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ORIGINAL RESEARCH

The Correlation Between RIN3 Gene Methylation and Cognitive Impairment in Parkinson's Disease

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Background: Parkinson's disease (PD) is the second most common neurodegenerative disorder, after Alzheimer's disease. Many individuals with PD experience cognitive impairment, significantly threatening both their physical and mental well-being. Research has shown that abnormal DNA methylation is closely linked to neurodegenerative conditions such as Alzheimer's and Parkinson's disease. The RIN3 gene, which encodes a guanine nucleotide exchange factor, plays a role in inhibiting amyloid-beta formation and affects protein endocytosis, both of which are linked to cognitive impairment. However, the potential connection between RIN3 gene methylation and cognitive impairment in Parkinson's disease has not yet been explored. This study aims to explore whether the methylation status of the RIN3 gene is connected to cognitive decline in Parkinson's patients, thereby shedding light on the gene's crucial role in the disease's development and identifying potential targets for diagnosing and treating cognitive impairment in this context.

Purpose: This study aims to explore whether the methylation status of the RIN3 gene is associated with cognitive impairment in Parkinson's disease and to further clarify the gene's significant role in the disease's pathogenesis.

Methods: This study involved 50 control subjects and 51 Parkinson's disease (PD) patients, who were assessed using a cognitive scale. Additionally, DNA methylation in whole blood was analyzed. The research compared RIN3 methylation levels between the PD group and the normal control group (NC), as well as between the subgroups of PD-Mild Cognitive Impairment (PD-MCI), PD-Normal Cognition (PD-NC), and the control group.

Results: The DNA methylation level of the RIN3 gene in the whole blood of patients with PD was lower than that in healthy controls (22.3%vs.23.6%, P=0.009). Moreover, individuals with PD-MCI had significantly lower RIN3 methylation levels than both the control group (21.3%vs.23.6%, P<0.001) and those in the PD-NC group (21.3%vs.23.3%, P=0.001).

Conclusion: RIN3 methylation is associated with PD-MCI. With appropriate lifestyle changes and clinical interventions, methylation may influence disease progression, suggesting that RIN3 gene methylation could serve as a predictor for the development of PD-MCI.

Keywords: Parkinson's disease, cognitive impairment, DNA methylation, RIN3 gene, amyloid β protein

Introduction

Parkinson's disease (PD) is a neurodegenerative disorder primarily affecting middle-aged and older adults, with its incidence ranking just below that of Alzheimer's disease.[1–4] At present, the precise causes and mechanisms underlying Parkinson's disease remain unclear. It is characterized by the degeneration of a significant number of dopaminergic neurons in the substantia nigra, along with the presence of remaining neuronal cell bodies, such as Lewy bodies, which are aggregates of alpha-synuclein protein.[5] Previous studies have shown that aging, environmental pollution, and genetics may be contributing factors to Parkinson's disease, causing the degeneration of dopaminergic neurons in the midbrain and a decrease in dopamine levels in the striatum, which ultimately leads to the onset of clinical symptoms. Despite these insights, the precise etiology of Parkinson's disease is still unknown, and effective treatments remain elusive.[6–9]

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The clinical symptoms of Parkinson's disease can be grouped into motor and non-motor symptoms, and cognitive impairment is a frequent non-motor problem among patients.[10,11] Cognitive dysfunction in Parkinson's disease is generally categorized into two primary forms: mild cognitive impairment (PD-MCI) and Parkinson's disease dementia (PDD). PD-MCI describes a syndrome in which patients report a subjective decline in cognitive abilities, noted by caregivers or observed by clinicians, and this decline is confirmed by neuropsychological testing. However, it does not significantly impact the patient's ability to live independently, and the severity of cognitive impairment does not meet the criteria for PDD.[12] In contrast, PDD is characterized by a gradual decline in cognitive function that becomes apparent about a year after the onset of Parkinson's disease, eventually resulting in considerable difficulties for the patient in carrying out daily tasks independently. Epidemiological research suggests that up to 40% of individuals with Parkinson's disease may experience mild cognitive impairment (PD-MCI), with a 30% incidence rate among newly diagnosed PD patients.[13] Studies have shown that PD-MCI serves as a distinct risk factor for the development of Parkinson's disease dementia (PDD).[14] As cognitive decline advances to PDD, it significantly impacts patients' social functioning and quality of life, highlighting the critical importance of early diagnosis and intervention for PD-MCI.[15–17] In contrast to the well-established treatments for the motor symptoms of Parkinson's disease, effective therapies for cognitive dysfunction remain unclear. At present, there is a notable absence of large-scale, randomized, double-blind controlled trials specifically investigating the treatment of PDD and PD-MCI. The cholinesterase inhibitor cabalatine is the only medication that has demonstrated effectiveness for cognitive impairment in PDD,[18,19] however, reliable data on both pharmacological and non-pharmacological treatments for PD-MCI remain limited. Consequently, there is a pressing need to identify new biomarkers and therapeutic targets.

