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Diffusion tensor imaging techniques show that parkin gene S/N167 polymorphism is responsible for extensive brain white matter damage in patients with Parkinson's disease

Jinqiu Yu [a,b,c,1], Jinying Shi [a,1], Lina Chen [a], Yingqing Wang [a], Guoen Cai [a], Xiaochun Chen [a,c], Weiming Hong [b,**,2], Qinyong Ye [a,c,d,*,2]

[a] Department of Neurology, Fujian Institute of Geriatrics, Fujian Medical University Union Hospital, Fuzhou, China
[b] Department of Neurology, Affiliated Sanming First Hospital, Fujian Medical University, Sanming, China
[c] Institute of Neuroscience, Fujian Key Laboratory of Molecular Neurology, Fuzhou, China
[d] Institute of Clinical Neurology, Fujian Medical University, Fuzhou, China

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ABSTRACT

Objective: To explore the influence of disease and genetic factors on the white matter microstructure in patients with PD. The white matter microstructural changes in the substantia nigra-striatum system were detected by diffusion tensor imaging (DTI) using the region of interest (ROI) and diffusion tensor tracer (DTT) methods.

Methods: Patients with primary Parkinson's disease (PD) without a family history of PD were selected and divided into PD-G/G and PD-G/A groups according to their parkin S/N167 polymorphism. Control groups matched for age, sex, and gene type (G/G and G/A) were also included. Three-dimensional brain volume imaging (3D-BRAVO) and DTI were performed. The microstructural changes in the substantia nigra-striatum system were evaluated by the ROI and DTT methods. The Mini-Mental State Examination (MMSE), Montreal Cognitive Assessment (MoCA), Hoehn-Yahr (H–Y) staging, and the third part of the Unified Parkinson's Disease Rating (UPDRS-III) scales evaluated the cognitive and motor function impairment in patients with PD. Independent samples *t*-test compared normally-distributed data, and the Wilcoxon rank sum test compared measurement or categorical non-normally distributed data. Multiple regression analysis was used to analyze the correlation between various DTI indicators and the MMSE, MoCA, UPDRS-III, and H–Y scores in the PD-G/G and PD-G/A groups. $P < 0.05$ was considered statistically significant.

Results: The white matter microstructural changes in the nigrostriatal pathway differed significantly between the PD or PD-G/A and the control group ($P < 0.05$)

The ROI method showed that the left globus pallidus radial diffusivity (RD) value was negatively correlated with the MMSE score ($r = -0.404$, $P = 0.040$), and the left substantia nigra (LSN)

* Corresponding author. Department of Neurology, Fujian Institute of Geriatrics, Fujian Medical University Union Hospital. 29 Xinquan Road, Fuzhou 350001, China

** Corresponding author. Department of Neurology, Affiliated Sanming First Hospital, Fujian Medical University, 29 Liedong Street, Sanming 365000, China.

E-mail addresses: hongweimin1@fjmu.edu.cn (W. Hong), unionqyye8@fjmu.edu.cn (Q. Ye).

[1] These authors contributed equally to this work.

[2] These authors are joint last authors.

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fractional anisotropy (FA) value was positively correlated with the MoCA score (r = 0.405, P = 0.040) and negatively with the H–Y stage (r = −0.479, P = 0.013).
The DTT method showed that the MMSE score was positively correlated with the right substantia nigra (RSN) FA value (r = 0.592, P = 0.001) and negatively with its RD value (r = −0.439, P = 0.025). The H–Y grade was negatively correlated with the number of fibers in the RSN (r = −0.406, P = 0.040). The UPDRS-III score was positively correlated with the mean diffusivity (r = 0.420, P = 0.033) and RD (r = 0.396, P = 0.045) values of the LSN, and the AD value of the RSN (r = 0.439, P = 0.025).
Conclusion: The DTI technique detected extensive white matter fiber damage in patients with PD, primarily in those with the G/A genotype, that led to motor and cognitivesymptoms.

1. Introduction

Parkinson's disease (PD) is a neurodegenerative disorder in middle-aged and older adults. The etiology, prevention, and treatment of PD have been the focus of much research. With the deepening of epidemiological and the rapid development of molecular and genetic research, PD has been widely considered to have complex causes [1], including aging, genetic and environmental factors, and their interactions [2]. With the rapid improvement in genetic testing, over 20 PD-related loci and 19 comprehensive pathogenic genes have been identified thus far, collectively classified as the PARK gene family. Among them, much research is dedicated to the parkin gene (*PARK2*), the primary cause of early-onset familial PD in young adults, which accounts for 10–20% of all cases.[3,4]

Hattori et al. first reported the parkin S/N167 polymorphism in 1998 [5]. Due to a G-to-A mutation in exon 4 of the parkin gene, codon 167 replaces serine (S) with asparagine (N), forming the S/N167 polymorphism. This is one of the most common mutation forms of the parkin gene. Some studies have shown that the S/N167 heterozygous (G/A) genotype might be a risk factor for sporadic PD [6], while a meta-analysis suggested that the effect of G/A genotype on the Western population was uncertain. For Eastern populations, polymorphism at the genotype and allele levels might increase the probability of developing PD, presumably due to the effect of allele A [7]. Environmental factors might contribute to PD development [8]. Recent research found differences between ethnic groups and regions. Therefore, it is of practical significance to study the parkin S/N167 polymorphism in local patients with PD.

Many studies have shown that advanced medical imaging technologies were useful for diagnosing and detecting characteristic changes in PD and have become an indispensable auxiliary examination method for the disease. In the mid-1990s, Bassar et al. [9] developed the diffusion tensor imaging (DTI) technique based on diffusion-weighted imaging (DWI). The water molecules' diffusion degree and direction can be accurately assessed by applying diffusion-sensitive gradient fields in more directions (≥6 directions) to water molecules. The diffusion capacity of water molecules is very sensitive to changes in the structural integrity of white matter fibers, the main white matter component. Therefore, microstructural changes in the white matter can be sensitively and quantitatively identified by measuring changes in the diffusion capacity of water molecules, especially when evaluating white matter changes during the early stage of PD. Current DTI research methods include the region of interest (ROI), voxel-based analysis (VBA), spatial statistics based on fiber tracer (TBSS), and derived fiber tracking technology. The most commonly assessed parameters in DTI are fractional anisotropy (FA), radial diffusivity (RD), axial diffusivity (AD), and mean diffusivity (MD). FA reflects the physical properties of the tissue. Its values, obtained at different times and using different imaging devices, are constant and comparable. Moreover, FA possesses rotational invariance, and its values are insensitive to changes in body position. Additionally, it has a relatively high signal-to-noise ratio, allowing for enhanced gray-white matter contrast and high-quality imaging. Therefore, FA is considered the most critical index in DTI research. The FA values range between 0 and 1, with values closer to 1 indicating better integrity of the white matter fiber bundle structure and values closer to 0 indicating a tendency of the water molecule motions toward isotropy. RD is related to the integrity, structure, and thickness of the myelin sheath; increased RD values suggest damage to the myelin sheath. AD reflects the axons' condition; the AD values decrease when the axon is damaged, or white matter fiber transmission consistency is disrupted. Changes in MD values are related to trends in RD and AD. Larger MD values suggest more free water molecules in the tissue, which is often associated with changes such as tissue degeneration and white matter rarefaction.

