

Subtype classification of Parkinson's disease based on cortical functional gradient heterogeneity and its neurotransmitter basis

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Abstract

Background

Patients with Parkinson's disease (PD) exhibit significant differences in the manifestation patterns, severity, and progression rate of motor and non-motor symptoms, demonstrating a strong clinical heterogeneity, which poses a key challenge to the precision diagnosis and treatment.

Purpose

The present study aimed to investigate cortical functional gradients in PD patients, seek effective classification for PD patients, and further elucidate the potential neuromodulation basis of clinical heterogeneity.

Materials and Method

We engaged a cohort of 99 PD and 104 normal controls (NC). Our examination focused on cortical gradient alterations in both whole brain gradient and large-scale network gradient in PD. Then, we performed hierarchical clustering analysis on PD patients by using individual gradient heterogeneity. Finally, we employed postmortem gene expression of cortices to investigate the relationships between neuromodulation basis and cortical gradient alterations.

Results

PD displayed abnormal global gradient alterations with increase and decrease coexistence in different cortices. Diminished focal gradients were observed in both dorsal attention and ventral attention networks. Furthermore, we found that PD patients could be divided into neuropsychiatric PD (NPPD) and non-NPPD, with NPPD exhibiting cognitive impairment and emotional disorders. Moreover, we identified notably correlations between the gradient changes of PD and NPPD and the different gene expression.

Conclusion

These results provide a new framework for PD classification and offer significant theoretical foundations for elucidating the intricate interplay between macroscopic functional gradient changes and microscopic neuromodulation roles for PD and NPPD.

Introduction

Parkinson's disease (PD) is a progressive neurodegenerative disease, involving the loss of dopamine producing neurons in the substantia nigra, accompanied by pathological accumulation of α -synuclein in many brain regions^(1–4). Motor impairment is considered as the main and classic symptom of PD, which can manifest as resting tremor, bradykinesia, muscular rigidity, and postural instability^(5–7). However, a growing body of evidence has demonstrated that PD is not an isolated disorder confined to the motor system, but a multisystem neurodegenerative disease^(8,9). Its non-motor symptoms, which are highly prevalent and widely distributed, can emerge as early as the pre-motor stage^(10,11). Therefore, patients with PD exhibit significant differences in the manifestation patterns, severity, and progression rate of motor symptoms and non-motor symptoms, demonstrating a strong clinical heterogeneity, which poses a key challenge to the precision diagnosis and treatment of the disease.

Previous studies had shown that the response rate of dopamine receptor agonists (e.g., pramipexole) was as high as 78% in patients with motor impairment-dominant PD, while the response rate of this drug was only 32% in mixed-type PD patients complicated with cognitive impairment^(9,12). It has also been reported in one study that the accuracy of predicting the 5-year quality of life in PD patients solely based on motor symptom staging (Hoehn-Yahr staging) was relatively low, while the prediction accuracy could be significantly improved after incorporating heterogeneous subgroup characteristics⁽¹³⁾. The above studies suggested that it might be necessary to scientifically classify patients based on their heterogeneous characteristics to achieve the goals of "precision diagnosis, targeted treatment, and individualized prognosis".

Heterogeneous motor and non-motor symptoms in PD are thought to arise from distinct cortical-subcortical neuromodulation deficits, and precise characterization of neural activity patterns via fMRI may facilitate subgroup stratification^(14,15). A hierarchical architecture is one of the fundamental organizational principles of the human brain⁽⁴⁾. Recently, a novel gradient-based approach has been introduced to define a non-linear decomposition of high-dimensional resting-state functional connectivity^(16–18). This method can be used to identify cortical functional hierarchies in the human brain. Its principal gradient spans the unimodal cortical regions (visual and sensorimotor areas) to the transmodal cortical regions (frontoparietal and default mode areas), which has been confirmed to correspond to the hierarchical synaptic distributions in postmortem brains⁽¹⁹⁾. Thus, exploring the changes in brain functional gradients in PD patients can be a good medium to analyze the relationship between heterogeneous motor and non-motor symptoms and its neuromodulation basis. As far as we know, currently no studies have reported whether and how the principal gradient of the functional connectome is disrupted in patients with PD and whether disruption of gradient provides could be used to classify the PD patients.

Previous studies have highlighted the significant role of genes in the human brain, primarily in shaping functional or structural connectivity across the cortices⁽²⁰⁾. Thanks to the Allen Human Brain Atlas (AHBA) microarray dataset, it has become feasible to obtain the relationship between human

neuroimaging and transcriptomes ⁽²¹⁾. Recently, the transcriptome-connectome association studies provide a critical chance to bridge the gap between the microscale transcriptome expression patterns and the macroscale cortical gradient ⁽²²⁾. So far, the clear association between the cortical gradient abnormality and the cortical gene-expression patterns for PD has not yet been established. Further studies are still needed to explore potential neurobiological regulatory mechanisms on the heterogeneous motor and non-motor symptoms of PD.

To address these issues, our study investigated changes of the functional gradients between 99 PD patients and 104 age-matched normal controls (NC), explored the possible PD classification based on gradient heterogeneity, and further sought associations between the neuromodulation basis and their cortical gradient changes. Specifically, we hypothesized that (1) the macroscale gradient was disrupted in patients with PD in both the global gradient and the focal gradient of 7 well-known functional systems; (2) the PD patients could be divided into different subtypes with different clinical symptoms; and (3) the changes of cortical gradient in PD or PD subtypes may associated with different gene expression profiles (inconsistent neuromodulation roles).

Materials and methods

Subjects

We included 99 patients with PD and 104 NC, recruited from our medical imaging center. Demographic information of all subjects was detailed in Table 1. All PD patients fulfilled the UK Parkinson's Disease Society Brain Bank criteria ⁽²³⁾. Individuals enrolled in the NC group were cognitively normal, free of neurological and psychiatric disorders, and had no family history of PD. All subjects performed both T1-weighted structural MRI scanning and blood oxygen level dependent (BOLD) functional MRI scanning. The unified Parkinson's disease rating scale (UPDRS), Hoehn-Yahr (HY) staging, Hamilton anxiety scale (HAMA), Hamilton depression scale (HAMD), and Mini-mental state examination (MMSE) were evaluated in PD patients. For PD patients, the collection of imaging and clinical data was all undergone in their "off" medication state. Our human participant study was based on the Helsinki Declaration and had been reviewed and approved by the institution review committee of local hospital. Informed consent forms were signed from all subjects before experiments.

Table 1
Demographic information of PD and NC.

<i>Clinical information</i>	<i>PD</i>	<i>NC</i>	<i>T/χ2</i>	<i>p</i>
Age (years)	60.53 ± 0.9149	60.84 ± 0.7151	0.2704	0.7871
Gender (M/F)	61/38	57/47	χ2 = 0.966	0.3257
UPDRS III	23.03 ± 1.313			
HY	2.303 ± 0.06727			
Handedness	99/0(R/L)	104/0(R/L)		
HAMD	5.879 ± 0.4897			
HAMA	6.222 ± 0.5498			
Education (years)	8.869 ± 0.3674	9.856 ± 0.3655		0.0584
LEDD (mg/day)	377.8 ± 33.89			
MMSE	26.67 ± 0.3376	28.08 ± 0.1993	-3.639	0.0003
T-tests were performed on age, education, and MMSE. Chi-square tests were performed on gender. LEDD=Levodopa equivalent daily dose, UPDRS=Unified Parkinson's disease rating scale, HY staging=Hoehn and Yahr staging, HAMA=Hamilton anxiety scale, HAMD=Hamilton depression scale, and MMSE=Mini-mental state examination.				

Imaging data acquisition

All participants were scanned on a GE Discovery MR750 3.0T MRI scanner. Earplugs and foam pads were used to reduce noise and head motion. Before scanning, subjects were told to keep their eyes closed, remain motionless, and not fall asleep. The resting-state BOLD functional data was collected using a gradient recalled echo echo-planar imaging sequence. Field of View = 240 × 240 mm², acquisition matrix = 64×64, Repetition Time = 2000 ms, Echo Time = 30 ms, Flip Angle = 90°, Slice Thickness = 4 mm without gaps, Voxel Size: 4.0 × 4.0 × 4.0 mm³, 33 interleaved axial slices cover the whole brain. The slices were parallel to the anterior/posterior commissure, and the entire scanning time was 480 seconds. The structural T1 data were acquired using a whole-brain 3D brain volume imaging sequence as follows: Repetition Time = 12 ms, Echo Time = 5.1 ms, Inversion Time = 450 ms, Flip Angle = 15°, Field of View = 240 × 240 mm², Matrix size: 256 × 256, Slice Thickness = 1 mm without gaps, Voxel Size: 1 × 1 × 1 mm³, and 172 continuous sagittal slices. The total scan time: 320 seconds.

