RESEARCH ARTICLE

# Cortical Macro- and Microstructural Changes in Parkinson's Disease with Probable Rapid Eye Movement Sleep Behavior Disorder

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**ABSTRACT: Background:** Evidence regarding cortical atrophy patterns in Parkinson's disease (PD) with probable rapid eye movement sleep behavior disorder (RBD) (PD-pRBD) remains scarce. Cortical mean diffusivity (cMD), as a novel imaging biomarker highly sensitive to detecting cortical microstructural changes in different neurodegenerative diseases, has not been investigated in PD-pRBD yet.
**Objectives:** The aim was to investigate cMD as a sensitive measure to identify subtle cortical microstructural changes in PD-pRBD and its relationship with cortical thickness (CTh).
**Methods:** Twenty-two PD-pRBD, 31 PD without probable RBD (PD-nonpRBD), and 28 healthy controls (HC) were assessed using 3D T1-weighted and diffusion-weighted magnetic resonance imaging on a 3-T scanner and neuropsychological testing. Measures of cortical brain changes were obtained through cMD and CTh. Two-class group comparisons of a general linear model were performed ($P < 0.05$). Cohen's $d$ effect size for both approaches was computed.
**Results:** PD-pRBD patients showed higher cMD than PD-nonpRBD patients in the left superior temporal, superior frontal, and precentral gyri, precuneus cortex, as well as in the right middle frontal and postcentral gyri and paracentral lobule ($d > 0.8$), whereas CTh did not detect significant differences. PD-pRBD patients also showed increased bilateral posterior cMD in comparison with HCs ($d > 0.8$). These results partially overlapped with CTh results ($0.5 < d < 0.8$). PD-nonpRBD patients showed no differences in cMD when compared with HCs but showed cortical thinning in the left fusiform gyrus and lateral occipital cortex bilaterally ($d > 0.5$).
**Conclusions:** cMD may be more sensitive than CTh displaying significant cortico-structural differences between PD subgroups, indicating this imaging biomarker's utility in studying early cortical changes in PD. © 2024 The Authors. *Movement Disorders* published by Wiley Periodicals LLC on behalf of International Parkinson and Movement Disorder Society.

**Key Words:** Parkinson's disease; rapid eye movement sleep behavior disorder; cortical mean diffusivity; cortical thickness; magnetic resonance imaging



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Rapid eye movement (REM) sleep behavior disorder (RBD) is characterized by a dissociation between REM sleep and wake that leads to dream enactment, with vocalizations and strenuous behaviors, because of the absence of muscle atonia during REM sleep.[1] It has been described as one of the most prolonged prodromal symptoms of Parkinson's disease (PD), with an average latency of 12–14 years before PD onset,[2] and is a common symptom in the motor phase of the disease.[3]

Polysomnography with synchronized audiovisual recordings (vPSG) is the gold standard instrument to confirm RBD.[4] Despite its utility, vPSG is expensive and not always available. Consequently, medical practitioners use validated questionnaires to screen RBD when clinical history of abnormal behaviors during the sleep state is present.[4] Specifically, the Innsbruck RBD Inventory (RBD-I) has demonstrated high specificity and sensitivity to detect both isolated and PD-related RBD,[5] also referred to as PD with probable RBD (PD-pRBD).

RBD in PD has been associated with more severe clinical outcomes, in terms of motor function and cognitive phenotypes. Thus, the presence of RBD in PD patients has been associated with more severe motor symptoms and motor complications and nonmotor symptomatology, substandard quality of life,[6] and higher rates of anxiety and depression, as well as lower scores in global cognitive function,[7,8] attention/processing speed,[9,10] executive functions,[8,9,11,12] and visuospatial abilities,[8,12] along with higher prevalence of mild cognitive impairment (MCI).[13]

Previous neuroimaging studies identified structural brain differences between PD-pRBD patients and PD patients without pRBD (PD-nonpRBD) using atlas-based volumetric measures, as well as voxel- (VBM), deformation- (DBM) and surface-based morphometry (SBM) in magnetic resonance imaging (MRI). These studies have reported reduced left thalamus volume in PD-pRBD compared with PD-nonpRBD,[12] whereas VBM studies reported a volumetric reduction in the thalamus,[14] putamen,[15] left lingual gyrus/cerebellum,[16] left posterior cingulate, and hippocampus[17] in comparison with PD-nonpRBD. These findings partially agree with one DBM study showing decreased volumes of PD-pRBD in the pontomesencephalic tegmentum, medullary reticular formation, hypothalamus, thalamus, putamen, amygdala, and anterior cingulate[18] when compared with PD-nonpRBD. On the contrary, SBM approaches have shown cortical thinning in PD-pRBD in the right perisylvian,[16] inferior temporal cortices.[16,19] This last study also showed volume shape contraction in the putamen.[16]

Preceding research has also assessed the impact of RBD on brain structure in PD patients in the motor phase of the disease.[12,14-19] However, there is currently a gap in studies focusing on gray matter (GM) microstructural changes. The collapse of the diffusion barriers of water molecules, due to neuronal damage, cellular death, or microstructural disorganisation,[20,21] can be measured using the metric of cortical mean diffusivity (cMD). This metric is measured using an SBM approach and has been proposed as a neuroimaging biomarker to detect those microstructural GM changes that predate macrostructural degeneration.[20] Nowadays, it is widely thought that cMD could be able to detect changes in extracellular water produced by the free diffusion of these molecules in several neurodegenerative diseases.[20-31] Previous works that have used this technique in PD demonstrated its sensitivity to detect longitudinal changes,[32] as well as correlations between GM microstructural changes and clinical[33] and biological biomarkers.[34,35] However, the differences in cMD between PD and healthy controls (HC) are not clear, and differences between PD subgroups have not been described. Studies focused on cortical microstructural changes in PD are limited, and furthermore, this matter has not been investigated in PD-pRBD patients yet.

