RESEARCH ARTICLE

Gait Analysis and Magnetic Resonance Imaging Characteristics in Patients with Isolated Rapid Eye Movement Sleep Behavior Disorder

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ABSTRACT: Background: Isolated rapid eye movement sleep behavioral disorder (iRBD) can precede neurodegenerative diseases. There is an urgent need for biomarkers to aid early intervention and neuroprotection.
Objective: The aim is to assess quantitative motor, cognitive, and brain magnetic resonance imaging (MRI) characteristics in iRBD patients.
Methods: Thirty-eight polysomnography-confirmed iRBD patients and 28 age- and sex-matched healthy controls underwent clinical, cognitive, and motor functional evaluations, along with brain MRI. Motor tasks included nine-hole peg test, five-times-sit-to-stand test, timed-up-and-go test, and 4-meter walking test with and without cognitive dual task. Quantitative spatiotemporal gait parameters were obtained using an optoelectronic system. Brain MRI analysis included functional connectivity (FC) of the main resting-state networks, gray matter (GM) volume using voxel-based morphometry, cortical thickness, and deep GM and brainstem volumes using FMRIB's Integrated Registration and Segmentation Tool and FreeSurfer.
Results: iRBD patients relative to healthy subjects exhibited a poorer performance during the nine-hole peg test and five-times-sit-to-stand test, and greater asymmetry of arm-swing amplitude and stride length variability during dual-task gait. Dual task significantly worsened the walking performance of iRBD patients more than healthy controls. iRBD patients exhibited nonmotor symptoms, and memory, abstract reasoning, and visuospatial deficits. iRBD patients exhibited decreased FC of pallidum and putamen within the basal ganglia network and occipital and temporal areas within the visuo-associative network, and a reduced volume of the supramarginal gyrus. Brain functional alterations correlated with gait changes.
Conclusions: Subtle motor and nonmotor alterations were identified in iRBD patients, alongside brain structural and functional MRI changes. These findings may represent early signs of neurodegeneration and contribute to the development of predictive models for progression to parkinsonism. © 2024 The Author(s). *Movement Disorders* published by Wiley Periodicals LLC on behalf of International Parkinson and Movement Disorder Society.

Key Words: isolated rapid eye movement sleep behavior disorder; magnetic resonance imaging; cognition

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Relevant conflicts of interest/financial disclosures: Funding from the Italian Ministero della Salute (grant number RF-2018-12366746).

Received: 31 January 2024; **Revised:** 20 May 2024; **Accepted:** 17 June 2024

Published online in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/mds.29911

Rapid eye movement (REM) sleep behavior disorder (RBD) is a parasomnia characterized by the loss of the physiological atonia of the skeletal muscle during REM sleep, leading to dream-enacting behavior with motor activity (often violent) and vocalization.[1] Polysomnography (PSG) is crucial for diagnosing RBD.[1] When it occurs without any other disease, it is defined isolated RBD (iRBD). Nevertheless, it often precedes motor or cognitive symptoms of neurodegenerative disorders and is now considered a prodromal stage of various clinically defined α-synucleinopathies. Over 90% of patients eventually convert to a neurodegenerative disease after 14 years (33% at 5 years), such as Parkinson's disease (PD) (43%) or dementia with Lewy bodies (DLB) (25%), and more rarely to multiple system atrophy (MSA).[2] Therefore, there is a growing interest in identifying biomarkers capable of predicting motor and/or cognitive signs and symptoms of neurodegeneration in iRBD. Identifying patients in the prodromal phase offers the unique opportunity for early neuroprotective therapy, potentially preventing the development of parkinsonism.[3] Several studies have proposed clinical and neuroimaging biomarkers, exploring subtle alterations in iRBD that could represent initial signs of parkinsonism.[2,4-12]

Simple motor assessments, like Movement Disorder Society Unified Parkinson's Disease Rating Scale (MDS-UPDRS), timed-Up-and-go (TUG) test, or Purdue pegboard test, have demonstrated the ability to detect changes up to 5 years before a PD diagnosis.[12,13] These evaluations can also be useful in tracking progression over time; for instance, patients with iRBD exhibited an estimated yearly increase of almost two points on the MDS-UPDRS-III.[14] Limited studies have thoroughly analyzed spatiotemporal gait changes in iRBD, revealing that motion analysis systems are more sensitive than clinical assessments in discriminating iRBD patients from healthy controls.[6,7,11] Indeed, iRBD subjects exhibited subtle changes in both upper- and lower-limb movements while walking (eg, gait asymmetry and variability), suggesting gait analysis as an α-synucleinopathies screening tool.[7,11,15,16] Challenging gait conditions such as fast walking and dual-task situations could be more sensitive in eliciting and detecting subtle alterations in iRBD,[7] as suggested in PD.[17] Studies on genetic, non-symptomatic PD individuals also indicated that arm-swing alterations may be promising early biomarkers of parkinsonism.[16]

Not only motor manifestations but also cognitive, behavioral, and autonomic alterations can be present in iRBD subjects because of the possible underlying α-synucleinopathy.[4] Cognitive impairments were reported in iRBD patients, with longitudinal studies also showing a worsening over time.[10]

Recently, various neuroimaging techniques have enhanced our understanding of iRBD pathophysiology and its connection to α-synucleinopathies.[4,5,18] Among them, magnetic resonance imaging (MRI) was proposed as a more accessible alternative to molecular imaging, revealing brain structural and functional alterations in iRBD.[5,8,9] Common findings include alterations in deep gray matter (GM) nuclei, cortical GM atrophy, and microstructural abnormalities in white matter.[5,8,9] However, structural MRI results are heterogeneous, likely due to different clinical characteristics of iRBD patients but also various MRI techniques.[5,18] A few resting-state (RS) functional MRI (RS-fMRI) studies in iRBD consistently reported reduced functional connectivity (FC) within the basal ganglia network and between the basal ganglia and the frontal, parietal, and temporal cortices.[5,9,19,20] Interestingly, FC is higher in iRBD patients compared to PD, suggesting a continuum of decline and a potential role for basal ganglia FC as a promising biomarker of prodromal parkinsonism.[5,21-23]

To the best of our knowledge, no studies have conducted a thorough assessment incorporating both structural and functional brain MRI alongside clinical motor/nonmotor evaluations and quantitative gait analysis to characterize iRBD patients. This study aimed at filling this gap by providing a comprehensive evaluation of neurological, neuropsychological, motor functional, and gait analysis features in PSG-confirmed iRBD patients. Additionally, we sought to identify the functional and structural neural correlates of subtle clinical changes using brain MRI.