Imaging processing

The resting state BOLD data was subjected to standard preprocessing using fMRI Prep 20.2.0 ^(24, 25). The first 10 volumes were discarded to achieve steady-state equilibrium. AFNI's 3dTshift was used to perform slice timing correction on the remaining volume, and mcflight was used to correct head motion ⁽²⁶⁾. Distortion correction was performed using a TOPUP implementation. This was followed by co-registration to the corresponding T1-weighted image using boundary-based registration (bbregister) with six degrees of freedom. Motion correcting transformations, field distortion correcting warp, BOLD-to-T1w

transformation and T1w-to-template (Montreal Neurological Institute, MNI) warp were concatenated and applied in a single step using antsApplyTransforms (ANTs) with Lanczos interpolation. Then, an eXtensible Connection Pipeline was used to take over the output of fMRI Prep⁽²⁷⁾ and perform subsequent processing. Sources of spurious variance (the six motion parameters, global signal, mean white matter, mean CSF signal with their temporal derivatives, quadratic expansion of six motion parameters, and tissues signals with their temporal derivatives) were removed through linear regression by using Scikit-Learn 0.24.2⁽²⁸⁾. Residual BOLD timeseries from this regression were then band-pass filtered to retain signals within the 0.01–0.08 Hz frequency band. Finally, the processed BOLD data was smoothed using Connectome Workbench with a gaussian kernel size of 6.0 mm (Full Width at Half Maximum, FWHM).

Processed functional time series were extracted from residual BOLD using Connectome Workbench for the Schaefer 400-parcellations resolution atlas. Corresponding pair-wise functional connectivity between all regions was computed for this atlas, which was calculated by Pearson's correlation. For each subject, a 400 × 400 weighted adjacency matrix was constructed.

Gradient analysis

Gradient analyses were performed using BrainSpace⁽²⁹⁾. By using diffusion embedding for dimensionality reduction, cortical gradients were derived separately from the functional adjacency matrix. This identified spatial axes of variation in connectivity across different areas, whereby cortical vertices that were strongly interconnected were closer together and vertices with little or no interconnectivity were farther apart^(30,31). We used normalized angle as a metric of distance similarity (values range from 0 to 1, with 1 denoting identical angle, and 0 opposing angle). The formula for calculating the normalized angle ($A(i, j)$) between two nodes i and j was as follows:

$$\angle(\vec{v}_i, \vec{v}_j) = 1 - \frac{|\vec{v}_i \cos^{-1}(\frac{\vec{v}_i \cdot \vec{v}_j}{|\vec{v}_i| |\vec{v}_j|})|}{\pi}$$

where $\cos$ was the cosine similarity function, v_i and v_j were the rows in the functional adjacency matrix corresponding to nodes i and j , respectively. First, we generated a group-level gradient component template from the average functional connectivity matrices of 1000 participants of Human Connectome Project (HCP) embedded in BrainSpace⁽³²⁾. Then, we aligned the gradient components of each subject to this group-level template. Finally, gradient components defined in connectivity space were mapped back onto the cortical surface (the Schaefer 400-parcellations). For each derived gradient, we calculated the variance explained by dividing the gradient's eigenvalue with the sum of the eigenvalues for all gradients (Fig. 1).

Subtype classification of PD based on individual gradient heterogeneity

In our study, each patient's gradient heterogeneity was defined as the difference between each patient and the mean gradient of NC subjects across the 400 cortical regions. Using individual gradient heterogeneity, hierarchical clustering analysis was performed to classify the PD patients (Fig. 1). This process was performed in R with the cluster package. Hierarchical clustering was used to assign patients to nested subgroups with similar patterns. The Euclidean distance was calculated between each pair of patients. Ward's minimum variance method was used to iteratively link pairs of patients in closest proximity, forming progressively larger clusters in a hierarchical tree. Finally, the NbClust package was used to determine the best number of clusters in the whole analysis.

Neurotransmitter receptor gene expression

Expression profiles for genes of dopaminergic, cholinergic, noradrenergic, and serotonergic receptors were extracted by using data from AHBA⁽³³⁾. We used the abagen toolbox embedded in BrainStat to calculate genetic expression data for the Schaefer 400 cortical regions⁽³⁴⁾. Specifically, the coordinates that provided by the alleninf package (<https://github.com/chrisgorgo/alleninf>) was used to obtain and update the MNI152 coordinates of all six donor tissue samples. Then, performing intensity-based filtering on the probes to remove probes that do not exceed background noise. For probes that indexed the same gene, selecting the probe with the highest differential stability among donors. Subsequently, the organizational samples with the regions were matched in the partitioning plan (the Schaefer 400 cortical regions). The expression values of each sample across genes and the expression values of each gene across samples were normalized by using the sigmoid function. Finally, 15,631 genes (over 20,000 initially included in the Allen atlas gene expression data) survived these pre-processing and quality assurance steps (Fig. 1). Expression profiles for 31 pre-selected genes (Fig. 5) encoding receptors for dopamine, acetylcholine, norepinephrine, and serotonin were then extracted for each of the 400 cortical regions.

Statistical analysis.

Statistical analyses in this study were performed using the SPSS statistical software (version 28: IBM Corp.) and MATLAB (version R2022b: MathWorks, Inc.). Demographics and clinical information were compared between PD and NC and between different subgroups of PD using t-tests or Mann–Whitney and chi-squared test (the D'Agostino–Pearson omnibus normality test was used to check the data normality). Statistical significance was set at $p < 0.05$ (two tailed).

For group gradient (included gradient 1 and gradient 2) comparisons, we used general linear model (GLM), with age and gender as covariates and comparisons of interest: (1) PD vs NC, the global cortical regions compared; and (2) PD vs NC, the 7 resting-state systems (visual, sensorimotor, dorsal attention, ventral attention, limbic, frontoparietal, and default mode) compared. We controlled for multiple comparisons by using FDR (Benjamini–Hochberg method, $q < 0.05$), across 400 cortical regions and across 7 resting-state systems, respectively.

Spearman correlations were calculated between the cortical differences in gradient (PD vs NC, PD subtype vs PD subtype, the vectors consisting of the mean difference at each of the 400 cortical regions) and the regional expression level of each of the chosen 31 neurotransmitter receptor genes at each of the 400 cortical regions. Results were FDR-corrected for multiple comparisons, $q < 0.05$, across 31 genes.

Results

Demographics and clinical variables

The demographic and clinical information were compared between the groups. We found that the PD cohort had significantly lower MMSE scores than the NC cohort ($p < 0.05$) (Table 1). And no significant differences were found in age, education, handedness and gender between the PD cohort and the NC cohort ($p > 0.05$) (Table 1).

Abnormal cortical gradient changes occur in PD

Similar to the previous studies, we focused our analyses on the first two principal gradients. The first principal gradient explained 15.8% (NC was 15.9%) of the variance for functional gradients and the second principal gradient 12.8% (NC was also 12.8%) for functional gradients in PD (According to the gravel plot, Fig. 2-A). The extreme values of gradient 1 were contracted in the PD group, which mainly occurred in the low-gradient sensorimotor system (According to Fig. 2-B&C, Fig. 3-C).

First, we examined how cortical gradient of whole brain (400 cortical regions) changes in PD. When compared with NC, 20 cortical regions of PD showed significantly reduced gradient 1 and 30 cortical regions of PD showed significantly increased gradient 1 (adjusting for age and gender, $p < 0.0053$, FDR corrected over 400 regions, $q < 0.05$). The cortical regions showing gradient 1 reduction in PD were mainly distributed in the middle frontal gyrus, supramarginal gyrus, cingulate gyrus, and precuneus. And the cortical regions with gradient 1 increasing in PD were mainly distributed in the precentral gyrus, paracentral lobule, cuneus, calcarine sulcus, and lingual gyrus (Fig. 2-F). And no significant differences were found in gradient 2 between the PD cohort and the NC cohort.

Subsequently, we continued to investigate how cortical gradient of the 7 resting-state systems changes in PD (Fig. 3-A). When compared with NC, 2 systems of PD showed significantly reduced gradient 1 (adjusting for age and gender, $p = 0.0079$ and $p = 0.002$, FDR corrected over 7 resting-state systems, $q < 0.05$). The systems showing gradient 1 reduction in PD were mainly distributed in the dorsal attention and ventral attention systems (Fig. 3-B, D). And no significant differences were found in gradient 2 in any system between the PD cohort and the NC cohort.