This study used the ROI and DTT methods of DTI to investigate the PD disease and gene (parkin S/N167 polymorphism) factors in various patient groups.

2. Methods

2.1. Study subjects

2.1.1. Source and grouping of case

We included patients with primary PD diagnosed between March 2018 and December 2019 b y two senior attending physicians following the 2015 MDS clinical diagnostic criteria for PD [10]. All patients underwent parkin S/N167 nucleotide polymorphism screening and cranial MRI that included 3D-BRAVO and DTI.

The study included 26 patients with PD (14 males and 12 females; 12 with G/G genotype and 14 with G/A genotype; mean age 65.5 ± 6.8 years) and 28 sex-, age-, and genotype-matched controls (12 males and 16 females; 15 with G/G genotype and 13 with G/A genotype; mean age 62.3 ± 6.0 years). Two patients with PD and the A/A genotype were excluded from the study. All patients were of Han Chinese descent.

2.1.2. Inclusion criteria

(1) Diagnosis was based on bradykinesia manifestations and resting tremor and/or myotonia or at least two supporting criteria with no warning signs and no apparent exclusion criteria.
(2) Onset age greater than 50 years with no relevant family history of PD.

2.1.3. Exclusion criteria

(1) History of a definite brain disease, including cerebrovascular disease, trauma, infection, poisoning, or autoimmune diseases, or significant abnormalities detected in intracranial MRI, including significant brain atrophy;
(2) Various secondary parkinsonism or parkinsonism-superimposed syndromes;
(3) Patients with severe dementia, anxiety, or depression;
(4) A history of diabetes, severe hypertension, alcohol use, smoking, and/or occupational exposure to harmful substances such as heavy metals and others that might affect white matter structural integrity.

2.1.4. Informed consent

The Ethics Committee of our hospital approved the study, and all participants or their families signed informed consent forms.

2.2. Clinical assessment

All subjects were clinically evaluated by neurology specialists using Hoehn-Yahr (H–Y) staging. The motor symptoms and cognitive function were assessed by the third part of the Unified Parkinson's Disease Rating Scale (UPDRS-III), Mini-Mental State Examination (MMSE), and Montreal Cognitive Assessment - Changsha Version (MoCA).

2.3. Parkin gene detection

2.3.1. Primary reagents

Venous blood was collected from the elbow of all subjects for parkin gene single-nucleotide polymorphism detection. Prime STAR HS (Premix), purchased from Baori Biotechnology (Beijing) Co., Ltd, was designed specifically for the parkin S/N167 assessment and synthesized by Shanghai Yingweijiki Company.

2.3.2. Sanger sequencing

The prepared reaction mixture (50 μL) contained 1 μL each of forward (10 μM) and reversed (10 μM) primers, 25 μL of Prime STAR HS (Premix), 5 μL of human genomic DNA (5 ng/μL), and 18 μL of ultrapure water. After PCR amplification, the amplified products were sent to Sangon Biotech (Shanghai) Co., Ltd. For Sanger DNA sequencing and gene polymorphism identification. HC-G/G and HC-G/A refer to the G/G and G/A genotype subgroups of the healthy control (HC) group, respectively. PD-G/G and PD-G/A refer to the G/G and G/A genotype subgroups of the PD group, respectively.

2.4. MRI data acquisition

A 3.0 T dual-gradient GE MR scanner was used. The primary sequences and scanning parameters were as follows: high-resolution T1WI brain structural imaging data were collected using a 3D-BRAVO with a repetition time (TR) = 8.7 m s, echo time (TE) = 3.42 m s, flip time (TI) = 400 m s, flip angle = 12°, matrix = 256 × 256, a field of view (FOV) = 240 × 240, number of slices = 180, and slice thickness = 1.1 mm; DTI scans were performed using an echo-planar imaging sequence and brain cross-sectional imaging, with the scanning slices parallel to the anteroposterior commissure line, TR = 6000 m s, TE = 65.7 m s, FA = 90°, matrix = 128 × 128, FOV = 240 × 240, number of slices = 55, slice thickness = 3 mm, continuous scanning without spacing, b values of 0 and 1000 s/mm^2, and 16 nonlinear diffusion-sensitive gradient directions. For patients on medication, MRI data collection was performed at least 12 h after medication discontinuation.

2.5. Image processing

2.5.1. Data preprocessing

2.5.1.1. Data preprocessing converted the scanned 3D-BRAVO T1W1 images and DTI data into the 4D NIFTI format using MRIcron software and its dcm2niigui conversion program. The DTI data gradient encoding file was generated while converting the DTI data to the *.Bvec and *.Bval 4D formats.

2.5.1.2. Data were imported into FSL software (FSL, v5.0.6, oxford university laboratory). The FDT toolkit (FMRIB's Diffusion Correction Toolbox) was used in the following manner: (1) the eddy current extraction command was successively operated to eliminate some of the head movements during FA data scanning and deformations caused by head movements and the eddy current; (2) the BET Brain

Extraction toolkit was used to strip the scalp and skull on the b0 images, removing all tissues except the white matter by setting the threshold to 0.2, and obtaining the mask (Mask); (3) the tensor was calculated following the DTIFIT instructions to generate diffusion tensor maps, and individual FA, RD, AD, and MD maps. FA pseudo-color maps were also generated.

2.5.2. Whole brain fiber reconstruction

The preprocessed data (FA map, Mask, and converted gradient magnetic field direction) were imported into Diffusion Toolkit software (Version 0.6.4.1) for whole-brain fiber tracking, setting the tracking parameters as follows: a threshold angle of 35°, an FA threshold of 0.2 after denudation, and converted gradient along the Z axis (invert Z). A deterministic fiber tracking method (FACT for cellulose extension algorithm) was used to generate files in the dti. trk format after whole brain fiber reconstruction.