Patients and Methods

Subjects

Thirty-eight patients with PSG-confirmed diagnosis of iRBD according to the International Classification of Sleep Disorders-3 criteria[1] were consecutively recruited at the Sleep Disorders Center, Division of Neuroscience, IRCCS San Raffaele Scientific Institute (Milan, Italy). Twenty-eight age- and sex-matched healthy controls were recruited among nonconsanguineous relatives and institute personnel and by word of mouth. Evaluations were performed at the Sleep Disorders Center and Neuroimaging Research Unit, IRCCS San Raffaele Scientific Institute (Milan, Italy). Inclusion criteria for patients and healthy controls were (1) right handedness, (2) monolingual native Italian speakers, (3) age 50 to 75 years, (4) Mini-Mental State Examination score ≥24, and (5) oral and written informed consent to study participation. Exclusion criteria were (1) secondary forms of RBD based on historical data, neurologic examination, and brain MRI findings; (2) history of (other) systemic, neurologic, psychiatric diseases, head injury, and cardiovascular events; (3) brain damage at routine MRI, including lacunae and extensive cerebrovascular disorders; and (4) alcohol and/or psychotropic drug abuse.

All participants underwent motor functional, gait analysis, and cognitive evaluations, and brain MRI.

Two iRBD patients could not complete the brain MRI scan because of unexpected claustrophobia, and 3 iRBD patients and 1 control subject refused to perform gait analysis due to personal reasons (characteristics of the subgroups included in each analysis are reported in the Supplementary Materials). No subjects were excluded due to brain alterations, excessive head motion, or excessive vascular lesions. Patients with iRBD also underwent PSG and neurological evaluations. Sleep disorders in healthy controls were assessed using the Pittsburgh Sleep Quality Index (PSQI),[24] Rapid Eye Movement Sleep Behavior Disorder Screening Questionnaire,[25] Epworth Sleepiness Scale (ESS),[26] Insomnia Severity Index,[27] STOP-Bang Questionnaire,[28] and International Restless Legs Study Group Severity Rating Scale.[29]

The local ethical standards committee on human experimentation approved the study protocol, and all participants provided written informed consent prior to study inclusion.

Neurological, Motor Functional, and Neuropsychological Evaluations

An experienced neurologist assessed sleep quality and motor/nonmotor impairments in iRBD patients using the MDS-UPDRS[30] and the Non-Motor Symptoms Scale (NMS).[31] Olfaction was assessed through clinical interview and the olfactory loss item of the NMS (>4 meaning reduced olfaction). An experienced physiotherapist administered the nine-hole peg test, the five-times-sit-to-stand test, and the 10-meter walking test. An experienced neuropsychologist performed a comprehensive cognitive assessment investigating global cognition, memory, attention and executive functions, language, visuospatial abilities, and mood. Supplementary Material presents details on the tests used.

Gait Analysis

A six-camera SMART-DX7000 (BTS Bioengineering, Italy) optoelectronic system was used to obtain spatio-temporal gait parameters. In particular, we acquired TUG to study the turning phase of gait and the 4-meter walking test (4MWT) to study straight walking parameters. Both TUG and 4MWT were also performed with a cognitive dual task (TUG-COG and 4MWT-COG). Moreover, we asked subjects to perform a gait initiation task to study the anticipatory postural adjustments (APA). A three-dimensional (3D) Kistler force platform type 9260AA was used to study APA during gait initiation and balance during steady stance. Supplementary Material presents details on the motion analysis protocol. Asymmetry between the right and left sides was calculated from upper- and lower-limb parameters as $\frac{\text{right}-\text{left}}{\max(\text{right};\text{left})} \times 100$. We calculated the dual-task cost (DTcost) as $\frac{\text{dual task}-\text{single task}}{\text{single task}} \times 100$.

MRI Study

Brain MRI scans were obtained using a 3.0-T MRI (Ingenia CX, Philips Medical Systems, Best, the Netherlands). 3D T1-weighted, 3D T2-weighted, fluid-attenuated inversion recovery, and RS-fMRI sequences were obtained.

The following analyses were performed: (1) whole-brain RS-FC analysis using MELODIC (Multivariate Exploratory Linear Optimized Decomposition into Independent Components)[32] in the FMRIB software library (FSL, version 5.0); (2) voxel-based morphometry analysis (VBM) using SPM12; (3) whole-brain vertex-by-vertex and regional cortical thickness analysis using FreeSurfer (version 6.0); (4) regional GM volume analysis of nucleus caudate, globus pallidus, putamen, thalamus, nucleus accumbens, amygdala, and hippocampus bilaterally using the FMRIB's Integrated Registration and Segmentation Tool in FSL; and (5) brainstem volumes (medulla oblongata, pons, midbrain, and superior cerebellar peduncle) using FreeSurfer (version 6.0). The Supplementary Material presents the complete MRI protocol and details on MRI analysis.

Statistical Analysis

Age, height, and education were compared between groups using analysis of variance (ANOVA) models (Bonferroni corrected for number of groups, $P < 0.05$) on rank-transformed data. We used a χ^2 test (Bonferroni corrected for number of groups, $P < 0.05$) to compare sex between groups. Neuropsychological, clinical, gait analysis, regional cortical thickness, and brain volume variables were rank transformed and compared between patients with iRBD and healthy controls using age-, sex-, and education-adjusted ANOVA models (Bonferroni corrected for number of groups, $P < 0.05$). Extreme outliers (values outside of the range: third quartile + 3*interquartile range; first quartile – 3*interquartile range) in neuropsychological, clinical, and gait analysis data were excluded from the analyses (subjects were excluded only from the analyses of parameters in which they were extreme outliers). Analyses were conducted using SPSS 25.

RS-fMRI data were compared using a general linear model, including 4D spatial maps of all participants as dependent variable. Analyses were restricted within the spatial RS network of interest using binary masks obtained by thresholding the corresponding Z map image ($Z > 2.3$). Nonparametric permutation tests (5000 permutations) were applied, and age, sex, and education were included as covariates. A family-wise error (FWE) correction for multiple comparisons was performed, implementing the threshold-free cluster enhancement using a significance threshold of $P < 0.05$.