DNA methylation modification serves as a vital epigenetic marker that significantly influences gene expression, chromatin stability, and various essential biological processes. Variations in methylation, known as methylation polymorphism, contribute to individual phenotypic differences and can also result in phenotypic abnormalities. Detecting methylation patterns is crucial for advancing research in biology and translational medicine. Previous studies have established a strong correlation between abnormal methylation patterns and neurodegenerative diseases, such as Alzheimer's disease (AD) and PD.[20] In PD, gene expression may be influenced by either hypermethylation or hypomethylation. Hypermethylation generally suppresses gene expression and primarily affects CpG islands within gene promoters, low methylation, on the other hand, typically promotes gene expression and is often associated with CpG sites located outside of the CpG islands. The RIN3 (Ras and Rab Interactor 3) gene is a member of the RIN family, found on human chromosome 14, encoding a guanine nucleotide exchange factor that plays a role in stabilizing the transport of GTP-rab5 from the plasma membrane to endosomes, which is crucial for cellular endocytosis.[21] This process negatively impacts the endocytic uptake of β-amyloid protein (Aβ).[22–24] Studies have shown that the RIN3 gene is significantly upregulated in AD,[25] with patients showing low methylation levels of this gene in whole blood samples.[26]

In conclusion, we hypothesize that the methylation status of the RIN3 gene may be linked to cognitive impairment in Parkinson's disease. Currently, there is no research investigating the link between RIN3 gene methylation and cognitive deficits in this context. This study aims to explore whether the methylation status of the RIN3 gene is associated with cognitive impairment in Parkinson's disease and to further clarify the gene's significant role in the disease's pathogenesis. Ultimately, this could enhance strategies for preventing and treating cognitive impairment in Parkinson's disease.

Patients and Methods

Participants

In this study, we enrolled 51 patients with Parkinson's disease (PD) and 50 control participants, matched for gender and age. The PD patients were recruited from the inpatient unit of the Department of Neurology at Qingdao Central Hospital, University of Health and Rehabilitation Sciences, between January 2022 and February 2023. Among the patients, 24

were diagnosed with Parkinson's disease mild cognitive impairment (PD-MCI), while 27 were classified as Parkinson's disease with normal cognition (PD-NC). All participants were of Han Chinese descent.

The inclusion criteria for patients with Parkinson's disease (PD) were as follows: all participants met the diagnostic criteria established by the International Society for Parkinson's and Movement Disorders (MDS),[27] confirmed by at least two neurologists with expertise in movement disorders. Additionally, participants were required to be between the ages of 40 and 75. The control group was selected from the Health Examination Center of Qingdao Central Hospital, University of Health and Rehabilitation Sciences.

The exclusion criteria for patients with Parkinson's disease (PD) included: (1) dementia related to Parkinson's disease, vascular dementia, or other neurodegenerative disorders such as Alzheimer's disease and dementia with Lewy bodies; (2) a revised Hoehn and Yahr (H-Y) score greater than III; (3) severe systemic or psychological illnesses; (4) contraindications identified through magnetic resonance imaging; and (5) secondary parkinsonism or parkinsonism-plus syndromes, including multiple system atrophy, progressive supranuclear palsy, corticobasal degeneration, and frontotemporal dementia.

Fifty healthy controls, matched for age and sex, were recruited. The exclusion criteria included: (1) the presence of mild cognitive impairment (MCI), (2) depression or dysthymic disorder (as defined in the American Psychiatric Association's DSM-IV), (3) significant neurological or systemic diseases, and (4) contraindications to MRI scanning. This study was approved by the Ethics Committee of Qingdao Central Hospital, University of Health and Rehabilitation Sciences, and was conducted in compliance with the principles outlined in the Helsinki Declaration. All patients provided written informed consent. For each participant, demographic and clinical data were collected, including demographic details, Hoehn and Yahr (H&Y) stage, Unified Parkinson's Disease Rating Scale (UPDRS) scores, Non-Motor Symptoms Questionnaire for Parkinson's Disease, Hamilton Anxiety Scale (HAMA), Hamilton Depression Scale (HAMD), and Montreal Cognitive Assessment (MoCA). The Montreal Cognitive Assessment (MoCA) and Mini Mental State Examination (MMSE) were used to assess the overall cognitive abilities of all participants. The cut-off point for MMSE is: illiteracy>17, primary education>20, and middle and higher education>24.

Parkinson's disease mild cognitive impairment (PD-MCI) was diagnosed based on the Level 1 diagnostic criteria established by the Movement Disorder Society Task Force, which does not classify PD-MCI into subtypes. PD-MCI is characterized by either subjective or objective cognitive impairment, indicated by a Montreal Cognitive Assessment (MoCA) score of less than 26.[28] In contrast, Parkinson's disease with normal cognition (PD-CN) was defined as having a MoCA score of 26 or higher, along with no reported difficulties in activities of daily living (ADL).