2.6. Data analysis

2.6.1. The ROI analysis method

The ROIs in this study were selected using a combination of manual drawing and brain template methods. Nigral ROI selection was made by hand drawing. ROIs in brain regions such as the putamen, globus pallidus, caudate nucleus, and thalamus were marked using the brain template method.

Fiber files of the reconstructed whole brain (dti.trk) were imported into TrackVis software (Version 0.6.1) for whole brain fiber display. The stripped brain dti image was simultaneously imported to facilitate nigral ROI localization. The substantia nigra mask was manually drawn at the midbrain level, where the substantia nigra and red nucleus could be clearly visualized. This process was repeated to reduce errors.

For the brain template method, we first used the parametric statistics mapping (SPM) 12 toolkit in the MATLAB (2013b) platform to obtain the standard spatial positions of the caudate nucleus, putamen, globus pallidus, and thalamus ROIs in the MNI standard space. Subsequently, we used the linear registration toolkit (FLIRT 6.0) in FSL software, combined with T1 images and one-step registration, to obtain better-matched spaces for each subject's spatial ROI, dti. trk, and MNI.

Finally, the FA, MD, AD, RD, and other values of each ROI were obtained using the statistics function in TrackVis software.

2.6.2. The DTT assay method

2.6.2.1. The nigrostriatal pathway. Nigral ROI selection method: the dti. trk file imported into TrackVis software for whole brain fiber display was combined with the DTI image after denudation as the bottom plate. A discoid ROI with a diameter of 3 mm was placed in the ventromedial aspect of the substantia nigra at the horizontal midbrain level, where the substantia nigra and red nucleus could be clearly visualized. This was combined with the FA pseudo-color map with color coding according to the fiber direction, where green represented fibers running anteroposteriorly and posteriorly). The discoid ROI direction was adjusted perpendicularly to the fiber course, slightly lower than the red nucleus level in the coronal view, and appropriately fine-tuned according to the tracking [11]. Striatal ROIs were obtained using the brain template method as above. The fiber tract passing through both ROIs was the dopamine projection fiber of the nigrostriatal pathway. Repeated operations were made to reduce errors.

Finally, we used the TrackVis software statistics function to obtain the fiber number, FA, RD, AD, and MD values of fiber bundles.

2.6.2.2. Neostriato-pallido-thalamic fiber tracts. Fiber tracking was performed after obtaining the ROIs of the neostriatum, globus pallidus, and thalamus using the brain template method. The neostriato-pallido-thalamic fiber bundles pass through the above ROIs. Finally, the various fiber bundle parameters were obtained using TrackVis software.

2.7. Statistical analysis

Demographic and clinical data were statistically analyzed using IBM SPSS Statistics for Windows, Version 25.0 (IBM Corp., Armonk, NY, USA). Measurement data are expressed as means ± standard deviations. Measurement data with normal distribution and equal variance were compared using an independent samples *t*-test. Non-normally distributed measurement or categorical data with uneven variance were compared using the Wilcoxon rank sum test. Multiple regression analysis calculated the correlation between various indicators in the PD and HC groups and the MMSE, MoCA, and UPDRS-III scores, and H–Y grade. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Comparison of demographic and clinical data

Fifty-four subjects were included in this study, 26 in the PD group and 28 in the HC group. Twenty-seven participants were in the G/G group (parkin gene S/N167 G/G) and 27 in the G/A group (parkin gene S/N167 G/A). Sex, age, and the MMSE score were similar between and within groups (Supplementary Table 1).

3.2. ROI analysis results

The left substantia nigra ROI is presented in Fig. 1.

3.2.1. Analysis of the gene-disease interaction

Two-way analysis of variance with simple effect analysis using disease and gene as independent variables was performed to identify interaction effects on the AD value of the right substantia nigra. The results of simple effect analysis indicated that patients with PD in the G/A group had the most significant difference in the AD value of the right substantia nigra than HC-G/A group, suggesting a higher degree of axonal injury (Tables 1 and 22and Supplementary Table 2).

Compared to the HC groups, the PD groups had significantly lower FA values in the substantia nigra and the left caudate nucleus, and higher values in the RD, AD, and MD of the left caudate nucleus, and RD values of the right putamen ($P < 0.05$). These results suggested more severe white matter fiber injury in multiple brain regions in the PD group, including the substantia nigra, caudate nucleus, and putamen.

The G/A group had significantly lower FA values in the right thalamus and higher AD values in the right globus pallidus than the G/G group ($P < 0.05$). Evidently, the G/A group exhibited more severe white matter fiber damage than the G/G group.

3.2.2. Comparisons within the disease and genetic groups

The PD-G/A and PD-G/G groups had similar FA, MD, AD, and RD values. Compared to the HC-G/G group, the HC-G/A group had significantly higher MD, RD, and AD values in the right substantia nigra, RD and AD values in the left putamen, and AD values in the