VBM group comparisons were tested using a two samples *t* test in SPM12, adjusting for total intracranial volume, age, sex, and education. Results were assessed at $P < 0.05$ FWE corrected for multiple comparisons. Vertex-by-vertex cortical thickness analysis was performed using ANOVA models in FreeSurfer adjusting for age, sex, and education ($P < 0.05$, FDR corrected for multiple comparisons).

Correlation analysis between MRI data (structural and functional) and clinical, neuropsychological, and gait analysis results were performed using partial correlation (Bonferroni corrected for number of groups, $P < 0.05$) controlled for age, sex, and education. The same correlation analysis was used to study the relationships between motor and nonmotor clinical variables, and between gait analysis features and disease duration in iRBD subjects.

Results

Clinical and Neuropsychological Findings

Patients with iRBD and healthy controls had similar sociodemographic variables (Table 1). Patients presented motor and nonmotor signs, as shown by MDS-UPDRS and NMS scores (Table 1). Olfactory loss was the most frequently reported nonmotor symptom (41%) followed by gastrointestinal and urinary symptoms (27%) (Table 1). iRBD patients compared to healthy controls exhibited poorer scores in sleep questionnaires (Table 1), and in cognitive tests assessing verbal and nonverbal memory (Rey's list immediate and delayed recall, and Benson's figure recall), abstract reasoning (Raven's progressive matrices), and visuospatial abilities (copy of Benson figure) (Table S1). Patients with iRBD performed worse in the nine-hole peg test and the five-times-sit-to-stand test relative to healthy controls and took more steps to walk 10 meters (Table 1). We found no correlations between neuropsychological and MRI findings.

Gait Analysis

Patients with iRBD exhibited an increased asymmetry of arm-swing amplitude during 4MWT and an increased double support time and stride length variability during 4MWT-COG relative to healthy controls (Fig. 1; Tables S2 and S3). Both iRBD patients and healthy controls exhibited an increased stride time during 4MWT-COG relative to 4MWT. Only iRBD patients exhibited a significantly worsened mean walking speed, stride length, stride time asymmetry, double support time, cadence, stride time variability during 4MWT-COG relative to 4MWT, and reduced mean and peak turning velocity during TUG-COG relative to TUG (Fig. 2; Tables S2–S4). Healthy controls exhibited an increased asymmetry of arm-swing amplitude during 4MWT-COG relative to 4MWT (Fig. 1; Table S3). Patients with iRBD relative to healthy controls exhibited a significantly greater DTcost on 4MWT mean walking speed, stride length, and stride time asymmetry (Fig. 2). We found no significant differences between patients with iRBD and healthy controls in TUG, steady stance, and APA parameters (Tables S4 and S5).

In iRBD patients, a poorer NMS score correlated with a worse ESS score ($r = 0.72$, $P < 0.001$) and UPDRS-I ($r = 0.93$, $P < 0.001$) and with a worse asymmetry of arm-swing amplitude during 4MWT ($r = 0.62$, $P = 0.01$). Moreover, a worse performance at Rey's list immediate recall correlated with a higher DTcost on walking speed ($r = 0.41$, $P = 0.04$) and stride length ($r = 0.45$, $P = 0.02$) during 4MWT. We did not observe correlations between disease duration and gait analysis variables.

Resting-State Functional Magnetic Resonance Imaging

Relative to healthy controls, iRBD patients exhibited a reduced RS-FC in the left pallidum/putamen within the basal ganglia network (Fig. 3A; Table S6) and in the left calcarine sulcus, left superior and bilateral middle occipital gyri, and left middle temporal gyrus within the visuo-associative network (Fig. 3B; Table S6). A reduced RS-FC within the basal ganglia network correlated with a higher stride time variability during 4MWT (Fig. 3A; $r = -0.50$, $P = 0.01$) in iRBD subjects. Moreover, in iRBD patients, a reduced RS-FC within the visuo-associative network correlated with a reduced arm-swing amplitude during 4MWT (Fig. 3B; $r = 0.50$, $P = 0.01$) and 4MWT-COG (Fig. 3B; $r = 0.52$, $P = 0.01$). No correlations with other clinical variables were observed. No differences in the other RS-fMRI networks (anterior and posterior salience, anterior and posterior default mode, auditory, sensorimotor, primary visual, precuneus, visuospatial, and left and right executive control networks) were observed in patients relative to controls.

Voxel-Based Morphometry

VBM analysis exhibited a reduced GM volume of the left supramarginal gyrus in patients with iRBD relative to healthy controls (Fig. 4; Table S7). We found no significant correlations between VBM, clinical, gait analysis, and fMRI results.

Cortical Thickness and Volumetric Analysis

Both vertex-by-vertex and regional cortical thickness analyses showed no significant differences between patients with iRBD and healthy controls. Subcortical and brainstem volumes also did not differ between groups (Tables S8–S10).

TABLE 1 *Sociodemographic and clinical characteristics of healthy controls and patients with iRBD*