DNA Extraction and Quality Control

All participants provided approximately 4 mL of peripheral venous blood in the early morning, which was collected in an ethylenediaminetetraacetic acid (EDTA) anticoagulant tube. The samples were then centrifuged at a radius of 12 cm and a speed of 2500 rpm for 10 minutes. The lower layer cells were collected for DNA extraction and stored at −80 °C for future use. The sample specifications required the genomic DNA to be intact and free from contamination. DNA quality was evaluated using a Nanodrop 2000 (NanoDrop Technologies, Wilmington, DE, USA), with a minimum concentration of $\geq$ 20 ng/μL and a total quantity of at least 400 ng. Sample purity standards were set at OD260/280 = 1.7 to 1.9 and OD260/230 $\geq$ 2.0. Integrity was verified by agarose gel electrophoresis, which showed clear main bands without significant dispersion or tailing.

Methylation Sequencing

DNA methylation analysis was carried out by Genesky Biotechnologies Inc. (Shanghai, China) utilizing MethylTarget sequencing technology, which is based on second-generation sequencing and allows for precise measurement of methylation levels at each CpG site. The EZ DNA Methylation™-GOLD Kit (ZYMO, CA, USA) was used to perform multiple bisulfite treatments on the extracted DNA. GeneCpG software was then employed to analyze the genome of the targeted region alongside the bisulfite-treated sequences. The PCR primers for RIN3_24 were: forward, 5′-

GAAGAGGTTGGTTTTGGTGGT-3′; reverse, 5′-CAACCCCAACCACAACCT-3′. For RIN3_25, the primers were: forward, 5′-TGGGAATTGATTTGGAGAGGA-3′; reverse, 5′-AAAACTAAAAATAACCTCCCCAAAC-3′. Following multiple rounds of PCR amplification (using the HotStarTaq polymerase kit from TaKaRa, Dalian, China) and the addition of specific tag sequences to the samples, with high-throughput sequencing conducted on the Illumina HiSeq platform (Illumina, CA, USA).

Statistical Methods

All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS, version 26.0, Chicago, IL, USA) for Windows. Continuous data are expressed as mean ± standard deviation (SD). For comparisons of categorical data between the normal control (NC) and Parkinson's disease (PD) groups, as well as among different PD subgroups and NC, the chi-square test (χ2-test) or Fisher's exact test was used. For normally distributed data, t-tests or one-way analysis of variance (ANOVA) were applied. For non-normally distributed continuous variables, non-parametric tests, such as the Mann–Whitney *U*-test or Kruskal–Wallis test, were used. In cases where significant differences were observed between PD subgroups and NC or among the PD subgroups, logistic regression (using SPSS 26.0) was employed to adjust for potential confounding factors. A p-value of less than 0.05 was considered statistically significant for all analyses.

Results

In this study, we investigated RIN3 methylation in whole blood samples from 101 participants (Figure 1). This included 51 Parkinson's disease (PD) patients in the case group (P group), which was further divided into 24 patients with cognitive impairment (PD-MCI group) and 27 patients without cognitive impairment (PD-NC group). The control group (NC group) consisted of 50 healthy participants. The clinical characteristics and RIN3 methylation levels of the PD groups (PD-MCI and PD-NC) as well as the NC group are presented in Tables 1 and 2.

A significant difference was observed in the overall RIN3 methylation levels between the 51 PD patients and 50 healthy controls (Table 3). The results showed that the total methylation level of the RIN3 gene was notably lower in the PD group compared to the control group (22.3%vs.23.6%, group difference = −0.013, P = 0.009) (Figure 2). This difference remained significant after adjusting for age and gender (adjusted P = 0.013) (Table 4).

The RIN3 methylation level in the PD-MCI group (n=24) was significantly lower than in the PD-NC group (n=27) (21.3%vs.23.3%, group difference = −0.02, P = 0.001) (Figure 3). This difference remained statistically significant even after adjusting for age and gender (adjusted P = 0.004) (Table 5).

The RIN3 methylation level in the PD-MCI group (n=24) was significantly lower than that in the NC group (n=50) (21.3%vs.23.6%, group difference = −0.023, P < 0.001) (Figure 4). This difference remained significant after adjusting for age and gender (adjusted P = 0.001) (Table 6).

No significant difference in RIN3 methylation levels was found between the PD-NC group (n=27) and the NC group (n=50) (23.3%vs.23.6%, group difference = −0.0035, P = 0.528) (Figure 5). After adjusting for age and gender, the difference was still not statistically significant (adjusted P=0.54) (Table 7).

