 \).

**Figure 5**

*Correlations between SuStaIn stage and disease severity in two Parkinson’s disease subtypes.* A: Correlations in discovery dataset; B: Correlations in validation dataset.

UPDRS: Unified Parkinson’s Disease Rating Scale; MDS: Movement Disorders Society. FDR corrected, \( p < 0.05 \). *\( p < 0.05 \); **\( p < 0.01 \); ***\( p < 0.001 \).
Figure 6

The differences in longitudinal prognosis between two Parkinson’s disease subtypes in the validation dataset.

MDS: Movement Disorders Society; UPDRS: Unified Parkinson's Disease Rating Scale. FDR corrected, $p < 0.05$. *$p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Supplementary Files

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- SupplementarmaterialsSuStaIn0720.doc