Characteristic	HC (N = 28)	iRBD (N = 38)	*P*, iRBD versus HC
Demographics			
Age (y)	63.86 ± 8.58 (51.14; 81.41)	65.76 ± 7.07 (51.88; 80.69)	0.36
Sex (M/F)	20/8	31/7	0.76
Height (m)	1.73 ± 0.09 (1.54; 1.90)	1.71 ± 0.08 (1.56; 1.89)	0.37
Education (y)	13.71 ± 3.97 (8; 27)	12.87 ± 4.11 (4; 18)	0.64
Sleep			
Disease duration (y)	–	6.45 ± 2.98 (5; 20)	–
Single Question Screen for RBD	0 ± 0 (0; 0)	1 ± 0 (1; 1)	<0.001
RBDSQ	0 ± 0 (0; 0)	9.76 ± 1.7 (6; 12)	<0.001
PSQI	3.04 ± 2.34 (0; 11)	6.42 ± 3.09 (1; 13)	<0.001
ESS	4.86 ± 3.53 (0; 15)	4.97 ± 4.12 (0; 21)	0.84
Clinical evaluations			
MDS-UPDRS-I	–	4.89 ± 3.50 (0; 13)	–
MDS-UPDRS-II	–	0.61 ± 1.35 (0; 7)	–
MDS-UPDRS-III	–	5.69 ± 2.49 (1; 11)	–
NMS	–	31.42 ± 22.09 (0; 68)	–
9HPT	21.28 ± 2.54 (16.39; 26.85)	23.98 ± 3.25 (16.50; 30.96)	0.001
10MWT—CS (s)	8.00 ± 0.97 (6.60; 10.77)	8.33 ± 1.05 (6.18; 10.87)	0.10
10MWT—MS (s)	6.05 ± 0.54 (5.23; 7.08)	6.18 ± 0.74 (4.60 ± 7.99)	0.41
10MWT—CS (steps)	13.93 ± 1.03 (12.00; 16.33)	14.53 ± 1.38 (11.67; 17.67)	0.02
10MWT—MS (steps)	12.19 ± 0.89 (11.00; 14.33)	12.57 ± 1.32 (10.30; 16.00)	0.18
5TSTS (s)	10.33 ± 2.11 (7.22; 15.47)	11.51 ± 2.55 (6.68; 16.20)	0.03
TUG (s)	9.29 ± 1.53 (6.91; 12.47)	9.53 ± 1.26 (7.40 ± 12.34)	0.47
TUG-COG (s)	10.20 ± 2.08 (6.76; 16.51)	11.23 ± 2.36 (8.02; 18.16)	0.14
Nonmotor symptoms			
Sleepiness	–	8%	–
Olfactory loss	–	41%	–
Gastrointestinal symptoms	–	27%	–
Urinary dysfunctions	–	27%	–
Sexual dysfunctions	–	23%	–
Orthostatic hypotension	–	3%	–

Note: Values are mean ± standard deviation (minimum; maximum). Categorical variables are reported as frequency. Age, height, and education were compared between groups using analysis of variance (ANOVA) models followed by post hoc pairwise comparisons (Bonferroni corrected for multiple comparisons, $P < 0.05$) on rank-transformed data. Age-, sex-, and education-adjusted ANOVA models (Bonferroni corrected for number of groups, $P < 0.05$) on rank-transformed data or χ^2 test for categorical variables on rank-transformed data Bonferroni corrected for multiple comparisons ($P < 0.05$) were used for the clinical variables.

Abbreviations: iRBD, patients with isolated rapid eye movement sleep behavior disorder; HC, healthy controls; N, number; M/F, male/female; RBDSQ, Rapid Eye Movement Sleep Behavior Disorder Screening Questionnaire; PSQI, Pittsburgh Sleep Quality Index; ESS, Epworth Sleepiness Scale; MDS-UPDRS-II or III, Movement Disorders Society Unified Parkinson's Disease Rating Scale Part II or Part III; NMS, Non-Motor Symptoms Scale; 9HPT, nine-hole pegboard test; 10MWT, 10-meter walking test; CS, comfortable speed; MS, maximal speed; 5TSTS, five-times sit-to-stand test; TUG, timed-up-and-go; TUG-COG, TUG with cognitive dual task.

Finally, as our results were influenced by patients with an MDS-UPDRS-III score >6 that was suggested as an indicator of increased risk of phenoconversion in the next few years,[33] we repeated the analysis excluding those subjects. Interestingly, we obtained the same gait analysis and MRI results, suggesting that our findings