Therefore, this study aims to characterize cortical microstructural changes in PD-pRBD and their relationship with cortical thickness (CTh).

Based on previous literature, we hypothesized that this novel neuroimaging SBM approach would be able to distinguish between subgroups of PD patients, the cortical microstructural changes being more extensive in PD-pRBD.

## Patients and Methods

### Participants

Participants in this study were recruited from the Parkinson's Disease and Movement Disorders Unit, at the Hospital Clínic, located in Barcelona (Catalonia, Spain). The total sample comprised 133 participants, 70 of whom were treated nondemented PD patients and the remaining 63 participants were age-matched HCs. Among the PD patients, the presence of probable RBD (pRBD) was assessed using the RBD-I,[5] with the cutoff set at 0.25, to classify this group in a subgroup of PD patients with the presence of clinical features of RBD (PD-pRBD) and another subgroup of PD patients without symptomatology of RBD (PD-nonpRBD), according to the literature.[5]

The inclusion criteria for PD patients were as follows: (1) fulfilling the UK PD Society Brain Bank diagnostic criteria for PD[36] and (2) no surgical treatment using deep brain stimulation. The exclusion criteria for patients were (1) presence of dementia according to the Movement Disorders Society criteria,[37] (2) PD age of onset below 40 years, (3) age at the time of assessment below 50 years, (4) missing data in the RBD-I inventory, (5) presence of severe psychiatric or neurological

comorbidities, (6) scores below 25 in the Mini-Mental State Examination[38] (MMSE), (7) claustrophobia, (8) pathological MRI findings other than mild white matter hyperintensities in long-Repetition Time (TR) sequences, and (9) MRI artifacts. The exclusion criteria for HCs also included the presence of (1) MCI and (2) pRBD according to the RBD-I.

This study was approved by the Bioethics Committee of the University of Barcelona (IRB00003099) and was conducted in accordance with the basic principles of the Declaration of Helsinki, among other relevant regulations and guidelines. Signed written informed consent was provided by all the participants of this study, after a complete explanation of the procedures involved.

## Neuropsychological Assessment

The participants included in this study, both HCs and PD patients, underwent a comprehensive neuropsychological assessment in accordance with the diagnostic criteria for MCI in PD proposed by the MDS Task Force.[39] The neuropsychological battery included (1) the Spanish version of the MMSE[38] as a screening test for assessing global cognition; (2) Vocabulary subtest of the Wechsler Adult Intelligence Scale, Third Edition (WAIS-III), to estimate premorbid functioning; (3) Boston Naming Test to assess language; (4) the oral version of the Symbol Digit Modalities Test, parts A and B of the Trail Making Test (TMT), digits (forward and backward) subtests (WAIS-IV), and Stroop color-word test to assess attention and working memory; (5) phonemic (letter "P") and semantic (animals) fluencies to assess executive functions; (6) Rey Auditory Verbal Learning Test to assess memory; and (7) the short version of the Benton Facial Recognition Test (BFRT), Benton Visual Form Discrimination, and Benton Judgment of Line Orientation to assess visuospatial and visuoperceptual abilities. The University of Pennsylvania Smell Identification Test was also included to assess olfactory abilities.

Furthermore, sociodemographic information and clinical features of participants were collected, including the date of PD diagnosis, years of PD duration, previous and current treatments, the severity of motor symptoms using part III of the Unified Parkinson's Disease Rating Scale[40] (UPDRS-III), and the Hoehn & Yahr (H&Y) scale to describe the progression of PD. Neuropsychiatric and PD-related nonmotor symptoms were also assessed using the Cummings Neuropsychiatric Inventory[41], Beck Depression Inventory 2nd edition (BDI-II), and RBD-I.[5]

Among participants, MCI was calculated using standardized $z$ scores adjusted for age, sex, and years of education and using the mean and standard deviations of the control group, and performing multiple regression analyses to obtain expected $z$ scores of the cognitive domains.[42,43] As mentioned previously, MCI presence was used for the inclusion/exclusion criteria.

## MRI Acquisition and Preprocessing

High-resolution three-dimensional T1-weighted images (Magnetization Prepared-RApid Gradient Echo [MPRAGE]) were acquired in the sagittal plane (TR = 2300 ms, Echo time [TE] = 2.98 ms, Inversion time [TI] = 900 ms, Acquisition time [TA] = 7′48″, 240 slices, Field-of-view [FOV] = 256 mm; 1-mm isotropic voxel), using a Trio Magnetom 3-T scanner located at the Centre de Diagnostic per la Imatge of the Hospital Clínic de Barcelona (Catalonia, Spain). The scanning protocol of this study also included T2-weighted images in axial orientation (TR = 3200 ms; TE = 563 ms; 512 × 307 matrix; flip angle, 120°; slice thickness, 0.8 mm; interslice gap, 1.5 mm), an axial FLAIR sequence (TR = 9000 ms; TE = 125 ms; TI = 2500; 250 × 171 matrix; flip angle, 150°; and slice thickness, 4 mm), and two sets of single-band spin-echo diffusion-weighted images in the axial plane with opposite phase encoding directions (TR = 7700 ms; TE = 89 ms; TA = 8′46″; FOV = 244 mm; isotropic voxel, 2 mm; number of directions = 30; $b$-value = 1000 s/mm$^2$; $b0$ value = 0 s/mm$^2$). These sequences allowed us to obtain gray and white matter structural data, along with clinically evaluating the effects of aging and microvascular changes.