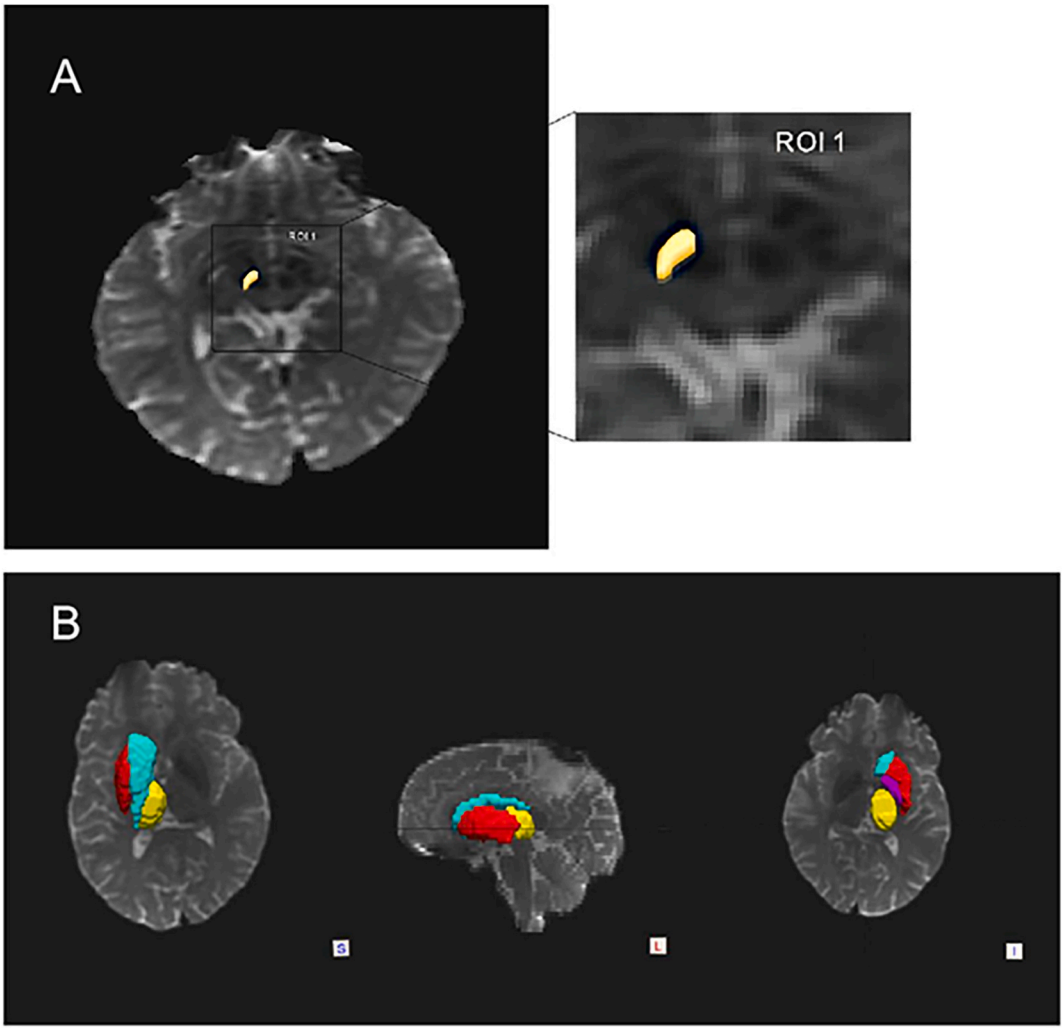

Fig. 1. The left substantia nigra region of interest (ROI) was demarcated using the manual drawing method (A). The right image shows a local magnification. (B) Spatial projection of the basal ganglia nuclei in the left hemisphere. Red, putamen; blue, caudate nucleus; purple, pallidum; yellow, thalamus. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 1
The results of gene-disease interaction analysis.

	FA		MD		RD		AD	
	F	P	F	P	F	P	F	P
Both disease and genetic factors								
RSN	2.995	0.09a	3.168	0.081	1.946	0.169	6.442	* 0.014
HC and PD								
LCN	5.063	* 0.029	5.482	* 0.023	5.671	* 0.021	5.086	* 0.029
RP	1.134	0.292	4.389	* 0.041	4.253	* 0.044	2.782	0.102
LSN	4.375	* 0.042	0.872	0.355	0.183	0.67	1.864	0.178
RSN	4.523	* 0.038	0.199	0.658	0.084	0.773	1.393	0.244
G/G and G/A in PD								
RGP	1.995	0.164	2.102	0.153	0.667	0.418	4.092	* 0.048
RT	4.728	* 0.034	0.057	0.813	0.106	0.746	0.007	0.936

Note: 1.Two-way ANOVA, main effect of gene factor. *P < 0.05 was considered statistically significant,** means POVA, main effect of gen.2.left caudate nucleus(LCN), right caudate nucleus (LCN), left globus pallidus(LGP), right globus pallidus(RGP), left putamen(LP), Right putamen(RP), left thalamus(LT), right thalamus(RT), left substantia nigra(LSN), right substantia nigra(RSN).

Table 2
Simple effect analysis of AD value of right substantia nigra.

Dependent variable: right substantia nigra							
Genetic factor	Disease factors	Disease factors	P	Disease factors	Genetic factor	Genetic factor	P
GG	HC	PD	0.343	HC	GG	GA	0.064
GG	PD	HC	0.343	HC	GA	GG	0.064
GA	HC	PD	* 0.011	PD	GG	GA	0.096
GA	PD	HC	* 0.011	PD	GA	GG	0.096

Note: multiple comparison adjustment: Stark method, with P < 0.05 considered statistically significant.

right putamen ($P < 0.05$). These results suggested more severe white matter fiber damage in the HC-G/A group than in the HC-G/G group.

The PD-G/G group had significantly higher AD values in the right putamen than the HC-G/G group ($P < 0.05$); the groups were similar in all other parameters. Compared to the HC-G/A group, the PD-G/A group had significantly lower FA values in the substantia nigra and higher MD and AD values in the right substantia nigra (Table 3 and Supplementary Table 3).

3.2.3. Correlation analysis between the ROI results and clinical indices in patients with PD

Age positively correlated with the MD, RD, and AD values in the thalamus and putamen. The MMSE scores negatively correlated with the RD values in the left globus pallidus. The MoCA scores positively correlated with the FA values in the left substantia nigra. The H–Y grades negatively correlated with the FA values in the left substantia nigra. No correlation was found between the UPDRS-III scores and the ROI results (Fig. 2 and Supplementary Table 4).

3.3. DTT analysis results

3.3.1. The nigrostriatal pathway

The fibers tracked in this study followed the anatomical route [12].(Fig. 3).

Analysis of variance with disease and gene as independent variables detected no interaction effect. The PD group had significantly lower FA values and higher MD and RD values than the HC group. The G/A group had significantly fewer left fibers, lower FA values,

Table 3
The Positive results of Comparison within different disease or genetic groups.

Comparison results of HC-G/G versus HC-G/A group								
	t/z	P	t/z	P	t/z	P	t/z	P
LP	0.691	0.496a	−1.727	0.084 b	−2.096	*0.036 b	−2.096	*0.036 b
RP	−0.806	0.420 b	−1.175	0.24 b	−0.622	0.534 b	−2.124	*0.043 b
RSN	−0.331	0.743a	−2.464	*0.014 b	−2.418	*0.016 b	−2.557	*0.011 b
Comparison results of HC-G/G versus PD-G/G group								
LP	0	1 b	−1.61	0.107 b	−1.025	0.306 b	−2.231	*0.035a
Comparison results of HC-G/A versus PD-G/A group								
LSN	2.332	*0.028a	−1.068	0.286 b	−0.582	0.56 b	1.532	0.138a
RSN	2.789	*0.01a	−2.087	*0.037 b	−0.825	0.409 b	−3.203	*0.001 b

Note: a: Independent sample *t*-test. b: Mann-Whitney *U* test. *P < 0.05 was considered statistically significant.