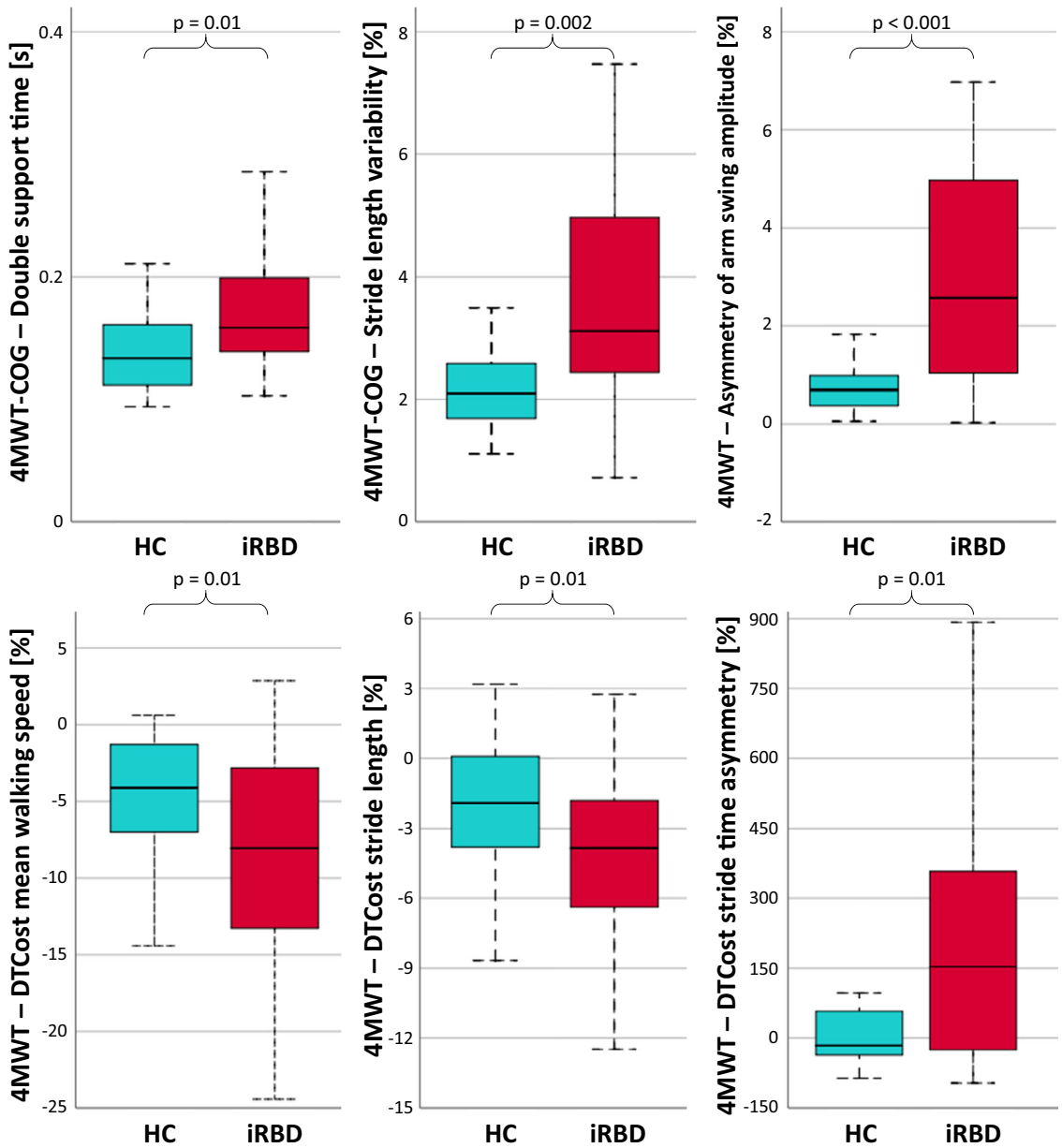

FIG. 1. Gait analysis parameters that were found to be significantly different between patients with iRBD and healthy controls. *P*-values refer to age-, sex-, and education-adjusted ANOVA (analysis of variance) models (Bonferroni corrected for number of groups, $P < 0.05$) on rank-transformed data. Variability was measured as coefficient of variation [(mean/standard deviation) × 100]. Asymmetry was calculated as [(left – right)/max(left; right)] × 100. DTcost was calculated as [(dual task – single task)/single task] × 100. 4MWT(-COG), 4-meter walking test (with cognitive dual task); DTcost, dual-task cost; HC, healthy controls; iRBD, patients with isolated rapid eye movement sleep behavior disorder; s, second. [Color figure can be viewed at wileyonlinelibrary.com]

are not a mere effect of the presence of subjects with MDS-UPDRS-III >6.

Discussion

In this study, we investigated the motor and non-motor clinical characteristics and their associated functional and structural neural correlates in a group of PSG-confirmed iRBD, with an average disease duration of ~6 years. As anticipated, the majority of patients already exhibited subtle signs of parkinsonism, the presence of autonomic failure and olfactory dysfunction, and low cognitive functioning. A notable strength of our study was the use of a motion analysis system, enabling us to objectively discern initial and subtle gait alterations that would not be easily identifiable through the mere visual observation. Moreover, the analysis of brain MRI data allowed us to identify RS-FC alterations of the basal ganglia and visuo-associative

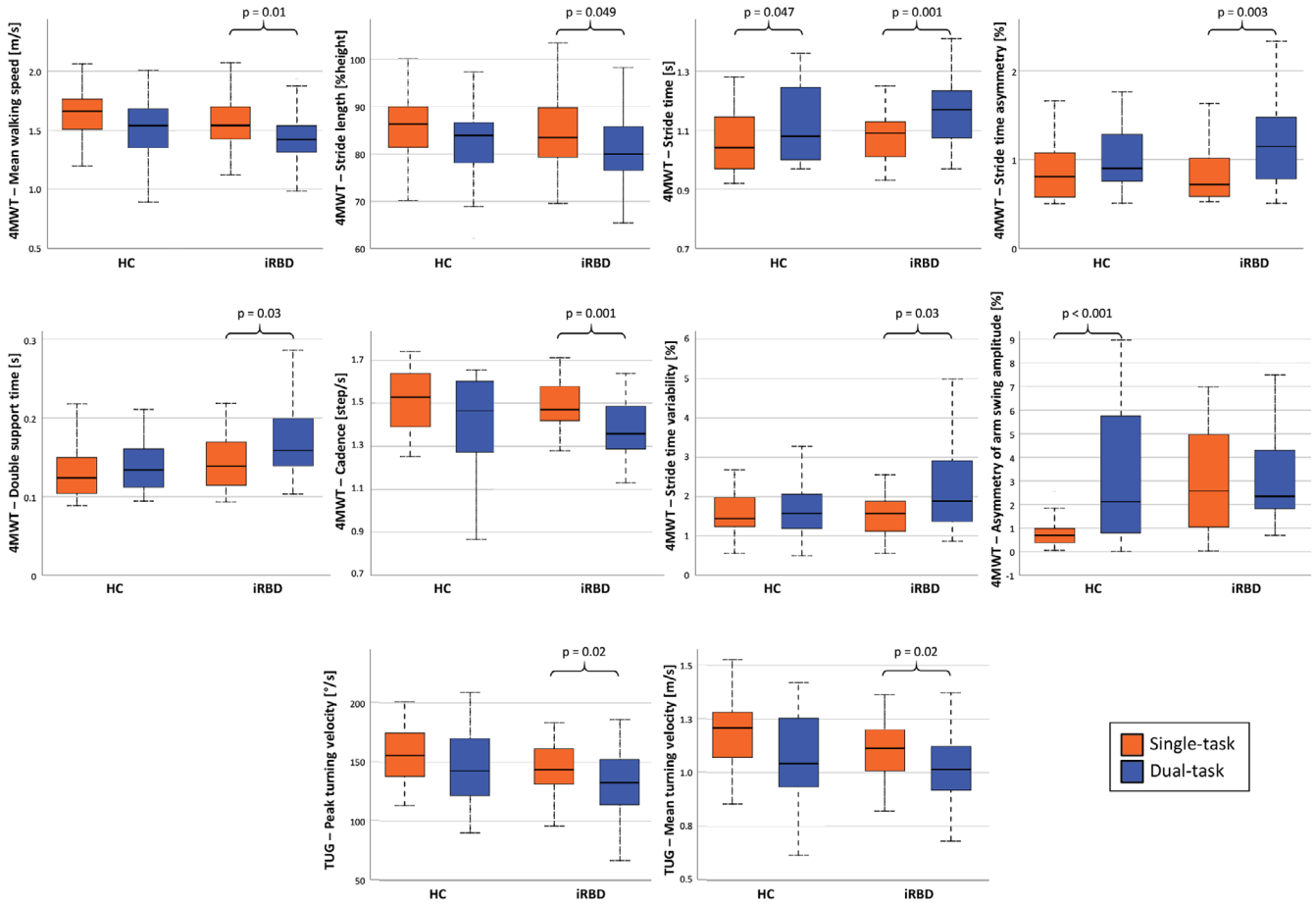

FIG. 2. Gait analysis parameters that were found to be significantly worsened by dual task in patients with iRBD and healthy controls (comparison between performance with and without the cognitive dual task). *P*-values refer to age-, sex-, and education-adjusted ANOVA (analysis of variance) models (Bonferroni corrected for number of groups, $P < 0.05$) on rank-transformed data. Variability was measured as coefficient of variation [(mean/standard deviation) × 100]. Asymmetry was calculated as [(left – right)/max(left; right)] × 100. 4MWT, 4-meter walking test; HC, healthy controls; iRBD, patients with isolated rapid eye movement sleep behavior disorder; m, meter; s, second; TUG, timed-up-and-go test. [Color figure can be viewed at wileyonlinelibrary.com]