PD can be divided into Neuropsychiatric PD (NPPD) and non-NPPD based on individual gradient heterogeneity

Using individual gradient 1 heterogeneity, hierarchical clustering was performed to discover clusters of patients by assigning them to nested subgroups with similar patterns. This unsupervised method objectively determined 15 clusters and 2 clusters as the optimal solutions. Thus, PD cohort was divided into two subgroups, subtype I with 39 PD subjects and subtype II with 60 PD subjects (Fig. 4).

Table 2
Comparison of clinical symptoms between NPPD and non-NPPD.

Clinical information	NPPD	Non-NPPD	T	p
UPDRS III	23.08 ± 1.737	22.95 ± 2.020	0.0498	0.9604
HY	2.358 ± 0.09807	2.218 ± 0.07963	1.02	0.3103
HAMD	4.600 ± 0.4802	2.949 ± 0.4602	2.352	0.0207
HAMA	7.150 ± 0.7760	4.795 ± 0.6726	2.13	0.0357
MMSE	25.97 ± 0.5114	27.74 ± 0.2667	-2.649	0.0094
T-tests were performed on UPDRS III, HY, HAMD, HAMA, and MMSE. UPDRS=Unified Parkinson's disease rating scale, HY staging=Hoehn and Yahr staging, HAMA=Hamilton anxiety scale, HAMD=Hamilton depression scale, and MMSE=Mini-mental state examination. NPPD showed significantly lower MMSE scores and higher HAMA and HAMD scores, while the scores in non-NPPD were at a normal level.				

To further explore the differences between the two subtypes, we compared the clinical symptoms between them. When compared the clinical information between the two subtypes, no significant difference was found for UPDRS III and HY. Interestingly, subtype II showed significant cognitive and emotional impairments compared to subtype I. Specifically, when compared with subtype I, subtype II showed lower MMSE scores ($p = 0.0094$, $t = 2.649$) (Table 2). And the HAMA and HAMD scores were found higher in subtype II, when compared to subtype I ($p = 0.0357$, $t = 2.13$) ($p = 0.0207$, $t = 2.352$) (Table 2). Based on the above results, we named subtype II as neuropsychiatric PD (NPPD) and subtype I as non-NPPD.

Neurotransmitter basis of cortical gradient changes between PD and NC, NPPD and non-NPPD

To further understand whether and how neuromodulation system played a role in the formation of gradient 1 changes pattern in PD and NPPD, we further investigated the relationship between maps of gene expression for neurotransmitter receptor genes (derived from post-mortem human brains) and cortical gradient 1 changes between PD and NC, NPPD and non-NPPD, respectively. We found that cortical gradient 1 changes between PD and NC showed significant correlations with cortical gene expression of dopaminergic, cholinergic, noradrenergic, and serotonergic receptors ($p < 0.0165$, FDR corrected over

31 genes, $q < 0.05$). Specifically, compared to NC, the pattern of gradient 1 changes in PD was associated with expression of DRD2, CHRNA2-4, CHRM4, ADRA1B, HTR2A, HTR1A, HTR7, and HTR1F (Fig. 5). And we found that cortical gradient 1 changes between NPPD and non-NPPD showed significant correlations with cortical gene expression of cholinergic receptor ($p = 0.0194$, $r = 0.1275$). Specifically, compared to non-NPPD, the pattern of gradient 1 changes in NPPD was associated with expression of CHRM5 (Fig. 5).

Discussion

In the present study, we revealed how the cortical functional gradient changes in patients with PD including the whole brain level and large-scale network level, proved that the PD patients could be further classified into NPPD and non-NPPD based on individual cortical gradient heterogeneity, and clarified that neuromodulation principles that drove the gradient alteration.

Evidence from many studies had consistently revealed that the cortical functional gradient varied spatially, with a gradual increase emerging along the cognitive representational hierarchy^(35, 36). Specifically, evolutionarily conserved unimodal regions such as visual and sensorimotor systems displayed relatively weaker gradients, whereas evolutionarily rapidly expanded transmodal regions such as frontoparietal and default mode systems displayed relatively stronger gradient^(37, 38). This gradient distribution pattern was also reproduced in our study (Fig. 2-E), both in the NC and PD group (lower gradient 1 distributed in visual and sensorimotor systems, moderate gradient 1 distributed in dorsal attention, ventral attention, and limbic systems, and higher gradient 1 distributed in frontoparietal and default mode systems). However, in patients with PD, cortices such as precentral gyrus, cuneus, and lingual gyrus, which belonged to visual and sensorimotor systems exhibited gradient 1 increase and cortices such as middle frontal gyrus, supramarginal gyrus, and cingulate gyrus, which belonged to dorsal attention, ventral attention, and default mode system exhibited gradient 1 decrease, when compared to NC. These changes to some extent indicated a less differentiated connectivity pattern between the unimodal and transmodal regions in PD patients, which further suggested that the brain of PD was undergoing a weakening of functional segregation. The same evidence could also be found in previous functional connectome study, where researchers found that PD patients showed a decrease in clustering coefficient (A graph theory parameter representing functional separation) compared to the NC⁽³⁹⁾.

Notably, compared to NC, the most pronounced differences in PD were observed in dorsal attention and ventral attention systems. The dorsal attention system is responsible for top-down attention functions (attention allocation), while the ventral attention system is primarily involved in bottom-up attention functions (attention transfer)^(40–42). Gradient 1 anomalies in these functional systems suggested that PD exhibited abnormal cortical hierarchical organization and might suffer information conversion disorders in tasks involving attention maintenance and switching.

Through the comparative study of cortical gradients between the groups, we realized that there might be strong individual cortical gradient heterogeneity in PD patients. Thus, by using the individual cortical gradient 1 heterogeneity, a further hierarchical clustering analysis was performed to classify the PD patients. And two subtypes were divided out in our study. Interestingly, when these two subtypes were compared, subtype II showed significantly lower MMSE scores and higher HAMA and HAMD scores, while the scores in subtype I were at a normal level, which indicated that PD patients from subtype II possessed more neuropsychiatric disorders. Many prior studies had also demonstrated that individuals with PD displayed substantial heterogeneity: some patients manifested neuropsychiatric symptoms, while others were devoid of these manifestations^(43, 44). Therefore, utilizing individual cortical gradient heterogeneity to distinguish between NPPD and non-NPPD held considerable clinical significance.

PD is currently regarded as a complex "disease spectrum" rather than a single entity, resulting from the combined effects of genetic diversity, differences in α -synuclein (α -syn) pathological characteristics, variations in the degree and scope of damage to dopaminergic and non-dopaminergic systems, as well as differences in individual protective factors^(45–48). This also explains why PD exhibits marked heterogeneity. Thus, to elucidate the potential principles of gradient 1 changes in PD and NPPD, we conducted correlation analysis between the cortical gradient 1 changes (PD vs NC, NPPD vs non-NPPD) and the gene expression of dopaminergic, cholinergic, noradrenergic, and serotonergic receptors. And a detected correlation between the cortical gradient changes (PD vs NC) and the gene expression of DRD2 indicated that dysregulation of the dopaminergic nervous system was partially responsible for gradient 1 changes in PD patients. This also indirectly suggested that the abnormal changes in gradient 1 to some extent represented the typical pathology of PD (dopamine deficiency). Interestingly, the cortical gradient 1 changes of PD vs NC were also found that correlated with the gene expression of CHRNA2, CHRNA2-4, CHRM4 (cholinergic receptor), ADRA1B (noradrenergic receptor), HTR1A, HTR2A, HTR7, and HTR1F serotonergic receptor). These were because impairment of PD not only involved motor damage to the dopaminergic system but also cognitive and emotional damage to non-dopaminergic systems, such as the cholinergic, noradrenergic, and serotonergic system^(49, 50). In our PD participants, more than half of the patients had varying degrees of cognitive and emotional impairment (based on MMSE, HAMD, and HAMA scores). We found that a detected correlation between the cortical gradient changes (NPPD vs non-NPPD) and the gene expression of CHRM5 indicated that dysregulation of the cholinergic nervous system was mainly responsible for gradient 1 changes in NPPD patients. This might suggest that CHRM5 is an intervenable molecular target and could serve as a novel strategy for treating the neuropsychiatric symptoms of NPPD. Previous studies had also shown that CHRM5 knockout mice exhibited dopamine dysregulation and depression-like and anxiety-like behaviors^(51, 52).