## CTh Processing

The estimation of CTh was performed using the automated FreeSurfer stream version 6.0 available at https://surfer.nmr.mgh.harvard.edu/. The procedures in FreeSurfer included removal of nonbrain data, registration to Talairach space, intensity normalization, tessellation of the GM and white matter boundaries, automated topology correction, and accurate surface deformation following intensity gradients to identify tissue borders. CTh was calculated as the distance between the white and GM surfaces at each vertex of the reconstructed cortical mantle (https://freesurfer.net/fswiki/FreeSurferMethodsCitation). Results for each subject were visually inspected, and the befitting manual corrections were also performed to ensure the accuracy of registration, skull stripping, segmentation, and cortical surface reconstruction (https://surfer.nmr.mgh.harvard.edu/fswiki/FsTutorial/TroubleshootingData).

## Diffusion MRI Processing

Diffusion MRI images were analyzed using a diffusion tensor imaging (DTI) approach with FMRIB's Diffusion Toolbox software from FSL (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/). The processing started with estimation and correction for susceptibility-induced distortions[44] (topup, https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/

topup), brain extraction using the Brain Extraction Tool[45] (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/BET), and distortion correction using eddy (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/eddy) that simultaneously corrects for eddy currents and subject motion.[46] Motion parameters were extracted pre- and post eddy currents correction to be compared across groups. These processing tools face common challenges of echo-planar imaging sequences.[47] Individual mean diffusivity (MD) maps were obtained using a diffusion model fit (DTIFIT).

### Cortical Mean Diffusivity Processing

Cortical diffusion MRI was processed using a surface-based DTI approach[21] using the FSL package (FMRIB's Software Library, https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/) and the FreeSurfer package, version 6.0. MD volumes were coregistered to the anatomic subject space, projected to the surface, normalized using a spherical registration to the FreeSurfer standard template, and finally smoothed. The diffusion images were corrected for motion effects, skull stripped, and DTI tensor fitted and projected to the brain surface. Before further analyses, a Gaussian kernel of 15-mm full width at half maximum was applied to the subjects' MD surface maps. Finally, the MD surface maps were used for statistical analyses.

### Statistical Analyses

Group analyses in sociodemographic, neuropsychological, and clinical data were performed using IBM SPSS Statistics 27.0.1.0 (2020, IBM Corp., Armonk, NY). Between-group comparisons were tested using analysis of variance (ANOVA) for continuous variables, using Levene's test to control variance heterogeneity, conducting post hoc comparisons using Bonferroni or Games–Howell corrections taking into account the equal variances assumption, and adding covariates when required. Categorical or dichotomous variables were analyzed using Pearson's $\chi^2$ test. Kruskal–Wallis test was performed to analyze variables that have violated the assumption of normality, followed by Mann–Whitney's test to do post hoc comparisons of these variables. Additionally, Student $t$ test or Mann-Whitney $U$ test was used to analyze group differences among patients regarding PD-related variables. The statistical significance threshold was set at $P < 0.05$.

Intergroup cMD and CTh analyses were conducted using a two-class vertex-wise general linear model (GLM) with FreeSurfer version 6.0, comparing all the groups included in this study (HCs and both subgroups of PD patients). Sex was included as a confounding variable running the cMD and CTh comparisons between HC and PD-pRBD, whereas disease duration was included as a covariate when both subgroups of PD patients were compared. Vertex-wise GLM was used to study the correlations between the cMD and cognitive measures. In all imaging analyses, cluster-extend corrections for multiple comparisons were tested using the Monte Carlo simulation, with 10,000 iterations implemented in FreeSurfer, to control false positives. The cluster-defining threshold was set at 1.3, in both directions (abs). Only the clusters that survived family-wise error (FWE) correction with the statistical significance threshold set at $P < 0.05$ were reported.

Vertex-wise Cohen's $d$ effect size was computed for cMD and CTh, limiting effect size results to the statistically significant regions obtained in the intergroup comparison analyses to represent the effect size of the topographical differences among HC, PD-pRBD, and PD-nonpRBD. Only moderate to high effect sizes were considered ($d > 0.5$).

## Data Sharing

The data that support the findings of this study are available from the corresponding author on reasonable request.

## Results

### Sample Composition and Demographics

The final sample consisted of 53 treated nondemented PD patients and 28 age-matched HCs. Following the cutoff established in the RBD-I, 22 PD patients were identified as pRBD and 31 PD patients as non-probable RBD (non-pRBD) patients. Seventeen PD patients and 41 HCs were excluded from the recruited sample. Figure 1 shows a flowchart with detailed steps until the final sample was composed and the reasons for exclusion.

Table 1 summarizes the sociodemographic and clinical characteristics of the participants. There were no differences between groups in age, years of education, premorbid intelligence (Vocabulary, WAIS-III), or BDI-II. Nevertheless, there were differences between groups in sex. Specifically, there were significantly more female participants in the HC group than in the PD-pRBD group, so it was introduced as a covariate in the analyses. The PD subgroups of patients were comparable in MDS-UPDRS-III, H&Y stage, levodopa equivalent daily dose (LEDD), and age at PD onset but significantly differ in PD duration. Both groups of PD patients showed statistically significant differences in olfaction in comparison with HCs. Only the PD-pRBD group showed significantly more neuropsychiatric symptoms than HCs. There were not significant differences in global motion within the diffusion-weighted sequence across the groups (Table S1).