networks, which correlated with clinical changes, together with a structural damage of the parietal lobe. To the best of our knowledge, this is the first study providing a comprehensive assessment of gait analysis alterations together with brain MRI hallmarks of PSG-confirmed iRBD.

Gait analysis results highlighted the presence of specific alterations of the locomotor pattern in iRBD patients, which might represent the underlying presence of a prodromal parkinsonism. Indeed, during straight gait we observed an asymmetry of arm-swing amplitude that has been already demonstrated in LRRK2-G2019S mutation carriers as a possible marker for prodromal PD.[16] Interestingly, a greater asymmetry of arm-swing amplitude correlated with worse nonmotor symptoms that may suggest a greater risk of phenoconversion in iRBD patients.[18] Additionally, as previously suggested,[16,17,34] walking under dual-task conditions revealed further challenges in gait that are typical in parkinsonian syndromes such as reduced mean walking speed, lower stride length and cadence, higher stride time asymmetry, double support time and stride time, and length variability. Dual task also worsened the ability to turn in iRBD patients relative to healthy controls. Indeed, iRBD exhibited a reduced mean and peak turning velocity during TUG-COG, which might represent reduced balance confidence during complex walking conditions. Overall, DTcost is higher in iRBD patients relative to healthy subjects, suggesting an earlier motor-cognitive overload as a result of a possible reduced motor-cognitive reserve. Further studies are needed to confirm these findings, but the presence of initial cognitive deficits in the mnemonic, visuospatial, and executive domains further corroborated our hypothesis supporting the use of dual-task walking/mobility assessments to highlight prodromal subtle gait changes in iRBD. It is worth noting that dual-task conditions can worsen gait parameters even in healthy subjects; thus the presence of a control group comparable for age is essential to interpret gait changes. For instance, in our study, both healthy controls and iRBD had an increased stride time during 4MWT-COG; thus, this

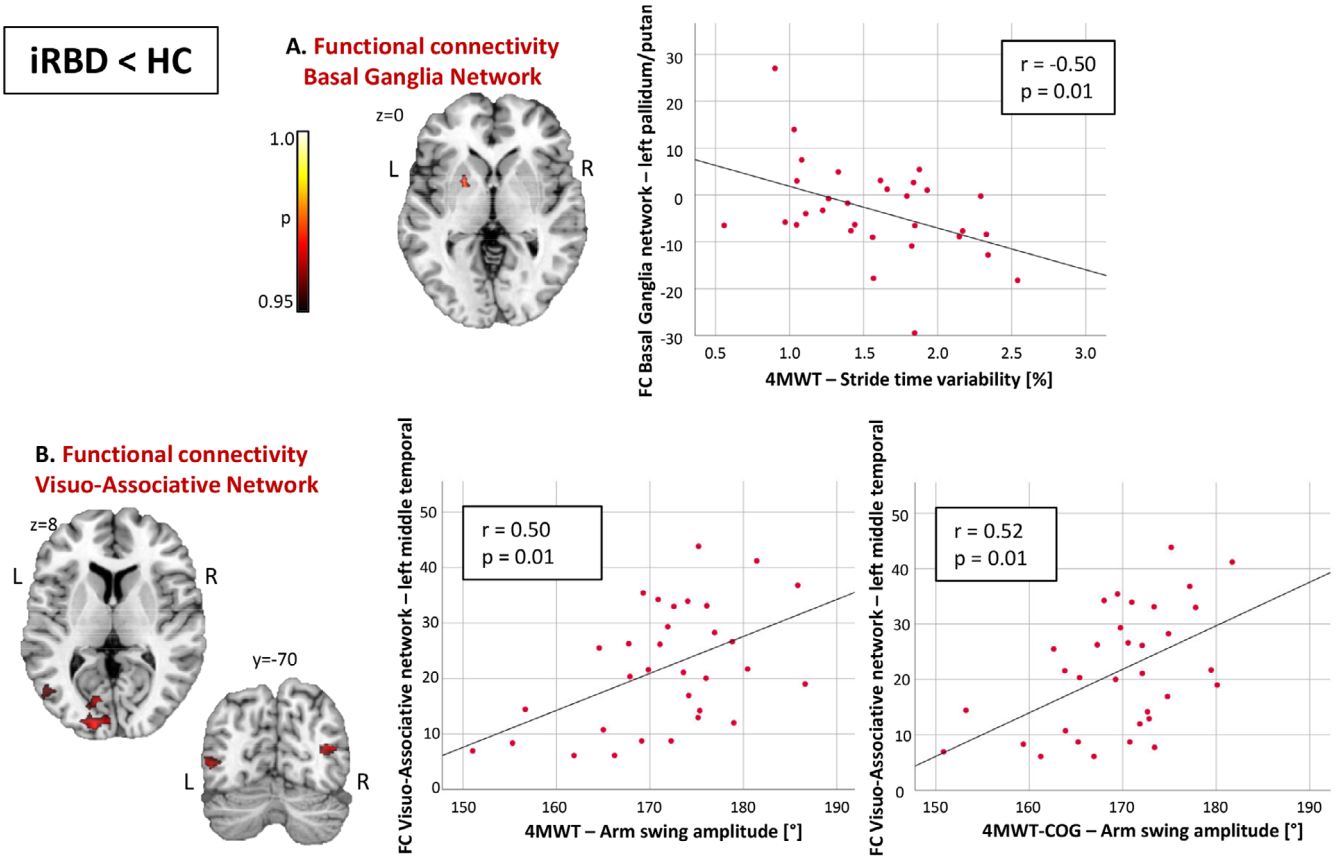

FIG. 3. Reduced functional connectivity within the basal ganglia (A) and visuo-associative (B) networks in patients with iRBD compared to healthy controls and correlations with clinical features. All findings are adjusted for age, sex, and education, and reported at $P < 0.05$ FWE (family-wise error) corrected. Results are shown on axial or coronal section of the Montreal Neurological Institute (MNI) standard brain (*z* or *y* MNI coordinates are reported). Color bars denote *P*-values. HC, healthy controls; iRBD, patients with isolated rapid eye movement sleep behavior disorder. [Color figure can be viewed at wileyonlinelibrary.com]