Several methodological considerations needed to be taken into account when interpreting the results of our study. First, although the impact of drug effect had been eliminated in patients (we conducted experiments during their "off" medication state), it is currently unknown whether long-term drug effect could affect functional gradient changes. Therefore, long-term drug effects might still be a potential factor affecting experimental results. Second, our study focused on cross-sectional data, although this provided useful insights, longitudinal studies in PD patients were still needed to provide further insights into the temporal pattern of cortical gradient changes in PD.

Taken together, our findings showed that functional gradients were severely disrupted in patients with PD across the cortex, with even more pronounced alteration in dorsal attention and ventral attention network. And we further discovered that PD patients could be divided into NPPD and non-NPPD by using

individual cortical gradient heterogeneity. What's more, we clarified the neurotransmitter basis of cortical gradient changes in PD and NPPD.

Abbreviations

AHBA
Allen Human Brain Atlas
antsApplyTransforms
ANTs
Blood Oxygen Level Dependent
BOLD
FDR
False Discovery Rate
Full Width at Half Maximum
FWHM
General Linear Model
GLM
Human Connectome Project
HCP
Hoehn-Yahr
HY
LOOCV
Leave-One-out Cross-validation
Mini-mental State Examination
MMSE
Montreal Neurological Institute
MNI
NC
Normal control
NPPD
neuropsychiatric PD
PD
Parkinson's disease
and UPDRS
Unified Parkinson's Disease Rating Scale.

Declarations

Conflict of interest

The authors disclose no conflicts of interest regarding this article. None of the authors have received any funding from any institution, including personal relationships, interests, grants, employment, affiliations, patents, inventions, honoraria, consultancies, royalties, stock options/ownership, or expert testimony for the last 12 months.

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Author Contribution

All authors of the paper have made significant contributions and are consistent with the content of the manuscript. Zhengye Cao, Song'an Shang, and Na Wang were in charge of project design; Hongying Zhang, and Song'an Shang was responsible for the inclusion of subjects; Zhengye Cao, Na Wang, and Hongying Zhang were in charge of data gathering and paper writing; Zhengye Cao was in charge of data analyses.

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Author roles

All authors of the paper have made significant contributions and are consistent with the content of the manuscript. Zhengye Cao, Song'an Shang, and Na Wang were in charge of project design; Hongying Zhang, and Song'an Shang was responsible for the inclusion of subjects; Zhengye Cao, and Na Wang were in charge of data gathering and paper writing; Zhengye Cao was in charge of data analyses.

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Data and code availability

statement

Some or all data, models, or code generated or used during the study are available from the corresponding author by request.

References

1. Pagonabarraga J, Kulisevsky J, Strafella AP, Krack P (2015) Apathy in Parkinson's disease: clinical features, neural substrates, diagnosis, and treatment. *Lancet Neurol* 14(5):518–531. 10.1016/s1474-4422(15)00019-8
2. Samii A, Nutt JG, Ransom BR (2004) Parkinson's disease. *Lancet* (London England) 363(9423):1783–1793. 10.1016/s0140-6736(04)16305-8
3. Tolosa E, Garrido A, Scholz SW, Poewe W (2021) Challenges in the diagnosis of Parkinson's disease. *Lancet Neurol* 20(5):385–397. 10.1016/s1474-4422(21)00030-2
4. Flint J, Kendler KS (2014) The genetics of major depression. *Neuron* 81(3):484–503. 10.1016/j.neuron.2014.01.027
5. Sarasso E, Agosta F, Piramide N, Gardoni A, Canu E, Leocadi M, Castelnovo V, Basaia S, Tettamanti A, Volontè MA, Filippi M (2021) Action Observation and Motor Imagery Improve Dual Task in Parkinson's Disease: A Clinical/fMRI Study. *Mov disorders: official J Mov Disorder Soc* 36(11):2569–2582. 10.1002/mds.28717
6. Schilder JC, Overmars SS, Marinus J, van Hilten JJ, Koehler PJ (2017) The terminology of akinesia, bradykinesia and hypokinesia: Past, present and future. *Parkinsonism Relat Disord* 37:27–35. 10.1016/j.parkreldis.2017.01.010
7. Reich SG, Savitt JM (2019) Parkinson's Disease. *Med Clin N Am* 103(2):337–350. 10.1016/j.mcna.2018.10.014
8. Kalia LV, Lang AE (2015) Parkinson's disease. *Lancet* 386(9996):896–912. 10.1016/s0140-6736(14)61393-3
9. Chaudhuri KR, Healy DG, Schapira AH (2006) Non-motor symptoms of Parkinson's disease: diagnosis and management. *Lancet Neurol* 5(3):235–245. 10.1016/s1474-4422(06)70373-8
10. Fanciulli A, Leys F, Falup-Pecurariu C, Thijs R, Wenning GK (2020) Management of Orthostatic Hypotension in Parkinson's Disease. *J Parkinsons Dis* 10(s1):S57–s64. 10.3233/jpd-202036
11. Munhoz RP, Tumas V, Pedroso JL, Silveira-Moriyama L (2024) The clinical diagnosis of Parkinson's disease. *Arq Neuropsiquiatr* 82(6):1–10. 10.1055/s-0043-1777775
12. Williams L, Butler JS, Thirkettle M, Stafford T, Quinlivan B, McGovern E, O'Riordan S, Redgrave P, Reilly R, Hutchinson M (2020) Slowed Luminance Reaction Times in Cervical Dystonia: Disordered Superior Colliculus Processing. *Mov Disord* 35(5):877–880. 10.1002/mds.27975
13. Kong D, Li C, Ma L, Du L, Jiang N, Zhao X, Zhang S, Zhao Z, Fang L, Du G (2024) Identifying genetic targets in clinical subtypes of Parkinson's disease for optimizing pharmacological treatment strategies. *Signal Transduct Target Ther* 9(1):320. 10.1038/s41392-024-02020-x
14. Wiesman AI, da Silva Castanheira J, Fon EA, Baillet S (2024) Alterations of Cortical Structure and Neurophysiology in Parkinson's Disease Are Aligned with Neurochemical Systems. *Ann Neurol* 95(4):802–816. 10.1002/ana.26871
15. Wiesman AI, da Silva Castanheira J, Fon EA, Baillet S Structural and neurophysiological alterations in Parkinson's disease are aligned with cortical neurochemical systems. *medRxiv* 2023. 10.1101/2023.04.04.23288137
16. Fornito A, Arnatkevičiūtė A, Fulcher BD (2019) Bridging the Gap between Connectome and Transcriptome. *Trends Cogn Sci* 23(1):34–50. 10.1016/j.tics.2018.10.005
17. Richiardi J, Altmann A, Milazzo AC, Chang C, Chakravarty MM, Banaschewski T, Barker GJ, Bokde AL, Bromberg U, Büchel C, Conrod P, Fauth-Bühler M, Flor H, Frouin V, Gallinat J, Garavan H, Gowland P, Heinz A, Lemaître H, Mann KF, Martinot JL, Nees F, Paus T, Pausova Z, Rietschel M, Robbins TW, Smolka MN, Spanagel R, Ströhle A, Schumann G, Hawrylycz M, Poline JB, Greicius MD (2015) BRAIN NETWORKS. Correlated gene expression supports synchronous activity in brain networks. *Science* 348(6240):1241–1244. 10.1126/science.1255905
18. Hawrylycz MJ, Lein ES, Guillozet-Bongaarts AL, Shen EH, Ng L, Miller JA, van de Lagemaat LN, Smith KA, Ebbert A, Riley ZL, Abajian C, Beckmann CF, Bernard A, Bertagnolli D, Boe AF, Cartagena PM, Chakravarty MM, Chapin M, Chong J, Dalley RA, David Daly B, Dang C, Datta S, Dee N, Dolbeare TA, Faber V, Feng D, Fowler DR, Goldy J, Gregor BW, Haradon Z, Haynor DR, Hohmann JG, Horvath S, Howard RE, Jeromin A, Jochim JM, Kinnunen M, Lau C, Lazarz ET, Lee C, Lemon TA, Li L, Li Y, Morris JA, Overly CC, Parker PD, Parry SE, Reding M, Royall JJ, Schulkin J, Sequeira PA, Slaughterbeck CR, Smith SC, Sodt AJ, Sunkin SM, Swanson BE, Vawter MP, Williams D, Wohnoutka P, Zielke HR, Geschwind DH, Hof PR, Smith SM, Koch C, Grant SGN, Jones AR (2012) An anatomically comprehensive atlas of the adult human brain transcriptome. *Nature* 489(7416):391–399. 10.1038/nature11405