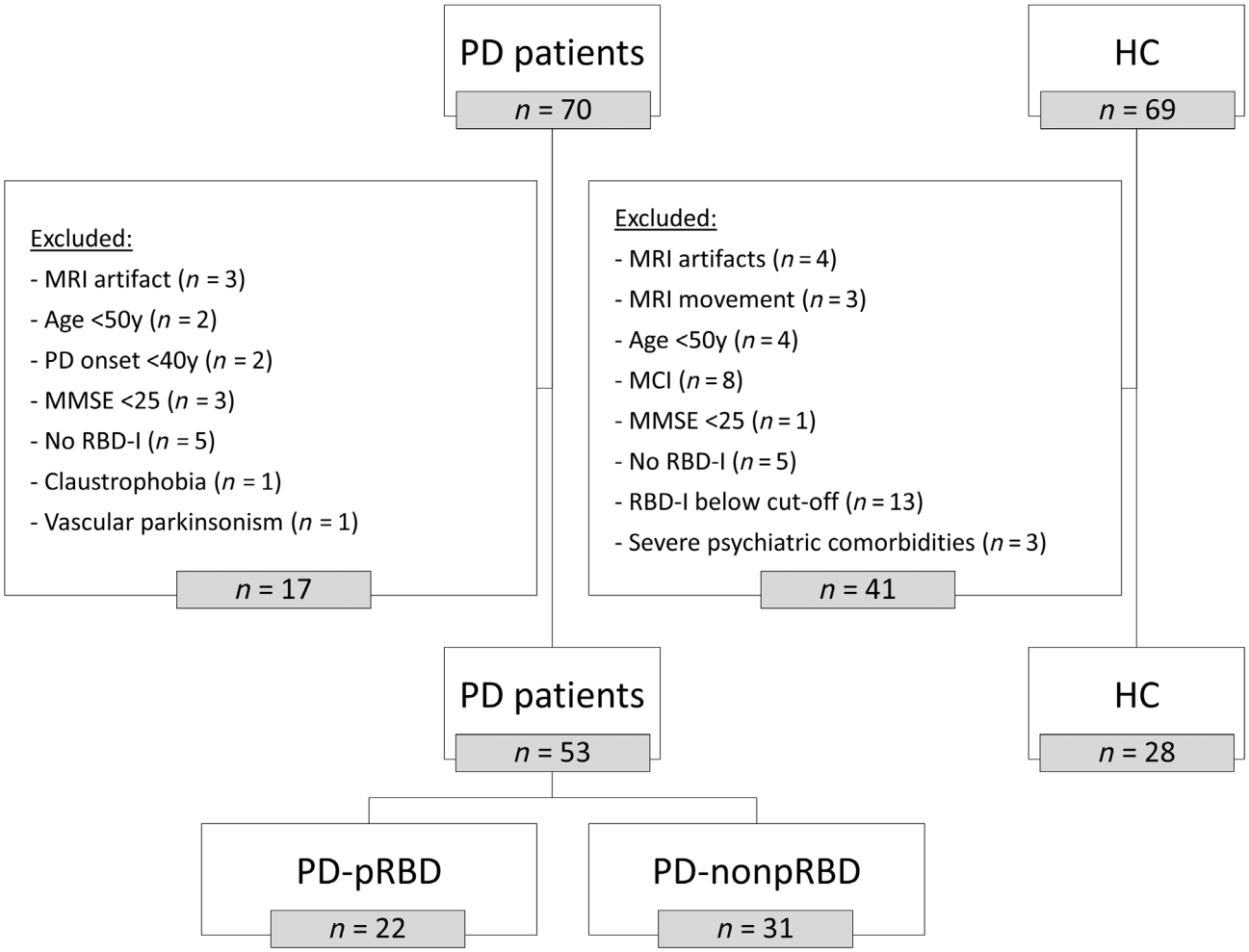


**FIG. 1.** Flowchart with detailed steps until the final sample composition. HC, healthy controls; MMSE, Mini-Mental State Examination; MRI, magnetic resonance imaging; PD, Parkinson's disease; PD-nonpRBD, Parkinson's disease patients without probable rapid eye movement sleep behavior disorder; PD-pRBD, Parkinson's disease patients with probable REM sleep behavior disorder; RBD-I, Innsbruck Rapid Eye Movement Sleep Behavior Disorder Inventory.

## Cortical Mean Diffusivity Group Comparisons

Figure 2 shows the effect sizes restricted to the significant regions in the intergroup cMD analysis maps ($P < 0.05$, FWE corrected) using the required covariates. The PD-pRBD group showed a widespread mainly posterior pattern of significantly increased cMD values in comparison with the PD-nonpRBD group, comprising the bilateral precuneus; occipital, isthmus, and posterior cingulate; pericalcarine cortices; the superior, middle, and inferior temporal, superior frontal, inferior parietal, precentral, postcentral, supramarginal, lingual, and fusiform gyri; the paracentral lobule; and the banks of the superior temporal sulcus spreading through the pars opercularis and the rostral middle frontal gyrus in the left hemisphere, as well as the parahippocampal and caudal middle frontal gyri, and the superior parietal and cuneus cortices in the right hemisphere. The PD-pRBD patients also showed larger cMD values in posterior regions than HCs, including the left precuneus, isthmus, posterior cingulate, cuneus and pericalcarine cortices, the right fusiform gyrus, along with the occipital cortex and the inferior parietal gyrus bilaterally. However, the PD-nonpRBD patients showed no differences in cMD when compared with HCs. Larger cMD values in the right fusiform gyrus in PD-pRBD patients in comparison with HCs, and in the lateral occipital and inferior parietal regions in PD-pRBD relative to PD-nonpRBD, survived a cluster-forming threshold of $P = 0.001$ (Table 2).