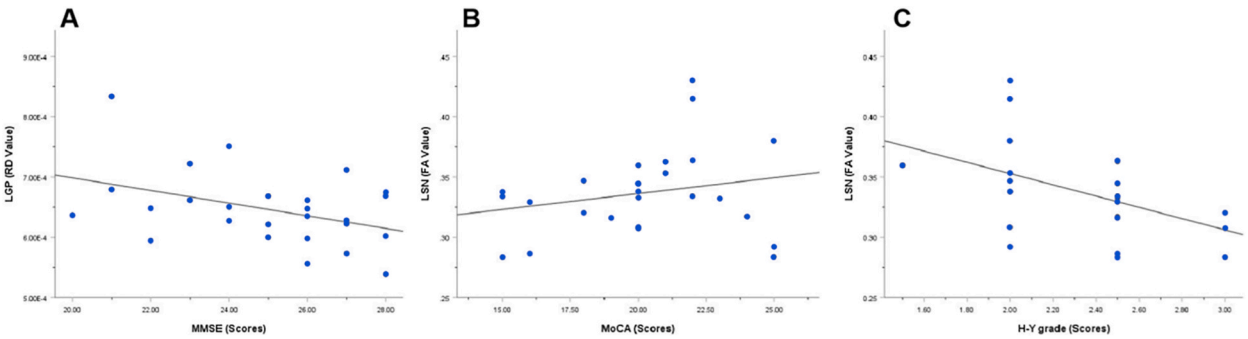

Fig. 2. Correlation analysis results associating ROI method findings and clinical indicators in patients with PD. Presented correlations between the RD values in the left globus pallidus and the MMSE scores (A) and FA values of the left substantia nigra and the MoCA scores (B) or the H–Y grades (C).

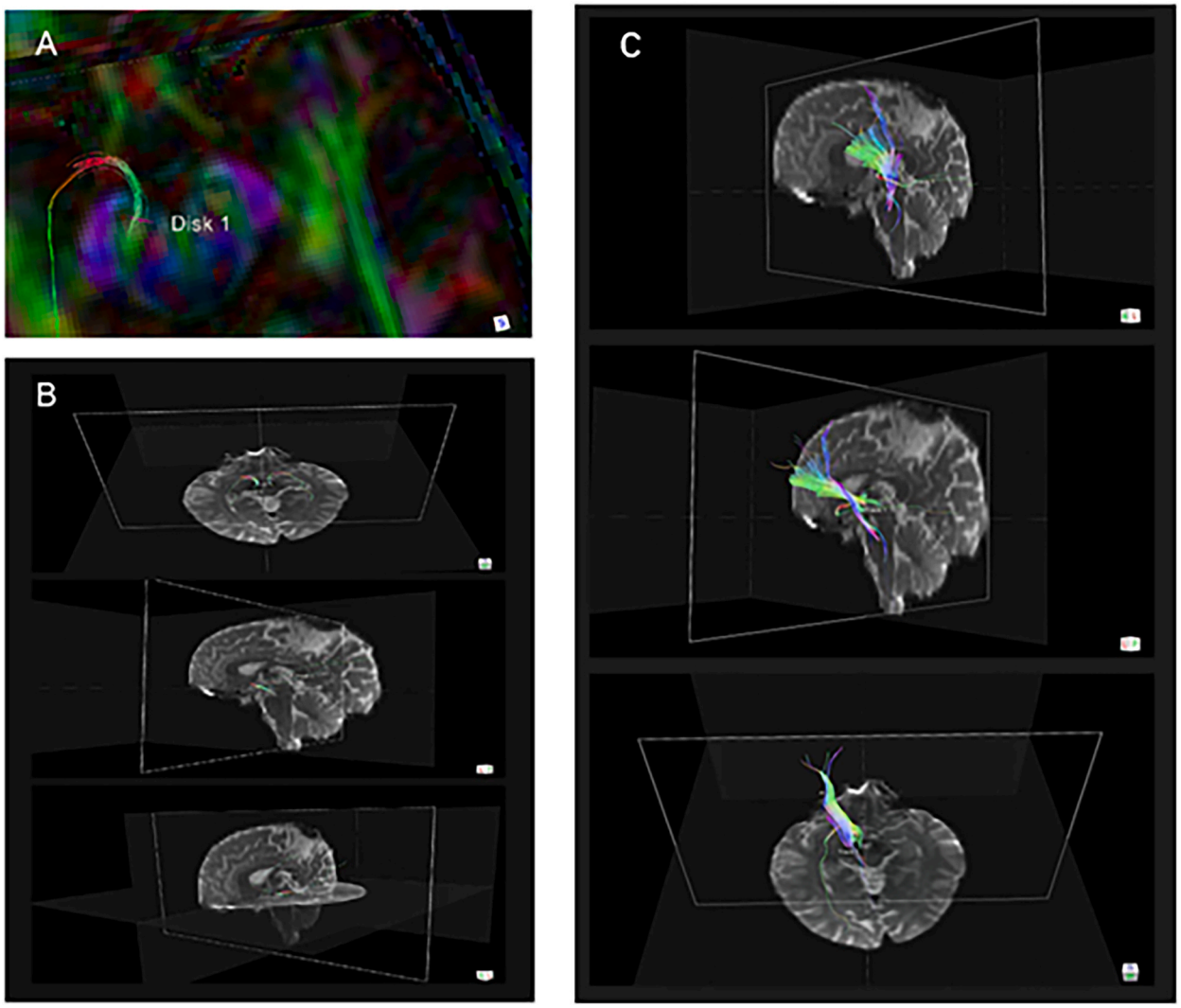

Fig. 3. A diagram of the neostriato-pallido-thalamic fiber pathway The 3D location of the left nigrostriatal dopamine projection fibers on the DTI (A) and FA pseudo-color (B) maps; (C) left neostriato-pallido-thalamic tractography results. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

and higher MD and RD values than the G/G group ($P < 0.05$). These results indicated more severe white matter fiber damage in the PD and G/A groups (Table 4 and Supplementary Table 5). No difference was detected between the HC-G/G and HC-G/A groups or the PD-G/G and PD-G/A groups.

Compared to the HC-G/G group, the RD values on the left side and the MD and RD values on the right side of the nigrostriatal pathway fibers were significantly increased in the PD-G/G group. Compared to the HC-G/A group, the FA value of nigrostriatal pathway fibers was decreased, and the RD value of the right side was increased in the PD-G/A group, with significant differences. These results indicate that there was more severe white matter fiber damage in the PD-G/G and PD- G/A group than in the HC group (Table 5).