There were no significant differences in gender, age, or education level between the PD-MCI group and the control group. However, significant differences were observed in MMSE, MoCA, HAMA, and HAMD scores between the two groups. The PD-MCI group had significantly lower MMSE and MoCA scores compared to the control group, while their HAMA and HAMD scores were significantly higher (Table 1).

When comparing the PD-MCI group with the PD-NC group, the PD-MCI group had significantly higher UPDRS-III scores (P = 0.02) and H&Y stages (P = 0.01), but significantly lower MMSE (P = 0.000) and MoCA scores (P = 0.000) (Table 2).

There were no significant differences in gender, age, education level, or disease duration between the PD-MCI and PD-NC groups. Additionally, no significant differences in MMSE and MoCA scores were found between the PD-NC and NC groups (P > 0.05). However, significant differences in MMSE and MoCA scores were observed between the PD-MCI group and both the PD-NC and NC groups (P < 0.05). Significant differences in HAMA and HAMD scores were also found between the PD-MCI group and the NC group, as well as between the PD-NC group and the NC group (P < 0.05).

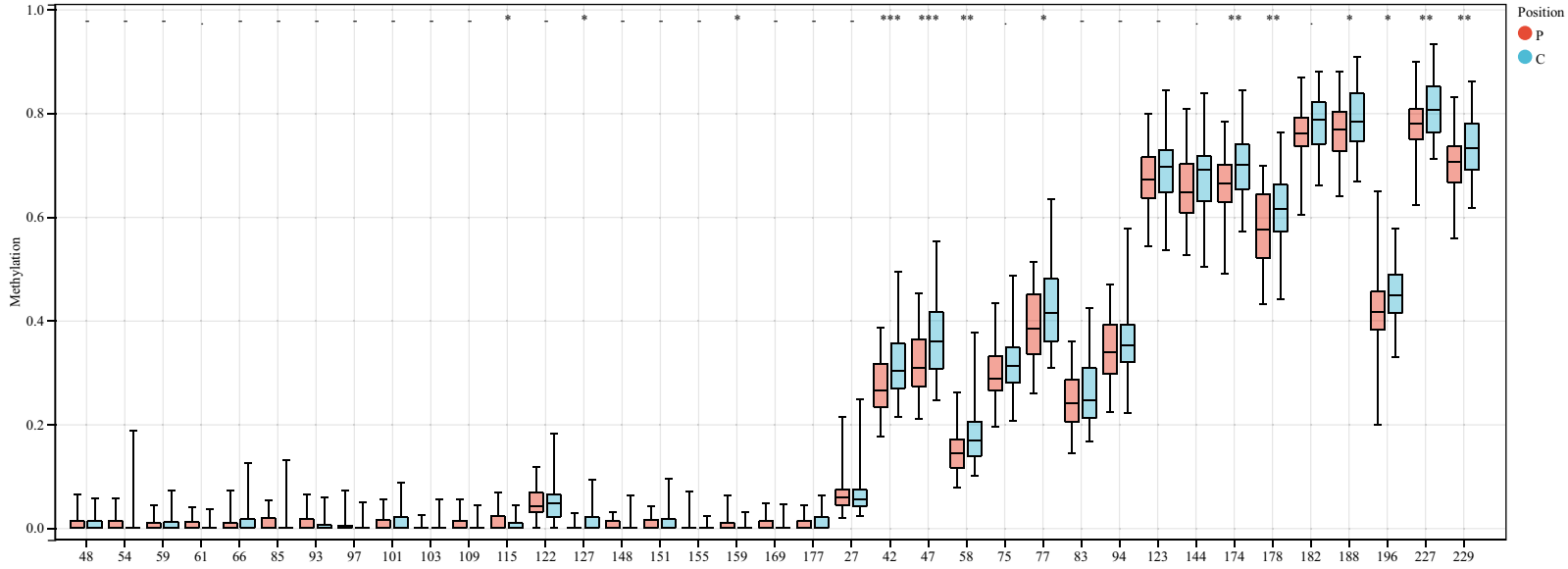

Figure 1 Compares methylation levels at measured sites in the case group (group (P)) and control group (group (C)), with the X-axis indicating the detected site and the Y-axis indicating the methylation level at each site. Students *t*-test was used to compare the groups. *refers to P<0.05, **refers to P<0.01, ***refers to P<0.001.