## Cortical Thickness Group Comparisons

Figure 3 shows the effect sizes restricted to the significant regions outputted in the intergroup CTh analysis maps ($P < 0.05$, FWE corrected) also controlling for the required variables in each comparison. In contrast to cMD, we found no differences in CTh when comparing both subgroups of PD patients. Nevertheless, the PD-pRBD patients showed cortical thinning compared

**TABLE 1** *Sociodemographic and clinical characteristics of the participants*

| | PD-nonpRBD, n = 31 | PD-pRBD, n = 22 | HC, n = 28 | Statistics | *P* |
|---|---|---|---|---|---|
| Age (y) | 64.74 (9.95) | 67.32 (8.73) | 67.39 (7.94) | $0.816^{F}$ | 0.446 |
| Sex, male (%) | 20 (64.5) | 19 (86.4) | 12 (42.9) | $10.052^{X2}$ | **0.007**[b] |
| Education (y) | 12.71 (5.37) | 12.32 (5.22) | 11.36 (3.93) | $0.966^{H}$ | 0.617 |
| Vocabulary (WAIS-III) | 47.00 (8.55) | 46.55 (7.80) | 44.57 (7.70) | $2.706^{H}$ | 0.259 |
| MDS-UPDRS-III | 15.47 (10.35) | 15.85 (6.68) | – | $-0.146^{t}$ | 0.884 |
| H&Y stage (1/2/2.5/3) | 6/16/1/6 | 2/10/0/8 | – | $3.123^{X2}$ | 0.373 |
| LEDD (mg) | 535.88 (281.78) | 693.88 (467.13) | – | $257.500^{U}$ | 0.271 |
| Age at PD onset (y) | 58.81 (10.67) | 57.25 (9.03) | – | $0.539^{t}$ | 0.592 |
| PD duration (y) | 6.29 (3.69) | 8.77 (5.00) | – | $-2.081^{t}$ | **0.043**[c] |
| BDI-II | 7.77 (7.01) | 10.55 (7.44) | 6.46 (4.74) | $4.184^{H}$ | 0.123 |
| NPI | 7.81 (8.67) | 10.09 (9.00) | 4.46 (7.22) | $8.283^{HU}$ | **0.016**[b] |
| UPSIT | 19.00 (6.88) | 17.55 (6.94) | 29.30 (4.43) | $28.158^{F}$ | **<0.001**[ab] |

Values denote mean (standard deviation) or numbers (frequencies). Statistical tests used: $^{F}$ANOVA, $^{X2}\chi^2$, $^{H}$Kruskall–Wallis, $^{t}t$ test, and $^{U}$Mann-Whitney. Post hoc comparisons: [a]HC versus PD-nonpRBD, [b]HC versus PD-pRBD, [c]PD-nonpRBD versus PD-pRBD. Statistically significant results in bold ($P$-value ≤ 0.05).
Abbreviations: PD-nonpRBD, Parkinson's disease patients without probable rapid eye movement sleep behavior disorder; PD-pRBD, Parkinson's disease patients with probable rapid eye movement sleep behavior disorder; HC, healthy control; WAIS-III, Wechsler Adult Intelligence Scale, Third Edition; MDS-UPDRS, Movement Disorder Society Unified Parkinson's Disease Rating Scale; H&Y, Hoehn & Yahr stage; LEDD, levodopa equivalent daily doses; PD, Parkinson's disease; BDI-II, Beck Depression Inventory 2nd edition; NPI, Neuropsychiatric Inventory; UPSIT, University of Pennsylvania Smell Identification Test.