19. Mesulam MM (1998) From sensation to cognition. *Brain* 121(Pt 6):1013–1052. 10.1093/brain/121.6.1013
20. Arnatkeviciute A, Fulcher BD, Oldham S, Tiego J, Paquola C, Gerring Z, Aquino K, Hawi Z, Johnson B, Ball G, Klein M, Deco G, Franke B, Bellgrove MA, Fornito A (2021) Genetic influences on hub connectivity of the human connectome. *Nat Commun* 12(1):4237. 10.1038/s41467-021-24306-2
21. Ritchie J, Pantazatos SP, French L (2018) Transcriptomic characterization of MRI contrast with focus on the T1-w/T2-w ratio in the cerebral cortex. *NeuroImage* 174:504–517. 10.1016/j.neuroimage.2018.03.027
22. Morgan SE, Seidlitz J, Whitaker KJ, Romero-Garcia R, Clifton NE, Scarpazza C, van Amelsvoort T, Marcelis M, van Os J, Donohoe G, Mothersill D, Corvin A, Pocklington A, Raznahan A, McGuire P, Vértés PE, Bullmore ET (2019) Cortical patterning of abnormal morphometric similarity in psychosis is associated with brain expression of schizophrenia-related genes. *Proc Natl Acad Sci U S A* 116(19):9604–9609. 10.1073/pnas.1820754116
23. Hughes AJ, Daniel SE, Kilford L, Lees AJ (1992) Accuracy of clinical diagnosis of idiopathic Parkinson's disease: a clinico-pathological study of 100 cases. *J Neurol Neurosurg Psychiatry* 55(3):181–184. 10.1136/jnnp.55.3.181
24. Chen Y, Hopp FR, Malik M, Wang PT, Woodman K, Youk S, Weber R (2022) Reproducing FSL's fMRI data analysis via Nipype: Relevance, challenges, and solutions. *Front Neuroimaging* 1:953215. 10.3389/fnimg.2022.953215
25. Gorgolewski K, Burns CD, Madison C, Clark D, Halchenko YO, Waskom ML, Ghosh SS (2011) Nipype: a flexible, lightweight and extensible neuroimaging data processing framework in python. *Front Neuroinform* 5:13. 10.3389/fninf.2011.00013
26. Jenkinson M, Bannister P, Brady M, Smith S (2002) Improved optimization for the robust and accurate linear registration and motion correction of brain images. *NeuroImage* 17(2):825–841. 10.1016/s1053-8119(02)91132-8
27. Ciric R, Rosen AFG, Erus G, Cieslak M, Adebimpe A, Cook PA, Bassett DS, Davatzikos C, Wolf DH, Satterthwaite TD (2018) Mitigating head motion artifact in functional connectivity MRI. *Nat Protoc* 13(12):2801–2826. 10.1038/s41596-018-0065-y
28. Abraham A, Pedregosa F, Eickenberg M, Gervais P, Mueller A, Kossaifi J, Gramfort A, Thirion B, Varoquaux G (2014) Machine learning for neuroimaging with scikit-learn. *Front Neuroinform* 8:14. 10.3389/fninf.2014.00014
29. Vos de Wael R, Benkarim O, Paquola C, Lariviere S, Royer J, Tavakol S, Xu T, Hong SJ, Langs G, Valk S, Misic B, Milham M, Margulies D, Smallwood J, Bernhardt BC (2020) BrainSpace: a toolbox for the analysis of macroscale gradients in neuroimaging and connectomics datasets. *Commun Biol* 3(1):103. 10.1038/s42003-020-0794-7
30. Margulies DS, Ghosh SS, Goulas A, Falkiewicz M, Huntenburg JM, Langs G, Bezgin G, Eickhoff SB, Castellanos FX, Petrides M, Jefferies E, Smallwood J (2016) Situating the default-mode network along a principal gradient of macroscale cortical organization. *Proc Natl Acad Sci U S A* 113(44):12574–12579. 10.1073/pnas.1608282113
31. Coifman RR, Lafon S, Lee AB, Maggioni M, Nadler B, Warner F, Zucker SW (2005) Geometric diffusions as a tool for harmonic analysis and structure definition of data: diffusion maps. *Proc Natl Acad Sci U S A* 102(21):7426–7431. 10.1073/pnas.0500334102
32. Glasser MF, Sotiropoulos SN, Wilson JA, Coalson TS, Fischl B, Andersson JL, Xu J, Jbabdi S, Webster M, Polimeni JR, Van Essen DC, Jenkinson M (2013) The minimal preprocessing pipelines for the Human Connectome Project. *NeuroImage* 80:105–124. 10.1016/j.neuroimage.2013.04.127
33. Hawrylycz M, Miller JA, Menon V, Feng D, Dolbeare T, Guillozet-Bongaarts AL, Jegga AG, Aronow BJ, Lee CK, Bernard A, Glasser MF, Dierker DL, Menche J, Szafer A, Collman F, Grange P, Berman KA, Mihalas S, Yao Z, Stewart L, Barabási AL, Schulkin J, Phillips J, Ng L, Dang C, Haynor DR, Jones A, Van Essen DC, Koch C, Lein E (2015) Canonical genetic signatures of the adult human brain. *Nat Neurosci* 18(12):1832–1844. 10.1038/nn.4171
34. Markello RD, Arnatkeviciute A, Poline JB, Fulcher BD, Fornito A, Misic B (2021) Standardizing workflows in imaging transcriptomics with the abagen toolbox. *Elife* 10. 10.7554/eLife.72129
35. Yao G, Luo J, Li J, Feng K, Liu P, Xu Y (2024) Functional gradient dysfunction in drug-naïve first-episode schizophrenia and its correlation with specific transcriptional patterns and treatment predictions. *Psychol Med* 54(15):1–13. 10.1017/s0033291724001739
36. Xia M, Liu J, Mechelli A, Sun X, Ma Q, Wang X, Wei D, Chen Y, Liu B, Huang CC, Zheng Y, Wu Y, Chen T, Cheng Y, Xu X, Gong Q, Si T, Qiu S, Lin CP, Cheng J, Tang Y, Wang F, Qiu J, Xie P, Li L, He Y (2022) Connectome gradient dysfunction in major depression and its association with gene expression profiles and treatment outcomes. *Mol Psychiatry* 27(3):1384–1393. 10.1038/s41380-022-01519-5
37. Yang C, Zhang W, Liu J, Yao L, Bishop JR, Lencer R, Gong Q, Yang Z, Lui S (2023) Disrupted subcortical functional connectome gradient in drug-naïve first-episode schizophrenia and the normalization effects after antipsychotic treatment. *Neuropsychopharmacology* 48(5):789–796. 10.1038/s41386-022-01512-0
38. Hu ML, Zong XF, Mann JJ, Zheng JJ, Liao YH, Li ZC, He Y, Chen XG, Tang JS (2017) A Review of the Functional and Anatomical Default Mode Network in Schizophrenia. *Neurosci Bull* 33(1):73–84. 10.1007/s12264-016-0090-1
39. Suo X, Lei D, Li N, Cheng L, Chen F, Wang M, Kemp GJ, Peng R, Gong Q (2017) Functional Brain Connectome and Its Relation to Hoehn and Yahr Stage in Parkinson Disease. *Radiology* 285(3):904–913. 10.1148/radiol.2017162929
40. Corbetta M, Shulman GL (2002) Control of goal-directed and stimulus-driven attention in the brain. *Nat Rev Neurosci* 3(3):201–215. 10.1038/nnr755
41. Slagter HA, Giesbrecht B, Kok A, Weissman DH, Kenemans JL, Woldorff MG, Mangun GR (2007) fMRI evidence for both generalized and specialized components of attentional control. *Brain Res* 1177:90–102. 10.1016/j.brainres.2007.07.097
42. Tosoni A, Capotosto P, Baldassarre A, Spadone S, Sestieri C (2023) Neuroimaging evidence supporting a dual-network architecture for the control of visuospatial attention in the human brain: a mini review. *Front Hum Neurosci* 17:1250096. 10.3389/fnhum.2023.1250096
43. Batzu L, Podlowska A, Gibson L, Chaudhuri KR, Aarsland D (2024) A general clinical overview of the non-motor symptoms in Parkinson's disease: Neuropsychiatric symptoms. *Int Rev Neurobiol* 174:59–97. 10.1016/bs.irn.2023.11.001
44. von Coelln R, Shulman LM (2016) Clinical subtypes and genetic heterogeneity: of lumping and splitting in Parkinson disease. *Curr Opin Neurol* 29(6):727–734. 10.1097/wco.0000000000000384