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Table 1 Clinical Characteristics and Levels of RIN3 Methylation of Parkinson's Disease Patients and Controls

	PD	NC	P value
Number	51	50	
Gender (male/female)[a]	28/23	26/24	0.843
Age (years)[b]	61.41±7.51	62.96±6.28	0.264
Education(years)[b]	9.35±2.89	9.32±2.19	0.949
Disease duration(months)	37.53±9.35	NA	NA
UPDRS-III	25.12±7.12	NA	NA
H&Y stage	2.17±0.55	NA	NA
MMSE score[c]	26.8±1.76	28.52±0.93	0.000
MoCA score[c]	24.27±3.07	27.08±1.12	0.000
HAMA score[c]	9.29±3.25	2.62±1.32	0.000
HAMD score[c]	9.84±3.43	3.82±1.44	0.000
Mean methylation level (%)[d]	22.31±2.29	23.60±2.57	0.009

Notes: [a]*P*-value were calculated using chi-square (χ^2) test; [b]*P*- values were calculated using unpaired *t*-test; [c]*P*-values were calculated using Kruskal–Wallis test; [d]*P*- values were calculated using Mann–Whitney *U*-test.

Abbreviations: PD, Parkinson's disease; NC, normal controls; H&Y stage, Hoehn and Yahr stage; UPDRS, Unified Parkinson's Disease Rating Scale; MMSE, Mini-mental State Examination, MoCA, Montreal Cognitive Assessment; HAMA, Hamilton Anxiety Scale; HAMD, Hamilton Rating Scale for Depression.

Table 2 Clinical Characteristics and Levels of RIN3 Methylation in PD-MCI, PD-NC and NC Group

	PD-MCI	PD-NC	NC		P value	
				PD-MCI vs.NC	PD-NC vs.NC	PD-MCI vs. PD-NC
Number	24	27	50			
Gender (male/female)[a]	14/10	14/13	26/24	0.809	1	0.78
Age (years)[b]	63.33±6.11	59.70±8.30	62.96±6.28	0.81	0.057	0.085
Education (years)	8.67±2.16	9.96±3.33	9.32±2.19	0.231	0.312	0.111
Disease duration (months)	39.63±9.54	34.85±8.37	NA	NA	NA	0.063
UPDRS-III[c]	27.54±7.52	22.96±6.09	NA	NA	NA	0.020
H&Y stage[c]	2.38±0.56	1.98±0.49	NA	NA	NA	0.010
MMSE score[d]	25.46±1.18	28.19±0.74	28.52±0.93	0.000	0.111	0.000
MoCA score[d]	24.27±3.07	26.81±0.92	27.08±1.12	0.000	0.297	0.000
HAMA score[d]	9.29±3.25	8.78±3.23	2.62±1.32	0.000	0.000	0.232
HAMD score[d]	9.84±3.43	9.33±3.33	3.82±1.44	0.000	0.000	0.265
Mean methylation level (%)[c]	21.26±2.0	23.25±2.13	23.60±2.57	0.000	0.550	0.001

Notes: [a]*P*-value were calculated using chi-square (χ^2) test, [b]*P*- values were calculated using unpaired *t*-test; [c]*P*- values were calculated using Mann–Whitney *U*-test; [d]*P*-values were calculated using Kruskal–Wallis test.

Abbreviations: PD, Parkinson's disease; NC, normal controls; PD-MCI, PD patients with mild cognitive impairment; PD-NC, PD patients with cognitively normal; NA, non-available; F, female; M, male; H&Y stage, Hoehn and Yahr stage; UPDRS, Unified Parkinson's Disease Rating Scale; MMSE, Mini-mental State Examination, MoCA, Montreal Cognitive Assessment; HAMA, Hamilton Anxiety Scale; HAMD, Hamilton Rating Scale for Depression.

Table 3 Information on Target DNA Methylation Sequencing

Target	Chr	Gene	mRNA	mRNA Strand	TSS	TES	Start	End	Length	Target Strand	Distance2TSS
RIN3-24	14	RIN3	NM_024832	+	92,513,780	92,688,994	92,513,748	92,513,546	203	-	−32
RIN3-25	14	RIN3	NM_024832	+	92,513,780	92,688,994	92,514,655	92,514,908	254	+	875

Abbreviations: Chr, chromosome; mRNA, mRNA closer to the product; mRNA strand, mRNA direction; TSS, transcription start site of mRNA; TES, transcriptional end site of mRNA; Start, starting position of the product on reference genomes; End, ending position of the product on reference genomes; Length, length of the product; Target strand, direction of the product; Distance2TSS, relative distance between product and TSS; a negative sign indicates that the site is upwards TSS.