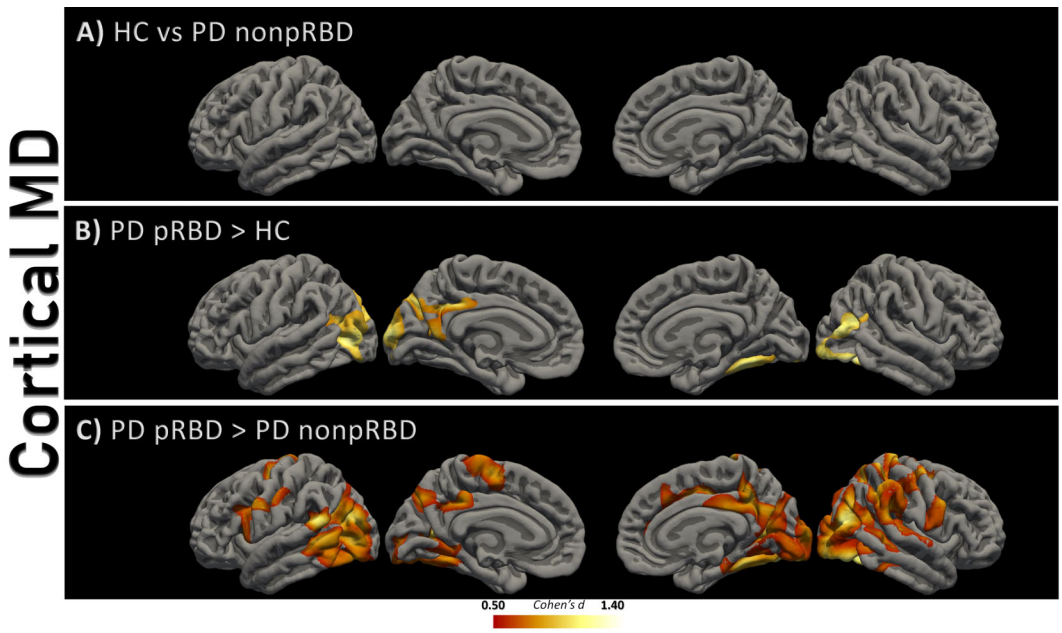


**FIG. 2.** Cortical mean diffusivity comparisons among PD-pRBD, PD-nonpRBD, and HC. (**A**) The PD-nonpRBD group showed no differences in cMD (cortical mean diffusivity) in comparison with HCs. (**B**) The PD-pRBD group showed increased cMD in comparison with HCs. (**C**) The PD-pRBD group showed higher cMD than PD-nonpRBD. Only clusters that survived cluster-extend Monte Carlo corrections for multiple comparisons ($P < 0.05$) are shown, represented with the effect sizes (Cohen's $d > 0.50$) restricted to the significant clusters. [Color figure can be viewed at wileyonlinelibrary.com]

with HCs in the left precuneus and superior parietal cortices, the lingual gyrus, the right inferior temporal gyrus, the entorhinal and occipital cortices, and pars opercularis, along with the cuneus cortex and the fusiform gyrus bilaterally. When a cluster-forming threshold of $P = 0.001$ was adopted, differences between these groups were restricted to the right pars opercularis (Table S2). The PD-nonpRBD group also showed cortical thinning compared with HCs in the left cuneus and precuneus cortices, the superior parietal,

**TABLE 2** *Neuropsychological performance of the participants*

| Domains | Tests | PD-nonpRBD, n = 31 | PD-pRBD, n = 22 | HC, n = 28 | Statistics | *P* |
|---|---|---|---|---|---|---|
| Global cognition | MMSE | 29.06 (1.26) | 29.05 (1.09) | 29.18 (0.95) | 0.109 | 0.897 |
| Executive functions | Fluency tests | | | | | |
| | Semantic (animals) | 16.90 (6.62) | 17.09 (6.56) | 20.46 (4.07) | 3.556 | **0.033**[a] |
| | Phonetic (letter P) | 15.61 (6.08) | 14.73 (4.31) | 15.61 (5.84) | 0.039 | 0.962 |
| Attention and working memory | Digit span | | | | | |
| | Forward | 5.32 (1.19) | 5.64 (1.22) | 5.61 (1.34) | 1.048 | 0.356 |
| | Backward | 3.97 (1.02) | 4.36 (1.26) | 4.14 (0.93) | 0.539 | 0.586 |
| | Forward minus backward | 1.35 (1.40) | 1.27 (1.32) | 1.46 (0.92) | 0.160 | 0.853 |
| | Stroop | | | | | |
| | Word | 89.40 (17.61) | 85.68 (15.30) | 96.29 (15.49) | 2.454 | 0.093 |
| | Color | 56.20 (14.05) | 51.00 (14.48) | 64.46 (10.24) | 3.991 | **0.023**[b] |
| | Word-color | 34.23 (11.87) | 29.55 (11.58) | 36.82 (9.39) | 2.725 | 0.072 |
| | TMT | | | | | |
| | Part A | 52.55 (35.43) | 51.50 (25.15) | 39.00 (11.16) | 4.219 | **0.018**[a] |
| | Part B | 148.72 (145.92) | 191.43 (220.82) | 95.25 (46.50) | 3.448 | **0.037**[b] |
| | B minus A | 97.41 (117.65) | 139.95 (201.13) | 56.25 (39.98) | 3.090 | 0.052[b] |
| | SDMT | 41.58 (14.92) | 43.41 (13.63) | 47.43 (8.53) | 1.813 | 0.170 |
| Memory | RAVLT | | | | | |
| | Total | 44.23 (10.51) | 42.77 (8.10) | 45.61 (6.24) | 0.143 | 0.867 |
| | Recall | 8.19 (3.72) | 8.41 (3.19) | 9.39 (2.06) | 0.962 | 0.387 |
| Visuospatial and visuoperceptual abilities | BJLO | 23.97 (5.75) | 23.77 (4.72) | 25.07 (3.49) | 2.519 | 0.087 |
| | BVFD | 29.42 (2.66) | 28.86 (3.20) | 29.18 (2.42) | 0.015 | 0.985 |
| | BFRT-short | 21.61 (2.65) | 21.32 (2.57) | 23.00 (1.89) | 4.640 | **0.013**[b] |
| Language | BNT | 13.58 (1.23) | 13.45 (1.01) | 13.68 (0.91) | 0.430 | 0.652 |

Values denote the mean (standard deviation) of raw scores. Statistical tests used: two-way ANOVA (analysis of variance), with group and sex as fixed factors. Post hoc comparisons: [a]HC versus PD-nonpRBD, [b]HC versus PD-pRBD, and [c]PD-nonpRBD versus PD-pRBD. Statistically significant results in bold (*P*-value ≤ 0.05).
Abbreviations: PD-nonpRBD, Parkinson's disease patients without probable rapid eye movement sleep behavior disorder; PD-pRBD, Parkinson's disease patients with probable rapid eye movement sleep behavior disorder; HC, healthy control; MMSE, Mini-Mental State Examination; TMT, Trail Making Test; SDMT, Symbol Digit Modalities Test; RAVLT, Rey Auditory Verbal Learning Test; BJLO, Benton Judgment of Line Orientation; BVFD, Benton Visual Form Discrimination; BFRT, Benton Facial Recognition Test; BNT, Boston Naming Test.

and fusiform gyri, as well as the right lateral fusiform and the bilateral occipital cortex. Figure S1 shows overlapping map between the statistically significant clusters of cMD and CTh results in the HC versus PD-pRBD comparison.