45. Bower JH, Maraganore DM, McDonnell SK, Rocca WA (2000) Influence of strict, intermediate, and broad diagnostic criteria on the age- and sex-specific incidence of Parkinson's disease. *Mov Disord* 15(5):819–825. 10.1002/1531-8257(200009)15:5%3C819::aid-mds1009%3E3.0.co;2-p
46. Schapira AH, Jenner P (2011) Etiology and pathogenesis of Parkinson's disease. *Mov Disord* 26(6):1049–1055. 10.1002/mds.23732
47. Nalls MA, Pankratz N, Lill CM, Do CB, Hernandez DG, Saad M, DeStefano AL, Kara E, Bras J, Sharma M, Schulte C, Keller MF, Arepalli S, Letson C, Edsall C, Stefansson H, Liu X, Pliner H, Lee JH, Cheng R, Ikram MA, Ioannidis JP, Hadjigeorgiou GM, Bis JC, Martinez M, Perlmutter JS, Goate A, Marder K, Fiske B, Sutherland M, Xiromerisiou G, Myers RH, Clark LN, Stefansson K, Hardy JA, Heutink P, Chen H, Wood NW, Houlden H, Payami H, Brice A, Scott WK, Gasser T, Bertram L, Eriksson N, Foroud T, Singleton AB (2014) Large-scale meta-analysis of genome-wide association data identifies six new risk loci for Parkinson's disease. *Nat Genet* 46(9):989–993. 10.1038/ng.3043
48. Berg D, Lang AE, Postuma RB, Maetzler W, Deuschl G, Gasser T, Siderowf A, Schapira AH, Oertel W, Obeso JA, Olanow CW, Poewe W, Stern M (2013) Changing the research criteria for the diagnosis of Parkinson's disease: obstacles and opportunities. *Lancet Neurol* 12(5):514–524. 10.1016/s1474-4422(13)70047-4
49. Petrova T, Orellana C, Jelic V, Oeksengaard AR, Snaedal J, Høgh P, Andersen BB, Naik M, Engedal K, Wahlund LO, Ferreira D (2020) Cholinergic dysfunction, neurodegeneration, and amyloid-beta pathology in neurodegenerative diseases. *Psychiatry Res Neuroimaging* 302:111099. 10.1016/j.pscychresns.2020.111099
50. Bohnen NI, Kaufer DI, Ivancio LS, Lopresti B, Koeppe RA, Davis JG, Mathis CA, Moore RY, DeKosky ST (2003) Cortical cholinergic function is more severely affected in parkinsonian dementia than in Alzheimer disease: an in vivo positron emission tomographic study. *Arch Neurol* 60(12):1745–1748. 10.1001/archneur.60.12.1745
51. Razidlo JA, Fausner SML, Ingebretson AE, Wang LC, Petersen CL, Mirza S, Swank IN, Alvarez VA, Lemos JC (2022) Chronic Loss of Muscarinic M5 Receptor Function Manifests Disparate Impairments in Exploratory Behavior in Male and Female Mice despite Common Dopamine Regulation. *J Neurosci* 42(36):6917–6930. 10.1523/jneurosci.1424-21.2022
52. Nunes EJ, Rupprecht LE, Foster DJ, Lindsley CW, Conn PJ, Addy NA (2020) Examining the role of muscarinic M5 receptors in VTA cholinergic modulation of depressive-like and anxiety-related behaviors in rats. *Neuropharmacology* 171:108089. 10.1016/j.neuropharm.2020.108089

Figures

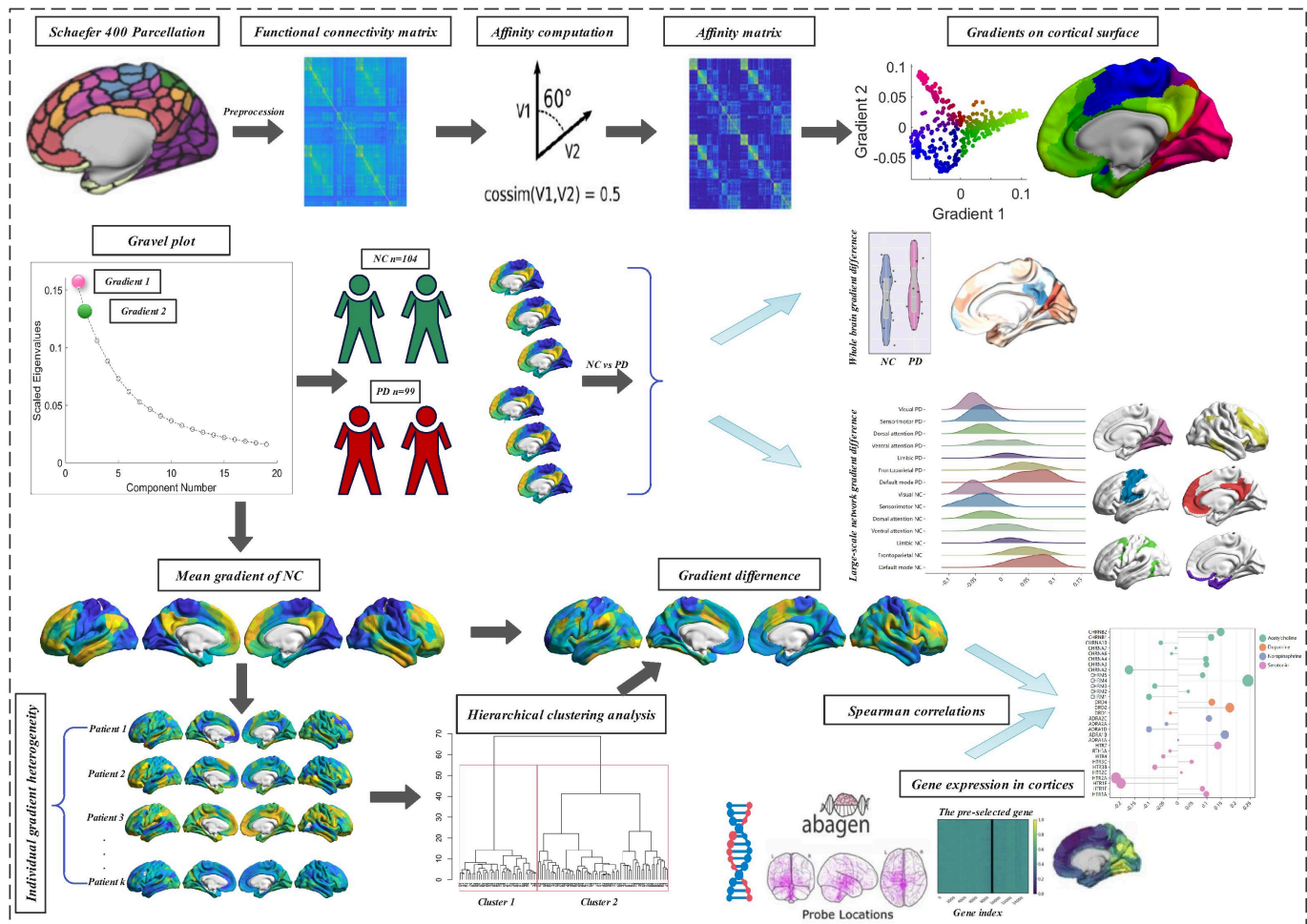


Figure 1

Overview of the study methodology.

Analyses were conducted using a whole brain Schaefer parcellation including 400 cortical regions. Gradients of connectivity covariance were constructed for each subject's functional connectivity matrix using diffusion map embedding, a non-linear compression algorithm that sorts nodes based on affinity (normalized angle was used as a measure of affinity). We focused our analyses on the first 2 principal functional gradients. The scores of each node for the first 2 gradients were shown in the kernel density plot. The variance size of the first 2 gradients could be seen in the gravel plot. Case-control gradient differences were compared for the first 2 gradients including the whole brain level and the large-scale network level. Each patient's gradient heterogeneity was defined as the difference between each patient and the mean gradient of NC subjects across the 400 cortical regions. Using individual gradient heterogeneity, hierarchical clustering was performed to discover clusters of patients by assigning them to nested subgroups with similar patterns. Expression profiles for genes were extracted by using data from AHBA. Mean group differences of gradient scores (PD vs NC, PD subtype vs PD subtype) and the gene expression might be projected back to the cortical surface. Then, we correlated the mean group difference of gradient scores with the pre-selected gene expression for each region.