3.3.2. The neostriato-pallido-thalamic fiber pathway

A diagram of the neostriato-pallido-thalamic fibers showed that the neostriatum, globus pallidus, and thalamus were anatomically close, and the fiber connection was dense. The fibers extended laterally to both sides, with one end radially connected to the cortex and the other partially connected to the substantia nigra and brainstem (Fig. 3).

The disease factor as the main effect in the analysis of variance indicated significantly lower left FA values and higher MD and RD values in the PD group ($P < 0.05$). These results suggested more severe white matter fiber damage in the PD than in the HC group. The gene factor as the main effect detected significant differences in the FA and RD values on the left side ($P < 0.05$). These results suggested more severe white matter fiber damage in the G/A group (Table 6 and Supplementary Table 6).

FA and higher MD and RD values than the HC-G/G group ($P < 0.05$). These results indicated that within the HC group, the HC-G/A group had more severe white matter fiber damage than the HC-G/G group. No difference was detected between the PD-G/G and PD-G/A groups. The PD-G/G group had significantly lower FA values and higher MD and RD values on the left side than the HC-G/G group. No difference was found between the PD-G/A and HC-G/A groups (Table 7).

3.3.3. Correlation analysis between the DTT results and clinical indices in patients with PD

The MMSE scores positively correlated with the FA values and negatively correlated with the RD values in the right nigrostriatal pathway. The H–Y grades negatively correlated with the number of fibers in the right nigrostriatal pathway. The UPDRS-III scores positively correlated with the MD and RD values in the left nigrostriatal pathway and the AD values in the right (Fig. 4 and Supplementary Table 7).

4. Discussion

We are unaware of reports about DTI studies conducted on parkin S/N167 polymorphism. This study used the ROI and DTT methods to investigate the nigrostriatal pathway and the entire white matter. The genetic imaging approach revealed the characteristics of white matter microstructural damage in patients with PD and evaluated the effects of parkin S/N167 polymorphism on the white matter. By comparing differences in white matter microstructure between the groups, some important imaging markers for early PD diagnosis are provided. This research highlights the importance of genetic imaging for assessing the effect of parkin S/N167 polymorphism in PD.

Consistent with the pathophysiological changes of PD, both ROI and DTT analyses showed that patients in the PD group had more severe white matter fiber damage in multiple nigrostriatal pathway-related brain regions than those in the HC group. Patients with PD first experience degeneration and necrosis of dopaminergic neurons in the substantia nigra, resulting in a gradual decrease in the number of nerve fibers emanating from neuronal axons and fibrous demyelination or gliotic changes. Subsequently, damage to other parts of the nigrostriatal pathway gradually occurs, accompanied by decreased FA values and increased RD and MD values. Previous studies have found that damage to the nigrostriatal pathway precedes the appearance of PD symptoms, so the combined assessment of

Table 4
The positive results of gene-disease interaction analysis and main effect of nigrostriatal pathway.

	main effect of disease				gene main effect			
	Group	Mean value	F	P	Group	Mean value	F	P
Left fiber count	HC	37.86	3.052	0.087	G/G	38.96	4.166	* 0.047
	PD	26.08			G/A	25.41		
FA Left	HC	0.49882329	9.289	* 0.004	G/G	0.49624781	5	* 0.03
	PD	0.47338185			G/A	0.47689959		
MD Left	HC	0.000770052	5.395	* 0.024	G/G	0.000770445	4.722	* 0.035
	PD	0.000801381			G/A	0.000799828		
RD Left	HC	0.000533124	10.111	* 0.003	G/G	0.000543977	2.138	0.15
	PD	0.000582061			G/A	0.000569396		
FA Right	HC	0.49328532	5.551	* 0.022	G/G	0.49099822	2.739	0.104
	PD	0.47388477			G/A	0.47689041		
MD Right	HC	0.000782832	6.955	* 0.011	G/G	0.000786788	2.947	0.092
	PD	0.000812303			G/A	0.000807255		
RD Right	HC	0.000543858	9.619	* 0.003	G/G	0.00054811	4.46	* 0.04
	PD	0.000578808			G/A	0.000573262		

Note: Analysis of variance. *P < 0.05 was considered statistically significant.

Table 5
Comparison of nigrostriatal pathways within different genetic groups.

	Group	t/z	P	Group	t/z	P
Left fiber count	HC-G/G vs PD-G/G	−0.976	0.329 b	HC-G/A vs PD-G/A	−0.85	0.395 b
FA Left		1.896	0.07a		2.401	*0.024a
MD Left		−2.009	0.055a		−1.55	0.134a
RD Left		−2.733	*0.006 b		−1.65	0.099 b
AD left		−0.578	0.569a		−0.528	0.602a
Right fiber count		−0.708	0.479 b		−0.121	0.903 b
FA Right		1.031	0.312a		2.571	*0.016a
MD Right		−2.127	*0.043a		−1.636	0.114a
RD Right		−2.218	*0.036a		−2.168	*0.04a
AD right		−1.355	0.188a		−0.569	0.574a

Note: a: Independent sample *t*-test, b: Mann-Whitney *U* test. *P < 0.05 was considered statistically significant.

Table 6
The positive results of gene-disease interaction analysis and main effect of nigrostriatal pathway.

	Main effect of gene factor				Main effect of disease factor			
	Group	Mean value	F	P	Group	Mean value	F	P
FA Left	G/G	0.48127219	7.254	* 0.01	HC	0.47931286	4.833	* 0.033
	G/A	0.46230004			PD	0.46368038		
MD Left	G/G	0.000777291	2.552	0.116	HC	0.000775275	4.976	* 0.03
	G/A	0.000792618			PD	0.000795379		
RD Left	G/G	0.000549159	6.663	* 0.013	HC	0.000549558	7.11	* 0.01
	G/A	0.000575614			PD	0.000576202		
MD Right	G/G	0.000789014	2.536	0.118	HC	0.000786591	5.35	* 0.025
	G/A	0.000805197			PD	0.000808429		
RD Right	G/G	0.000559806	3.855	0.055	HC	0.000557855	6.757	* 0.012
	G/A	0.000579114			PD	0.000581958		

Note: Analysis of variance. *P < 0.05 was considered statistically significant.

Table 7
Comparison of Neostriatal-pallidal-thalamic cellulose within different genetic groups.