### Neuropsychological Performance

Table 2 describes the neuropsychological differences between groups. There were no intergroup differences between the two PD subgroups of patients regarding cognitive functions. In comparison with HCs, PD-pRBD patients showed worse performance in the Stroop test (color), part B of TMT, and the short version of BFRT, whereas PD-nonpRBD patients scored lower in semantic fluency and took more time doing part A of TMT.

Regarding cortical microstructural correlates of neuropsychological performance in PD-pRBD patients, there were no statistically significant results.

## Discussion

In this study, we identified cMD increase in PD-pRBD patients and demonstrated the usefulness of this

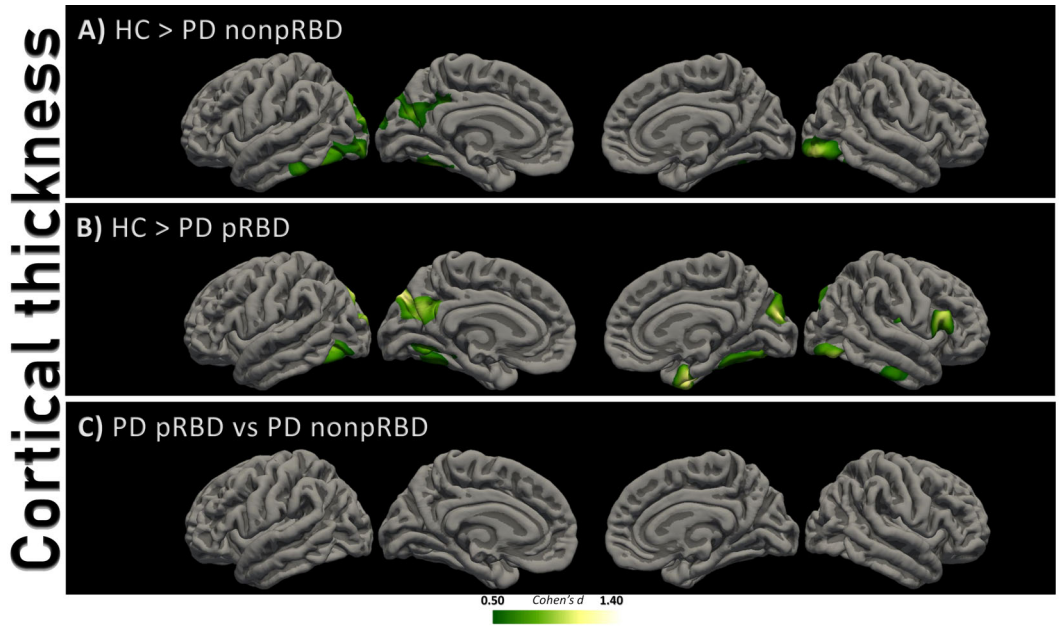


**FIG. 3.** Cortical thickness comparisons among PD-pRBD, PD-nonpRBD, and HC. (**A**) The PD-nonpRBD group showed cortical thinning in comparison with HCs. (**B**) The PD-pRBD group showed cortical thinning in comparison with HCs, overlapping with some of the cMD (cortical mean diffusivity) results. (**C**) The PD-pRBD group showed no differences in cortical thickness in comparison with the PD-nonpRBD group. Only clusters that survived cluster-extend Monte Carlo corrections for multiple comparisons ($P < 0.05$) are shown, represented with the effect sizes (Cohen's $d > 0.50$) restricted to the significant clusters. [Color figure can be viewed at wileyonlinelibrary.com]

approach to measure cortical brain changes. Our results showed that PD-pRBD patients have a mainly posterior pattern of increased cMD in comparison with HCs and PD-nonpRBD patients. The PD-pRBD group showed a pattern of cortical thinning that partially overlaps with cMD increases, these cortical microstructural changes being more extended and showing higher effect sizes. These findings suggest that the cMD approach could be more suitable and sensitive than CTh to distinguish between clinical phenotypes among PD patients and add evidence suggesting that RBD symptomatology in PD patients is related to a more severe PD phenotype.