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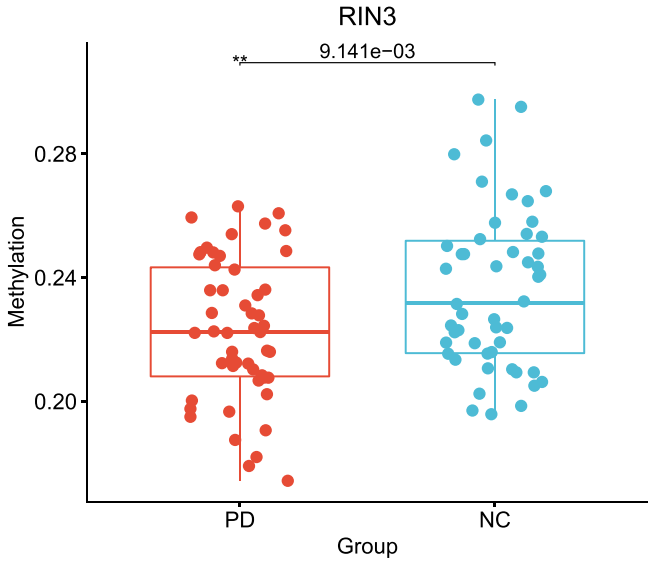

Figure 2 Shows the difference in RIN3 methylation levels between the case group (group PD) and the control group (group NC). The RIN3 gene was relatively hypomethylated in group PD. Students *t*-test was used to compare the groups. **9.141e-03 refers to P=0.009141.

Discussion

This study indicates that low methylation of the RIN3 gene is associated with PD and PD-MCI. Research on the relationship between PD-MCI and gene methylation is currently scarce, which prompted the initiation of this study. The MMSE and MoCA are commonly used cognitive assessment scales in clinical practice, known for their sensitivity in screening mild cognitive impairment.[28–31] The MMSE includes five components: memory, orientation, attention and calculation, recall, and language ability, with a total score range of 0–30 points. Normal scores are defined as >17 for illiterate individuals, >20 for those with middle school education, and >24 for those with high school or higher education. The MoCA assesses various domains, including language, visuospatial abilities and orientation, attention and executive function, and memory. It also has a total score of 0–30 points, with a normal value of 26 or above. PD-MCI is defined as subjective or objective cognitive impairment with a MoCA score of <26, while PD with normal cognition (PD-NC) is defined as a MoCA score of ≥26.[12]

In this study, we observed that the incidence of PD-MCI was 47.1% (24/51), which aligns with findings reported in other studies.[32–34] DNA methylation, an essential epigenetic marker, plays a key role in regulating gene expression, maintaining chromatin stability, and other fundamental biological processes.[35] In PD, gene expression can occur in two forms: hypermethylation and hypomethylation. Hypermethylation typically suppresses gene expression and affects CpG islands in gene promoters, while hypomethylation promotes gene expression and often involves CpG sites outside of

Table 4 Differences in RIN3 Methylation Levels Between Parkinson's Disease Patients and Healthy Controls

Gene	*P* value	Groupdiff	Adj. *P* value
RIN3	0.009	−0.013	0.013

Note: Students *t*-test was used to compare the groups.
Abbreviations: Gene, gene name; Groupdiff, difference in mean methylation between the case and control groups; Adj. P-value, P-value after adjusting for age and gender.

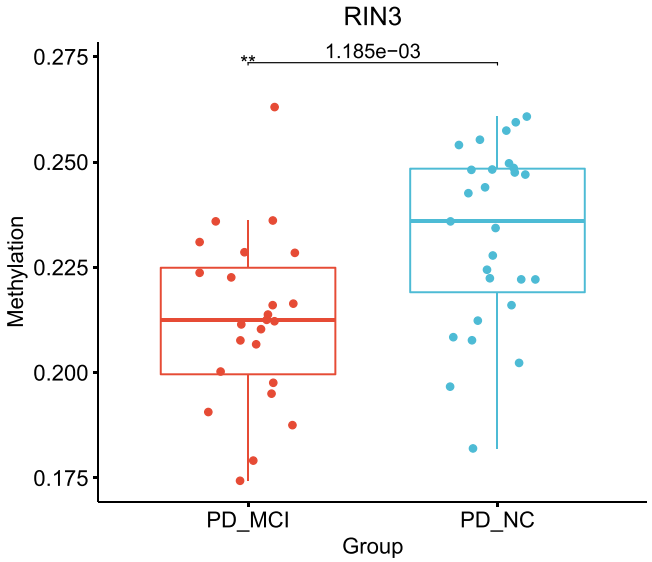

Figure 3 Compares the RIN3 methylation levels between the group with cognitive impairment after PD-MCI and the group without cognitive impairment after PD-NC. The RIN3 gene was hypomethylated in the former. Students *t*-test was used to compare the groups. **1.185e-03 refers to P=0.001185.

CpG islands. We examined the association between RIN3 methylation and PD/PD-MCI. Our findings indicate that, compared to the healthy control group, the RIN3 gene is hypomethylated in the whole blood of patients with PD/PD-MCI.