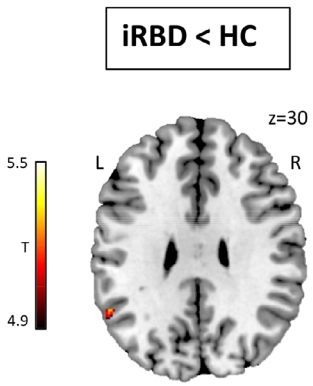

FIG. 4. Reduced gray matter volume at voxel-based morphometry (VBM) in patients with iRBD compared to healthy controls and correlations with functional MRI (magnetic resonance imaging) metrics. All findings are adjusted for age, sex, and education and shown at $P < 0.05$ FWE (family-wise error) corrected. Results are shown on an axial section of the Montreal Neurological Institute (MNI) standard brain (*x*, *y*, and *z* MNI coordinates are reported). Color bars denote *T*-values. HC, healthy controls; iRBD, patients with isolated rapid eye movement sleep behavior disorder. [Color figure can be viewed at wileyonlinelibrary.com]

variation could be considered an effect of the dual task. Moreover, only healthy subjects exhibited an increased asymmetry of arm-swing amplitude during 4MWT-COG relative to 4MWT. This result must be interpreted in terms of the presence of arm asymmetry in the walking test without dual task in subjects with iRBD: individuals with iRBD do not exacerbate their asymmetry because they are already asymmetric in the simpler condition, whereas it is normal for healthy subjects to exhibit a mild asymmetry of the upper-limb amplitude during dual-task gait. Interestingly, gait analysis changes were not correlated with disease duration, suggesting that, since the prodromal phases of parkinsonism, disease duration is not sufficient to explain sign/symptom severity.

Although nonpathological according to the Italian normative data, we noted lower scores in patients compared to controls in tests related to memory, executive functions, and visuospatial abilities. These observations align with a recent meta-analysis that reported that patients with iRBD exhibit significantly poorer performance in these cognitive domains relative to healthy controls.[10] Furthermore, a separate study indicated that assessments targeting attention, executive functions, and memory proved most effective in differentiating prodromal DLB patients from individuals with normal cognition.[35] Additionally, tests evaluating attention, executive functions, and visuospatial abilities were

found to be particularly useful differentiating prodromal DLB from parkinsonism in RBD patients.[35]

iRBD patients exhibited a diminished FC within the basal ganglia network, specifically in the left pallidum and putamen. This result aligns with prior studies[5,9] and supports the presence of an ongoing pathological process in the nigrostriatal pathway, which is typical of parkinsonian syndromes. This finding holds particular significance due to its specificity, as we employed a whole-brain approach rather than the seed-based method proposed in previous studies.[5,19,20] Notably, the reduced RS-FC within the basal ganglia correlated with an increased stride time variability during straight gait in iRBD. Such an increased variability is a common gait alteration seen in patients with parkinsonism and can manifest in the early stages of the disease.[36] Moreover, it has also been associated with cognitive decline in subjects with mild cognitive impairment.[37]

In addition to the involvement of dopaminergic pathways, the cholinergic system has been suggested to play a role in the manifestation of locomotor and cognitive deficits in parkinsonism, contributing to the presentation of complex gait signs such as freezing of gait.[38] We did not observe alterations in the midbrain, but future studies should delve into the analysis of basal forebrain and midbrain nuclei, such as the pedunculopontine nucleus, the nucleus basalis of Meynert, and the laterodorsal tegmental nucleus, to elucidate whether cholinergic system could be involved even in the prodromal phases of parkinsonian syndromes.[38] Moreover, also extramotor cortical regions (ie, frontal, parietal, and occipital) were suggested to contribute to gait alterations in α-synucleinopathies.[38] We found decreased RS-FC of the visuo-associative network in iRBD patients, which was correlated with a reduced movement amplitude of the upper limb during gait. As previously suggested by several studies, there is a strict relation between gait and cognitive domains, and our findings further support the role of cognitive areas in modulating motor behavior.[38,39] Previous studies established a correlation between reduced FC in temporal and parietal areas and cognitive decline in executive-attentive functions in iRBD, suggesting the involvement of posterior cortical networks even in the prodromal phase of parkinsonian syndromes.[40] Although we did not observe a relationship between visuo-associative FC alterations and the cognitive functioning of our iRBD patients, as previously mentioned, we did note that these patients, when compared to healthy controls, exhibited poorer performances in visuospatial abilities, nonverbal memory, and executive functions, which are associated with temporoparietal and occipital regions.[41] The involvement of posterior cortical areas is further substantiated by the reduced volume of the left supramarginal gyrus in iRBD patients. Parietal and occipital atrophy and thinning have been previously reported in iRBD patients, suggesting that these areas might be particularly vulnerable to iRBD and could possibly represent a preclinical manifestation of PD or DLB.[5,8,42] We did not observe correlations between MRI changes and clinical variables other than gait parameters, suggesting that gait analysis data might detect subtle yet significant changes. These changes, rather than scores obtained in other clinical assessments, might be particularly responsive to FC alterations of RS networks that are usually involved in parkinsonian syndromes.[38]

Notably, our MRI findings were mainly in the left hemisphere, suggesting its increased vulnerability to iRBD. Considering that clinical signs were equally distributed in our sample, lateralization of MRI findings may indicate early susceptibility of the dominant hemisphere, possibly due to higher metabolic demand and excitatory projections, leading to accelerated neurodegeneration.