First, our analyses based on cMD have shown a notable increase in PD-pRBD patients in comparison with PD-nonpRBD patients mainly in posterior regions, including the left precuneus and superior temporal sulcus but also involving more anterior but limited regions such as the precentral gyrus and superior frontal gyrus in the left hemisphere or the postcentral gyrus, paracentral lobule, and caudal middle frontal gyrus in the right hemisphere. Cortical microstructural increases detected through cMD have been commonly interpreted as a sign of underlying degeneration,[25] reflecting the disruption of microstructural water barriers, intracellular disorganization, neuronal damage, and cellular death.[20,21,26] This is the first study assessing cMD in PD-pRBD patients, so there are no previous results concerning cMD in a sample with RBD in PD, with neither probable nor confirmed RBD diagnosis using vPSG. That being the case, the mainly posterior pattern of cortical microstructural changes found in the PD-pRBD group would be consistent with previously reported cerebral posterior thinning in the inferior temporal,[16,19] fusiform, supramarginal, and superior temporal gyri,[16] as well as atrophy on lingual gyrus[16] based on VBM and cingulate cortex[17,18] based on DBM, but showing a more extended pattern of degeneration. Considering previous evidence, CTh analyses seem to be less sensitive to PD-pRBD cortical changes. In line with that, our CTh results have shown no cortical macrostructural changes in the between-PD group contrast. Previous studies suggested the suitability of using cMD to compare and contrast early subtle variations in different subgroups of the same neurodegenerative or psychiatric disease.[20] In this regard, studies focused on different neurodegenerative diseases have reported statistically significant differences in cMD between different stages of the same pathological continuum (Alzheimer's disease,[21] amyotrophic lateral sclerosis-frontotemporal dementia,[26] Huntington's disease,[28] multiple sclerosis[31]) or subgroups of the reported disease (Alzheimer's disease,[24] primary progressive aphasia[27]) and HC, as well as in schizophrenia subtypes.[48] Focusing on PD, no studies have developed comparisons among PD subgroups in cMD. Concretely, only one previous work showed cMD differences among groups cross-sectionally, with increased cMD in PD patients in comparison with HCs.[33] However, the sample included in such study showed different ages among groups, along with older PD patients, higher scores of motor

symptoms, and higher LEDD compared with our study. Moreover, the study was focused on a correlational approach and did not report cMD or CTh between-group differences clearly.

Second, the contrast between PD-pRBD and HC has shown both a difference in a posterior pattern of microstructural changes in PD-pRBD patients and cortical thinning partially overlapping posterior regions with increased cMD. Several cMD studies focused on different neurodegenerative diseases have reported similar results, emphasizing this overlap between an increased cMD and a decreased CTh.[21,24-27] This fact has been considered as a face validity marker of cortical integrity or degeneration, providing evidence of this inverse association between cMD and CTh as a continuum of the same pathological process.[49] Furthermore, some of these authors claimed a higher sensitivity of cMD than CTh,[20,25,27] in some cases related to specific cerebral regions.[24] Although a few studies suggested cortical microstructural changes as precursors to cortical macrostructural changes,[20,26,27] our results cannot confirm this conclusion. With regard to our study, PD-nonpRBD patients have shown cortical thinning in comparison with HCs in the lateral occipital cortex bilaterally and the left fusiform gyrus, with no differences between the two groups in cMD. Nevertheless, the CTh contrast showed only medium effect sizes, $0.5 < d < 0.8$, whereas the cMD contrasts resulted in large effect sizes, $d > 0.8$, suggesting the lower practical significance of the CTh contrast results.

Additionally, the results obtained analyzing the neuropsychological data among groups in the current study are consistent with previously reported literature in PD-related RBD, highlighting the lower performance of the PD-pRBD group in tests that implied attention/processing speed,[9,10] executive functions,[8,9,11,12] and visuospatial[8-12]/visuoperceptual[9,10] abilities. Although sleep apnea could affect the cognitive performance of the participants, only 2 PD-pRBD patients were using continuous positive airway pressure in our sample. Furthermore, we did not find significant correlations between neuropsychological performance and microstructural and macrostructural changes. The lack of such a relationship could be because PD-pRBD patients had not yet developed substantial cognitive impairment. Therefore, it is plausible that subtle structural brain changes precede cognitive impairment. Considering the PD-pRBD group presumably will show significant cognitive impairment over time, future studies should focus on whether the cMD changes are predictive of future cognitive decline.

To the best of our knowledge, this is the first study assessing cMD in PD-related RBD. We included between-group comparisons with well-matched HC as well as a comprehensive neuropsychological examination. Other aspects that strengthen our study are the implementation of a multimodal neuroimaging approach that comprises two previously validated surface-based techniques to study cortical macro- and microstructural changes in different neurodegenerative diseases, the use of an improved method to obtain measures of cMD[21] which is capable of overcoming previously reported methodological and processing concerns related to the cortical microstructural change[20] computation, and the computation of vertex-wise Cohen's $d$ effect sizes to measure the intensity of our cMD and CTh results. Nevertheless, we must be also aware of the limitations of our study. Although we have used a validated screening questionnaire for RBD with excellent sensitivity (0.914) and specificity (0.857),[5] both for isolated RBD and PD-related RBD, the diagnosis of PD-pRBD of our patients has not been confirmed using vPSG. In further studies, these findings should be replicated using bigger samples, including PD patients with confirmed RBD diagnosis and other biomarkers of degeneration. Furthermore, it would be interesting to investigate cMD changes in well-characterized RBD patients without PD to know if this measure is present in the prodromic stages of PD. However, this approach involves a complex design, and taking into account the conversion rates of RBD patients, it would be necessary to have a large sample of patients. Even so, the inclusion of an isolated RBD group should also be considered, to infer when changes in cMD begin. Besides, longitudinal studies are needed to better understand the relationship between cortical macro- and microstructural changes in PD clinical subtypes.

To sum up, we have found significant brain cortical macro- and microstructural differences between the PD subgroups, the PD-pRBD phenotype being more prone than PD-nonpRBD to greater and wider cortical involvement, at both macro- and microstructural levels. Considering our results, we can also conclude the suitability of cMD as a neuroimaging marker to distinguish subtle brain cortical differences between PD clinical subtypes. ■

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### Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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## Supporting Data

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.