	Group	T	P	Group	T	P
Left fiber count	HC-G/G vs HC-G/A	−0.173	0.864	G/G-HC vs G/G-PD	0.07	0.945
FA Left		2.707	*0.012		2.576	*0.016
MD Left		−1.841	0.077		−2.556	*0.017
RD Left		−2.797	*0.01		−4.311	*0
AD left		0.927	0.362		0.067	0.947
Right fiber count		−0.379	0.708		0.596	0.557
FA Right		2.118	*0.044		1.859	0.075
MD Right		−2.393	*0.024		−2.03	0.053
RD Right		−2.694	*0.012		−2.305	*0.03
AD right		−1.017	0.318		−1.127	0.27

Note: Independent T-test. *P < 0.05 was considered statistically significant.

damage to the nigrostriatal pathway could help in early diagnosis [13,14] and differential diagnosis [15] of PD.

Previous PD-related ROI analysis studies have mostly focused on the substantia nigra region. Yoshikawa et al. [16] compared the FA values of multiple ROIs along the line between the substantia nigra and the lower part of the neostriatum (roughly along the nigrostriatal pathway) between patients with PD and healthy controls and found that the FA values of the substantia nigra decreased most significantly in PD. In mouse model, Boska et al. [17] found damage to the substantia nigra site precedes that to the striatum and other regions. Moreover, decreased FA changes can already be observed at the initial stage of substantia nigra damage. A recent meta-analysis [18] proposed that decreased FA values in the substantia nigra could be used as a diagnostic marker for PD, consistent with the results of this study that objectively demonstrated that the dopaminergic neurons in the substantia nigra were damaged the most severely during PD development. Therefore, the degree of decrease in FA values in the substantia nigra could be used as a reference criterion for the early diagnosis of PD. Most previous studies have also found significant changes in MD values in the substantia nigra of patients with PD [19,20]; however, the change in the MD values found in this study was insignificant. The MD values are related to the overall trend of AD, and RD and MD values reflect the overall degree of water molecules' diffusion. Increased MD values indicate cell degeneration and increased cell membrane permeability, making it suitable to measure the degree of overall damage to cellular tissues. The overall damage to white matter fibers in patients at the early stages of PD is relatively mild, and the disturbance of the white matter microstructural integrity is limited. Such mild changes might show a decrease in the FA values without

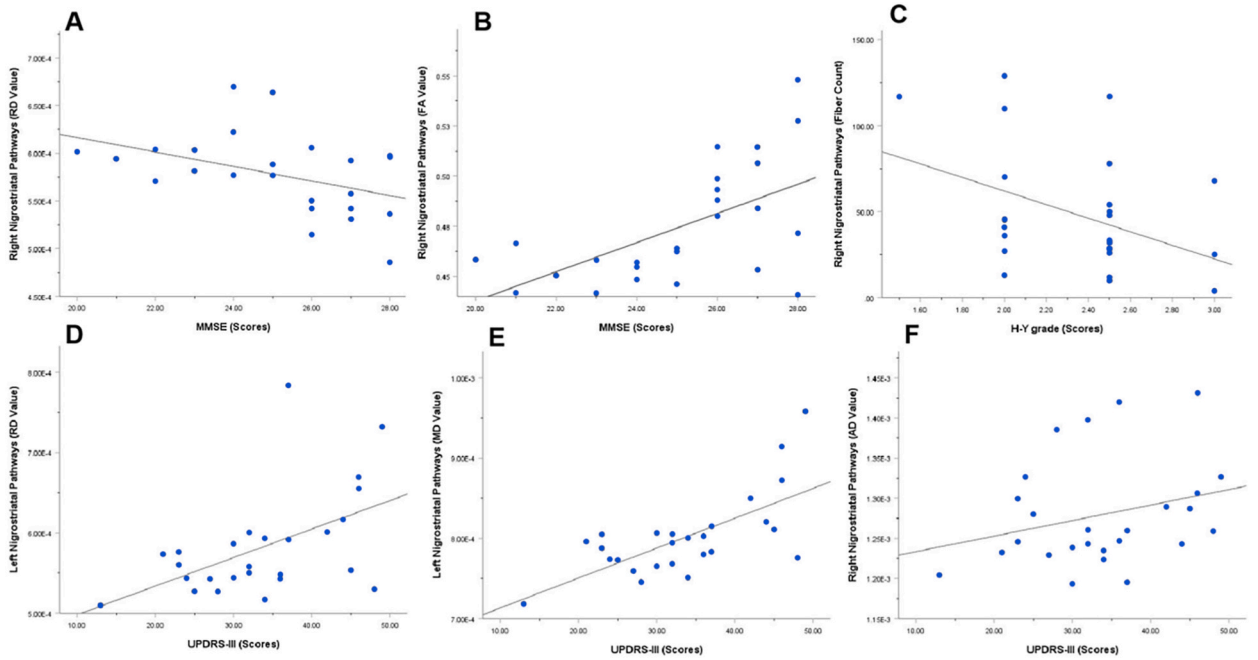

Fig. 4. Correlation analysis results for the associations between clinical indicators in patients with PD and the DTT findings. Correlation between the MMSE scores and the FA (A) and RD (B) values in the right nigrostriatal pathway. Correlation between the H–Y grades and the number of fibers in the right nigrostriatal pathway (C). Correlations between the UPDRS-III scores and the RD (D) and MD (E) values in the left nigrostriatal pathway and the AD values in the right (F).

significant changes in the AD or MD values. Therefore, the role of MD values in early PD diagnosis is less significant than that of FA values. Furthermore, a meta-analysis [21] indicated that changes in the MD values of the substantia nigra are not suitable as imaging markers for PD diagnosis, and no association with PD was confirmed in some patients with altered MD values in this region. Compared to the HC group, DTT analysis found lower FA values and higher AD and MD values in the PD group, indicating microstructural damage to the bilateral fibers in the PD group. The PD group also had fewer fibers than the HC group. Decreased fiber number or destruction of the fiber myelin sheath structure can decrease the FA values, so that they can be used as a reference standard for PD diagnosis. In conclusion, the degree of decrease in the FA values could be used as a reference standard for early PD diagnosis, regardless of whether ROI is used to analyze the parts related to the substantia nigra-striatum system or DTT is used to track and analyze the nigrostriatal pathway; of these, the degree of change in the FA values in the substantia nigra when assessed by the ROI method is of particularly great significance.