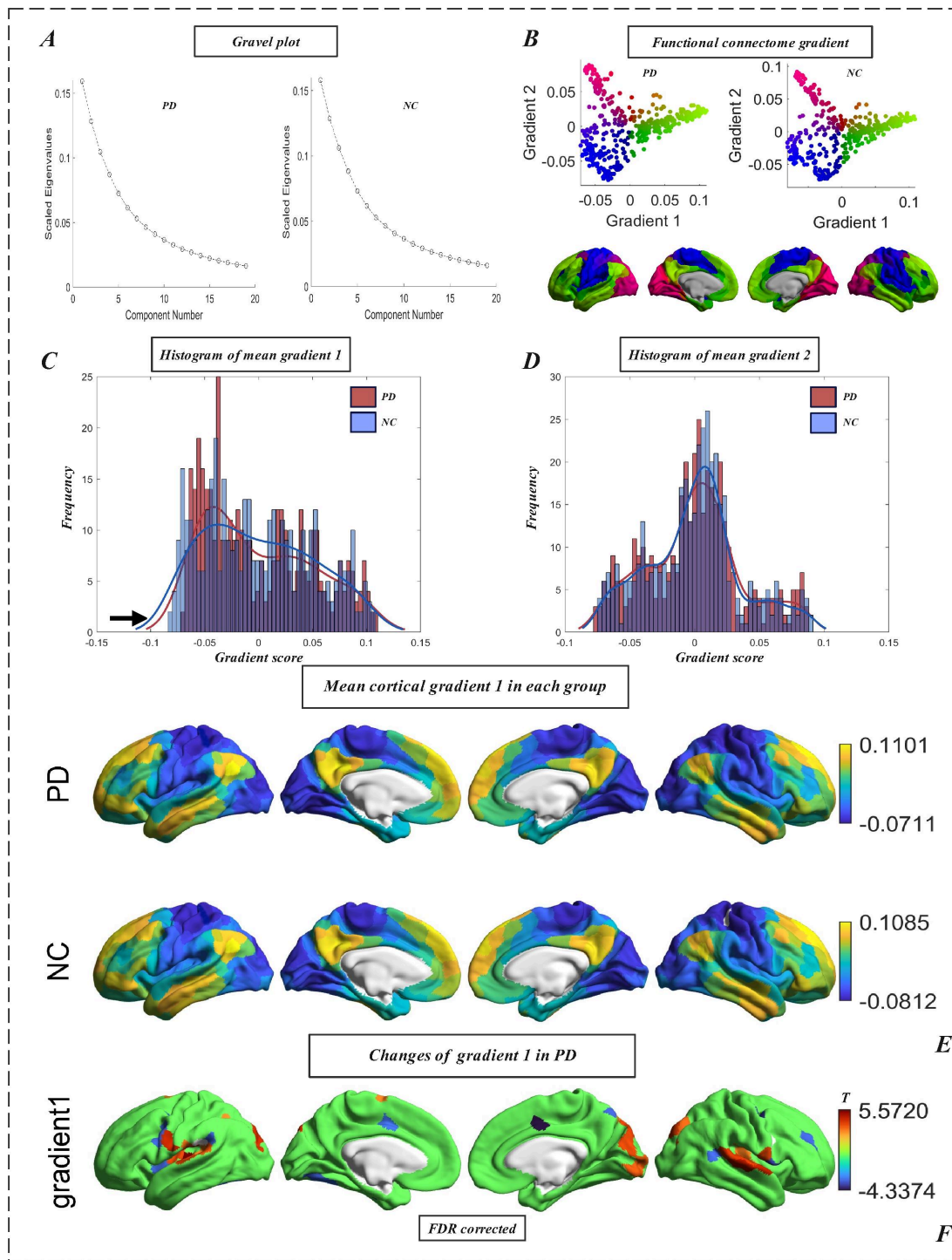


Figure 2

Gradients comparisons between NC and PD in the whole brain.

A The gravel plot for annotating the variance size of each gradient in PD and NC group. **B** The kernel density plot for presenting the first two principal gradients. **C-D** Distribution of mean gradients of gradient 1 and gradient 2 in PD and NC. Seen from **B** and **C**, the extreme values of gradient 1 were contracted in the PD group, which mainly occurred in the low-gradient sensorimotor system. **E** Spatial pattern of gradient 1 in NC and PD. The gradient1 varied widely across the cortex. Default mode and frontoparietal exhibited relatively high gradient1, intermediate gradient 1 were mainly exhibited in limbic, dorsal attention, and ventral attention, while visual and sensorimotor showed relatively low gradient 1. Both NC and PD showed the same distribution pattern. **F** Gradient changes for each node across the brain in PD. Whole brain comparisons of gradient were performed between NC and PD, with age and gender included as covariates. Multiple comparison correction using FDR ($q < 0.05$, across 400 nodes)

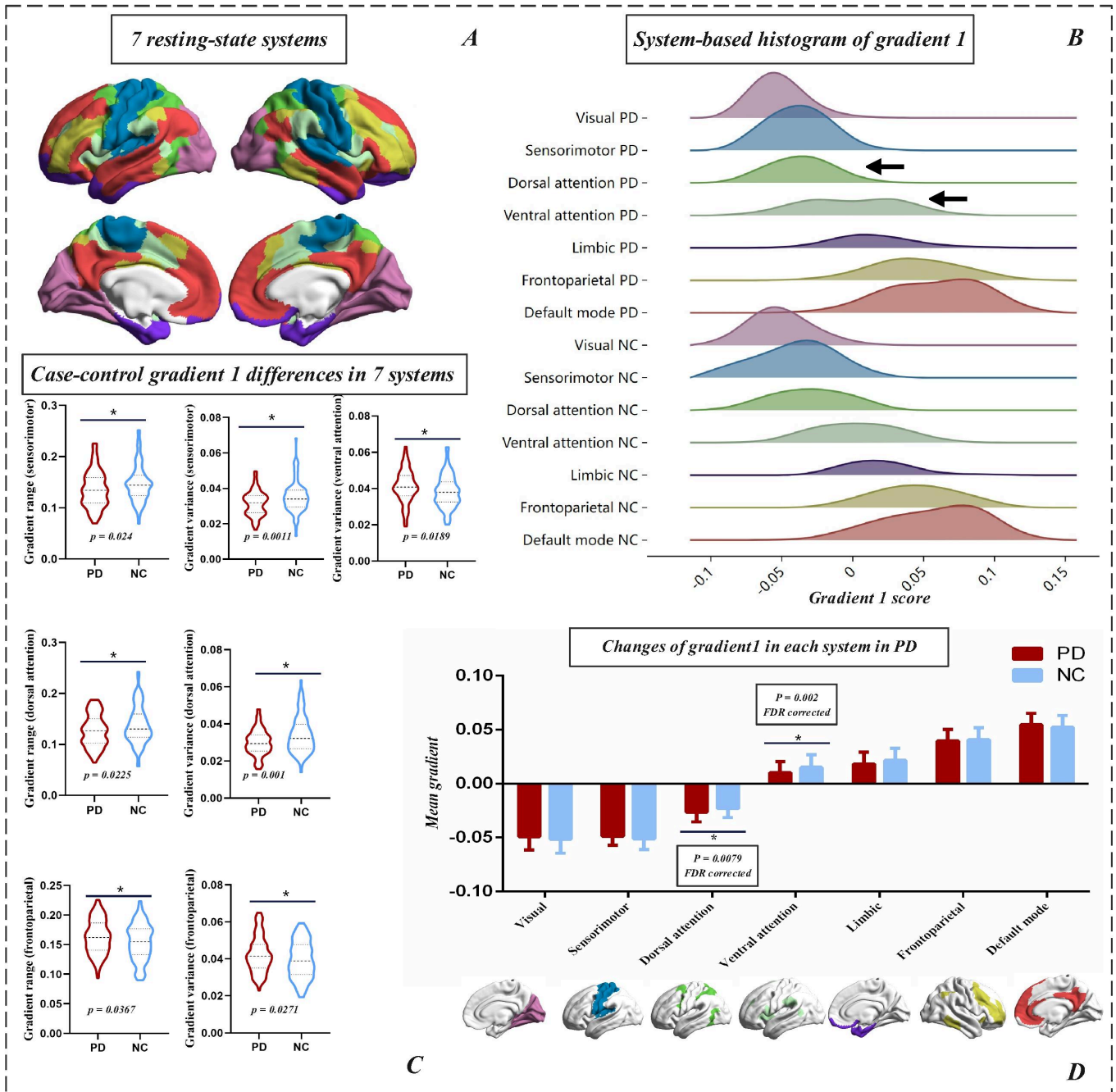


Figure 3

Gradients comparisons between NC and PD in the 7 resting-state systems.