Shen et al demonstrated that elevated RIN3 expression levels could impact its transport function, potentially resulting in an accumulation of alpha-beta proteins within neurons.[25] Similarly, Boden reported that early-onset Alzheimer's disease (AD) patients exhibit low methylation levels of the RIN3 gene in whole blood, leading to increased RIN3 expression.[26] Furthermore, another study confirmed that abnormal methylation of the RIN3 gene and its loci is linked to early cognitive impairment following transient ischemic attacks (TIA) or minor strokes.[24] Based on this evidence, we hypothesize that patients with Parkinson's disease (PD) or PD-related mild cognitive impairment (PD-MCI) may also show aberrant methylation of the RIN3 gene. Given that methylation levels are influenced by environmental factors and can be quantitatively measured, they hold promise as key components in developing predictive models and potential intervention strategies.

Previous research has indicated that Aβ42 levels in the cerebrospinal fluid of patients with Alzheimer's disease (AD) and vascular dementia (VAD) are significantly lower compared to the control group.[36] Additionally, the concentration of Aβ in red blood cells is higher in patients with vascular dementia (VD) than in controls, and this elevated blood Aβ level may contribute to vascular amyloidosis, further impairing cognitive function.[37] Autopsy studies have revealed that patients with cognitive impairment in Parkinson's disease (PD) often exhibit a combination of complex pathological changes in the cortex, including significant deposition of synaptic nucleoproteins, AD-like pathological features, and

Table 5 Differences in RIN3 Methylation Levels Between PD-MCI and PD-NC

Gene	*P* value	Groupdiff	Adj. *P* value
RIN3	0.001	−0.020	0.004

Note: Students *t*-test was used to compare the groups.
Abbreviations: Gene, gene name; Groupdiff, difference in mean methylation between the case and control groups; Adj. P-value, P-value after adjusting for age and gender.

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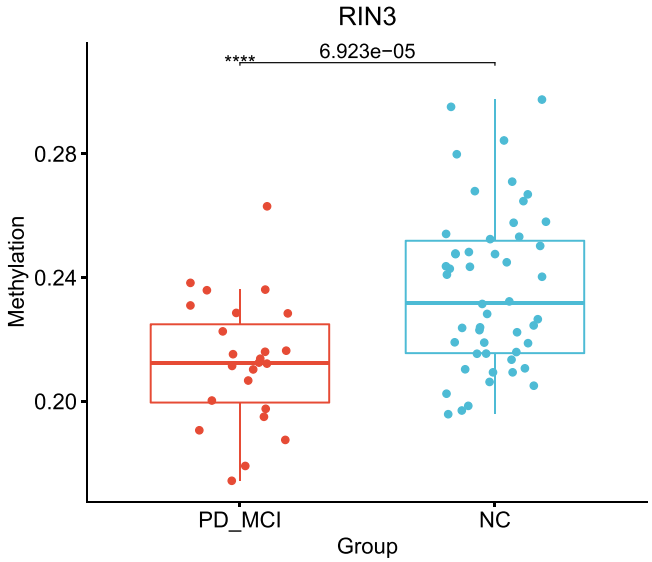

Figure 4 Compares the RIN3 methylation levels between the group with cognitive impairment after PD-MCI and the group without cognitive impairment after NC. The RIN3 gene was hypomethylated in the former. Students *t*-test was used to compare the groups. ****6.923e-05 refers to P=0.00006923.

subcortical microvascular lesions. The hallmark pathological characteristic of AD is the accumulation of β-amyloid (Aβ) and the presence of neurofibrillary tangles.[38,39] Studies show that over one-third of PD patients have Aβ deposits in the cortex. However, this protein's accumulation appears to be an intermediate process, necessitating further investigation into the underlying molecular mechanisms. Another autopsy study found that nearly 60% of patients with Parkinson's disease dementia (PDD) exhibit pathological features of Lewy bodies and Aβ plaques. Notably, only 3% of patients present with all three pathological features: Lewy bodies, Aβ plaques, and neurofibrillary tangles. Additionally, PD patients with both Aβ plaques and Lewy body deposits tend to experience more rapid cognitive decline and shorter survival rates.[40]

RIN3, a gene that encodes a protein, belongs to the RIN family. The protein it produces is a recently discovered binding protein that acts as a Rab5 guanine nucleotide exchange factor (Rab5-GEF).[41] It has been observed that the upregulation of RIN3 leads to enhanced activation of Rab5, which in turn disrupts endocytic trafficking and signaling processes, resulting in excessive deposition of Aβ peptides in the cerebral vasculature and brain tissue.[41,42] In addition, it was discovered that RIN3 interacts with both BIN1 and CD2-associated protein (CD2AP) to modulate APP trafficking and processing, a process linked to enhanced tau hyperphosphorylation. High RIN3 gene expression has been shown to reduce Aβ protein metabolism.[22,23] Moreover, abnormal expression of the RIN3 gene has been linked to Alzheimer's disease (AD), Studies have reported reduced methylation levels of RIN3 in whole blood from AD patients.[25,26] A recent study showed that TIA/MIS patients had lower RIN3 methylation levels compared to controls, with those experiencing early cognitive decline exhibiting even more pronounced hypomethylation. These findings suggest that RIN3 methylation

Table 6 Differences in RIN3 Methylation Levels Between PD-MCI and Healthy Controls

Gene	*P* value	Groupdiff	Adj. *P* value
RIN3	<0.001	−0.023	0.001

Note: Students *t*-test was used to compare the groups.
Abbreviations: Gene, gene name; Groupdiff, difference in mean methylation between the case and control groups; Adj. P-value, P-value after adjusting for age and gender.