As a protective gene, mutations in parkin mostly cause cell destruction. This genetic imaging study of S/N167 polymorphisms revealed more severe white matter microstructural damage in the G/A group than in the G/G group, confirming this view. The ROI analysis found more severe white matter microstructural damage in the right substantia nigra and putamen regions of the HC-G/A group than in the HC-G/G group. Furthermore, the damage in the substantia nigra of the PD-G/A group was more severe than in other sites and that of the HC-G/A group, suggesting that the G/A genotype had a more significant effect on the substantia nigra than other sites. While DTT analysis found no difference between the HC-G/G and HC-G/A or PD-G/G and PD-G/A groups, it found significant differences between the PD-G/G and HC-G/G and PD-G/A and HC-G/A groups. These results indicated that the effect of PD factors on the nigrostriatal pathway was more significant than that of the G/A factors. The difference between these two analyses might be related to the parkin gene distribution. Previous studies have found that the parkin gene was mainly distributed in the substantia nigra and neocortex [22]. Therefore, white matter fiber damage caused by a parkin gene mutation might appear in the substantia nigra earlier than in other regions and be more severe, as confirmed by the ROI analysis. This phenomenon is because the parkin gene is distributed in the substantia nigra more than in other parts of the nigrostriatal pathway, as shown by the DTT analysis. While the G/A genotype causes minor direct damage to the nigrostriatal pathway, it might disrupt the nigrostriatal pathway indirectly by causing damage to the substantia nigra, accelerating the nigrostriatal pathway degeneration rate, and increasing the risk of PD.

The nigrostriatal and cortico-neostriato-pallido-thalamo-cortical (CTC) pathways are extensive fibrous connections between various parts of the extrapyramidal system, which are closely related to the occurrence and development of PD. This study used ROI analysis to compare the putamen, globus pallidus, caudate nucleus, thalamus, etc., while the DTT analysis tracked the neostriato-pallido-thalamic fibers. Both analysis methods found more severe white matter fiber damage in the PD group than in the HC group. The neostriato-pallido-thalamic pathway is part of the CTC. It is divided into direct and indirect pathways according to whether it passes through the subthalamic nucleus. The fiber tracking results primarily reflected the fiber connections from the striatum to the medial pallidum to the thalamus (direct pathway) because the deflection angle threshold selected for fiber tracking was 35°. The direct

pathway is related to motor regulation. Animal experiments found that stimulating the direct pathway in mice improved the gait freezing; activation or inhibition of the direct pathway could regulate the mice's motor ability [23]. DTT analysis found no difference in comparisons involving the nigrostriatal pathway in the HC-G/G group, while a comparison of the neostriatal-pallido-thalamic fibers revealed more severe white matter fiber damage in the HC-G/A group than in the HC-G/G group. The reason for this difference is that, in addition to the substantia nigra, the neostriatal-pallido-thalamic pathway also receives many projection fibers from the neocortex, a brain region with considerable parkin gene distribution. Parkin gene mutations might cause damage to the substantia nigra, neocortex, and the white matter fiber tracts associated with them. The difference between the PD-G/G and HC-G/G groups was more significant than that between the PD-G/A and HC-G/A groups, which might be because PD factors masked the influencing effect of the G/A genotype.

ROI analysis detected an association between the nigrostriatal pathway or globus pallidus and impaired cognitive function, while substantia nigra damage affected the motor function of the patients. Previous studies also found that the H–Y grades were negatively correlated with the FA values in the left substantia nigra [24]. DTI analysis suggested that the nigrostriatal pathway destruction was closely related to the decline in motor and cognitive function [25]. A PET study alsofound that patients with PD but no cognitive impairment exhibited reduced levels of dopamine release in the dorsal caudate nucleus when performing spatial-related actions than the normal group [26]. It has been suggested that the nigrostriatal pathway can influence cognitive performance by modulating the electrical activity of the ventral pallidum. As an important part of the cortico-basal ganglia circuit, the globus pallidus is closely related to the cerebral cortex, thalamic network, and the limbic system, and therefore, to executive ability, language function, memory ability, emotional perception, and other cognitive functions [27], consistent with this study. The nigrostriatal pathway, an important component of the extrapyramidal motor nerve conduction pathway, is also the site of early damage in PD. This damage is closely related to motor function impairment. Therefore, we applied the DTT technique to assess the nigrostriatal pathway, which indirectly reflects the degree of damage to the motor and cognitive functions and the evaluation index used for prognosis assessment. Due to the lack of specific biological or imaging findings for PD diagnosis, physicians rely on clinical manifestations and their experience. However, misdiagnosis or missed diagnosis is likely to occur in early PD due to the striatal function compensatory mechanism; the typical dyskinesia is manifested only after over 50% of the dopaminergic neurons in the substantia nigra are damaged, and dopamine content in the striatum dropped by over 80% [28]. The timing, profile, and rate of cognitive decline vary greatly among individuals with PD, making it hard to predict which patients are at increased risk of early and rapid cognitive decline. Early diagnosis can ensure neuroprotective measures are taken and targeted treatment is administered as early as possible, thereby delaying the progression of PD and the development of PD-related cognitive impairment and improving the patients' quality of life. Early diagnosis is also important for a better prognosis and clinical management. Therefore, exploring specific biological or imaging markers of early PD is important.

The limitation of the ROI and DTT methods is that they can only detect regional changes, while they cannot reveal changes in the entire brain. In the absence of a reasonable predictor for the lesion site, or deviation between the selected ROI position and the actual spatial position, results might be false negative or false positive. However, it is undeniable that the ROI and DTT methods can independently select the brain regions to study, an unmatched advantage over other methods used to study specific brain regions and evaluate the damage to the substantia nigra and nigrostriatal pathways. Our findings can provide a reference for early PD diagnosis and disease evaluation.

5. Conclusions

This study evaluated the damage to the nigrostriatal pathway and characterized global white matter injury comprehensively in patients with PD. By comparing images of patients with the S/N167 G/A heterozygous variant of the parkin gene with other genotypes and phenotypes, we confirmed by imaging that this mutation was associated with greater white matter fiber damage and PD. The ROI method found that white matter fiber damage was prevalent in multiple brain regions of the nigrostriatal pathway in patients with PD, with substantia nigra damage being the most significant. The G/A genotype was associated with more severe damage in the substantia nigra, somewhat increasing the risk of PD occurrence. Using the DTT method, we found that patients with PD had severe white matter fiber damage in the nigrostriatal pathway. The damage was characterized by changes in diffusion parameters such as FA and a decrease in the number of fibers. The damage to fiber tracts in the nigrostriatal pathway of patients with the G/A genotype might occur indirectly through disruption of the substantia nigra. The neostriato-pallido-thalamic fiber bundles in patients with PD also presented severe white matter fiber damage. This was particularly apparent in patients with the G/A genotype, suggesting possible involvement of the parkin gene mutation, which also destroys the neocortex and the white matter fibers projecting from it. Measuring changes in the FA values in the nigrostriatal pathway, particularly in the substantia nigra region, could help in early PD diagnosis.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e18395.

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