A Parcellation of the cortex into the 7 resting-state systems: visual (pink), sensorimotor (blue), dorsal attention (green), ventral attention (cyan), limbic (purple), frontoparietal (yellow), and default mode (red). **B** Distribution of 7 subnetwork-based functional gradients. Arrows in **B** indicate the direction of the significant differences between PD and NC. **C** Statistical comparisons of gradient 1 metrics in the 7 systems. **D** Gradient 1 changes for each resting-state system in PD. Comparisons of gradient 1 were performed for each system between NC and PD, with age and gender included as covariates. Multiple comparison correction using FDR ($q < 0.05$, across 7 systems).

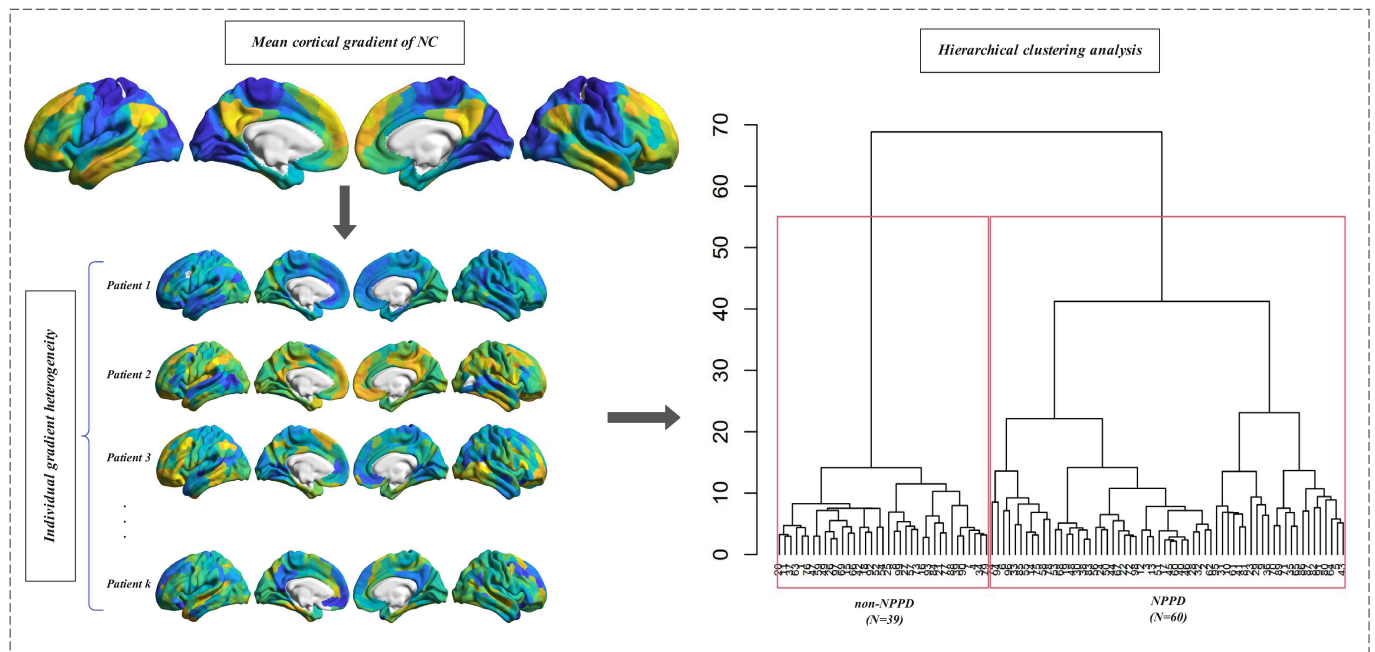


Figure 4

Subtype classification of PD based on individual gradient heterogeneity.

In our study, each patient's gradient heterogeneity was defined as the difference between each patient and the mean gradient 1 of NC subjects across the 400 cortical regions. Using individual gradient heterogeneity, hierarchical clustering was performed to discover clusters of patients by assigning them to nested subgroups with similar patterns. This unsupervised method objectively determined 15 clusters and 2 clusters as the optimal solutions. Thus, PD cohort was divided into two subgroups, non-NPPD with 39 PD subjects and NPPD with 60 PD subjects. NPPD possessed more neuropsychiatric disorders, as shown in **Table 2**.

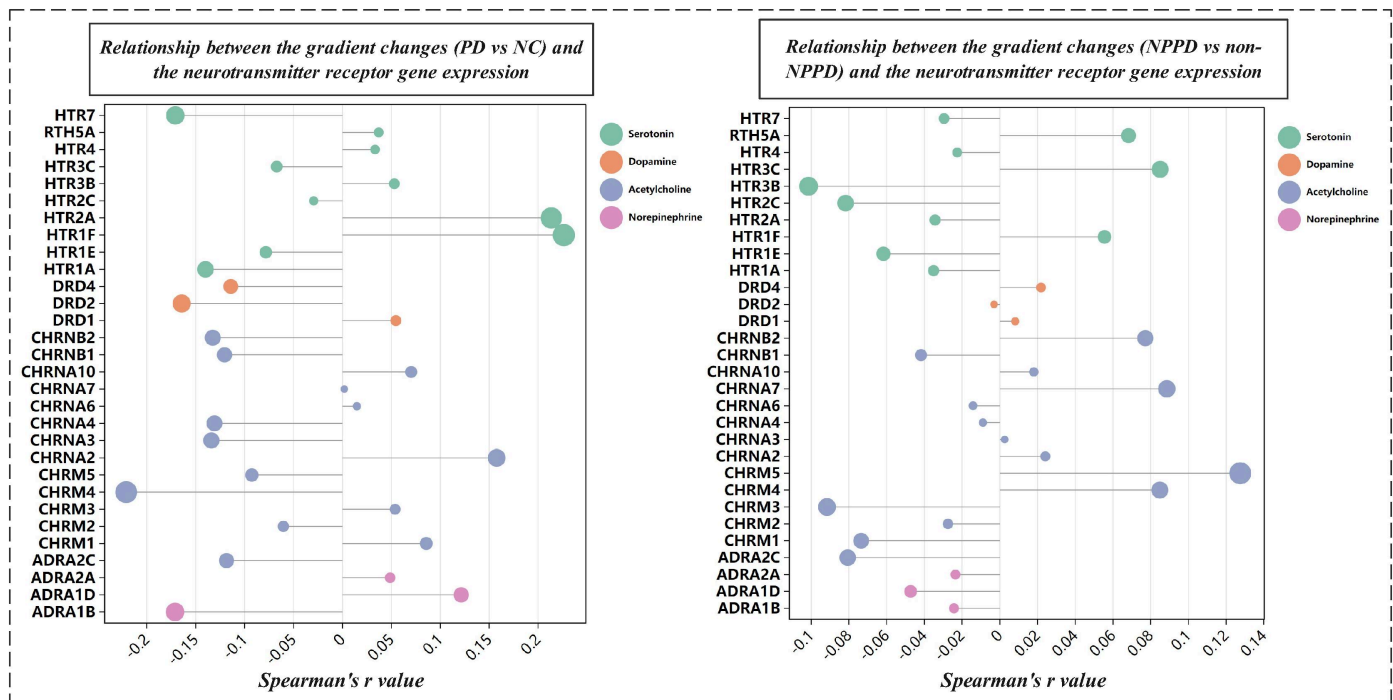


Figure 5

Correlations between the gradient changes (PD vs NC, NPPD vs non-NPPD) and the gene expression.

Spearman correlations between regional cortical expression of adrenergic, cholinergic, serotonergic, and dopaminergic receptors and differences in gradient seen between PD and NC, NPPD and non-NPPD. Results color coded according to receptors: green: serotonergic receptors, orange: dopaminergic receptors, pink: adrenergic receptors, purple: cholinergic receptors. Sticks with longer length and balls with larger volume indicate statistically significant relationships (FDR corrected $q < 0.05$).