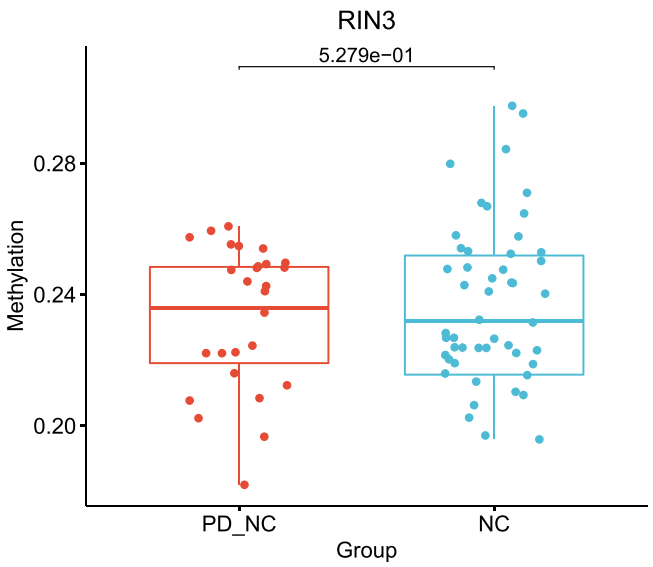

Figure 5 Compares the RIN3 methylation levels between the group with cognitive impairment after PD-NC and the group without cognitive impairment after NC. The RIN3 gene was hypomethylated in the former. Students *t*-test was used to compare the groups. 5.279e-01 refers to P=0.5279.

levels could serve as a potential predictor for early cognitive impairment following TIA/MIS.[24] Consequently, it is likely that early cognitive decline in PD patients is also related to disrupted RIN3 gene expression. Given that methylation is a crucial factor in regulating gene expression, this study indicited that abnormal methylation of the RIN3 gene is associated with early cognitive impairment in PD. Based on this data, RIN3 could be a useful biomarker for PD-MCI, and understanding its role could pave the way for targeted interventions, such as epigenetic-based therapies or early diagnostic tools, to improve patient outcomes. Further research could investigate how RIN3 methylation could inform the development of personalized treatment strategies, optimizing care based on individual genetic and epigenetic profiles.

This study has certain limitations. First, the sample size is small, which limits the statistical power and generalizability of the findings. In future research, we plan to recruit a larger cohort of PD patients to enhance the robustness of our findings. Second, while MMSE and MoCA were used to assess cognitive function, these tools may not fully capture all relevant cognitive domains. Meanwhile, relying on a single score from each scale, which may introduce bias.[43,44] More comprehensive neuropsychological assessments could provide a clearer picture of the relationship between RIN3 methylation and specific types of cognitive impairment. Third, the study's cross-sectional design means that it only captures data at a single point in time. In the future, longitudinal studies would be needed to track changes over time and better understand the progression of cognitive decline in relation to RIN3 methylation.

Table 7 Differences in RIN3 Methylation Levels Between PD-NC and Healthy Controls

Gene	*P* value	Groupdiff	Adj. *P* value
RIN3	0.53	−0.0035	0.54

Note: Students *t*-test was used to compare the groups.
Abbreviations: Gene, gene name; Groupdiff, difference in mean methylation between the case and control groups; Adj. P-value, P-value after adjusting for age and gender.

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Conclusion

Compared to the healthy control group, the RIN3 gene in the whole blood of Parkinson's disease (PD) patients exhibited reduced methylation. Furthermore, the methylation levels of the RIN3 gene in PD-MCI were significantly lower than those observed in both the control group and the PD patients without cognitive impairment (PD-NC). RIN3 methylation is strongly associated with PD-MCI. Methylation levels can be influenced by environmental factors, and this process is reversible without affecting the gene sequence. Through appropriate lifestyle changes and/or clinical interventions (such as a healthy diet, regular exercise, cognitive training, etc), methylation can be influenced, which may further impact the progression of the disease. Therefore, there is potential for the development of methylation as a therapeutic and intervention target for PD-MCI.

Ethical Statement

This study was approved by the Ethics Committee of Qingdao Central Hospital, University of Health and Rehabilitation Sciences, and was conducted in compliance with the principles outlined in the Helsinki Declaration. All patients provided written informed consent.

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Disclosure

The authors report no conflicts of interest relating to this manuscript.

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