We found no significant differences between patients with iRBD and healthy controls in TUG, steady stance, and APA parameters, as well as in cortical thickness, subcortical, and brainstem volumes. One possible explanation could be that our patients had a relatively short disease duration that may not be sufficient to observe alterations that are typical of clinically manifested parkinsonisms. Moreover, patients may exhibit heterogeneous brain damage based on the aggressiveness of the underlying pathology and the possibility to develop different parkinsonisms (ie, PD, DLB, MSA). Recent emerging evidence suggested the possible presence of distinct clinical subtypes of iRBD, but further studies are needed to define if specific iRBD subtypes can explain different disease trajectories.[4,18] We are following longitudinally our iRBD sample to study the conversion to clinically defined α-synucleinopathies. We aim to determine whether the integration of neurological, neuropsychological, gait analysis, and MRI measures facilitates the identification of iRBD phenotypes and the prediction of their conversion to parkinsonism. Currently, no validated biomarkers exist for this prediction, necessitating further studies with diverse biomarker combinations.

This study was not without limitations. The sample size was relatively small, and we performed an exploratory analysis without a strict apriori hypothesis; considering the exploratory nature of our study and the wide number of clinical variables, we presented clinical results corrected for the number of group comparisons to limit type 2 error. Additionally, to minimize the potential influence of confounding factors, we adjusted the analysis for age, sex, and education, as these variables can significantly impact motor, cognitive, and neuroimaging outcomes. Moreover, we analyzed the FC within the main RS networks obtained from the independent component analysis (MELODIC process). Future studies could expand the current knowledge in this field employing more sophisticated connectome-based methodologies. Another important point to address is that all our iRBD patients were treated with clonazepam and/or

melatonin to manage nocturnal motor symptoms, following the clinical practice guidelines of the American Academy of Sleep Medicine.[43] However, considering the very low dosage of clonazepam (mean: 0.3 ± 0.2 mg), we did not expect any significant effect on clinical and fMRI data. Furthermore, our sample included 10 patients with a UPDRS-III score >6, which has been suggested to correlate with a higher likelihood of phenoconversion.[33] To rule out any influence from these subjects, we repeated all the analyses without them, and results remained unchanged.

In conclusion, our study offered a compelling and thorough insight into clinical, gait analysis, and neuroimaging alterations in patients with iRBD. Interestingly, our findings demonstrated subtle gait changes that may signify the initial signs of neurodegeneration, coupled with functional brain alterations in basal ganglia. These data, when collected longitudinally and in larger samples, hold promise as potential biomarkers for predicting and monitoring the course of iRBD. Future investigations, employing machine learning techniques, will hopefully identify the most relevant features to predict the phenoconversion from iRBD to specific parkinsonisms, significantly enhancing the management of patients in clinical settings. ■

Author Contributions

Elisabetta Sarasso: Conception and design; Data acquisition; Data analysis; Manuscript drafting; Manuscript editing. **Andrea Gardoni:** Data acquisition; Data analysis; Manuscript drafting; Manuscript editing. **Sara Marelli:** Conception and design; Data acquisition; Data analysis; Manuscript drafting; Manuscript editing. **Roberta Balestrino:** Data acquisition; Data analysis; Manuscript editing. **Lucia Zenere:** Data acquisition; Data analysis; Manuscript drafting; Manuscript editing. **Alessandra Castelnuovo:** Data acquisition; Data analysis; Manuscript editing. **Massimo Malcangi:** Data acquisition; Data analysis; Manuscript editing. **Silvia Basaia:** Data analysis; Manuscript drafting; Manuscript editing. **Andrea Grassi:** Data acquisition; Data analysis; Manuscript editing. **Andrea Tettamanti:** Conception and design; Data analysis; Manuscript editing. **Elisa Canu:** Conception and design; Data acquisition; Data analysis; Manuscript drafting; Manuscript editing. **Luigi Ferini-Strambi:** Conception and design; Data analysis; Manuscript editing. **Massimo Filippi:** Conception and design; Data analysis; Manuscript editing. **Federica Agosta:** Conception and design; Data acquisition; Data analysis; Manuscript editing.

Data Availability Statement

The dataset used and analyzed during the current study is available from the corresponding author upon request to qualified researchers (i.e., affiliated to a university or research institution/hospital).

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Supporting Data

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(1) Research project: A. Conception, B. Organization, C. Execution; (2) Statistical analysis: A. Design, B. Execution, C. Review and critique; (3) Manuscript: A. Writing of the first draft, B. Review and critique.

E.S.: 1A, 1B, 1C, 2A, 2B, 2C, 3A, 3B
A.G.: 1B, 1C, 2A, 2B, 2C, 3A, 3B
S.M.: 1A, 1B, 1C, 2C, 3A, 3B
R.B.: 1C, 2C, 3B
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Full financial disclosures of all authors for the preceding 12 months

E.S., E.C., S.B., S.M., and R.B, have received research support from the Italian Ministry of Health. A.Ga., L.Z., M.M., A.Gr., A.C., and A.T. have nothing to disclose. L.F.-S. is field editor of the journal *Sleep Medicine* journal, associate editor of the *Journal of Alzheimer's Disease*, and member of the editorial board of the *European Journal of Neurology*, and *Behavioral Neurology*; L.F.-S. has received speaker honoraria from Biprojet, Idorsia, Italfarmaco, and Takeda, and receives or has received research support from the Italian Ministry of Health and the Italian Ministry of University and Research. M.F. is editor in chief of the *Journal of Neurology* and associate editor of *Human Brain Mapping*, *Neurological Sciences*, and *Radiology*; received compensation for consulting services from Alexion, Almirall, Biogen, Merck, Novartis, Roche, and Sanofi; received speaking activity fees from Bayer, Biogen, Celgene, Chiesi Italia SpA, Eli Lilly, Genzyme, Janssen, Merck-Serono, Neopharmed Gentili, Novartis, Novo Nordisk, Roche, Sanofi, Takeda, and Teva; and is an advisory board member of Alexion, Biogen, Bristol-Myers Squibb, Merck, Novartis, Roche, Sanofi, Sanofi-Aventis, Sanofi-Genzyme, Takeda; he provided scientific direction of educational events for Biogen, Merck, Roche, Celgene, Bristol-Myers Squibb, Lilly, Novartis, Sanofi-Genzyme; he receives research support from Biogen Idec, Merck-Serono, Novartis, Roche, Italian Ministry of Health, the Italian Ministry of University and Research, and Fondazione Italiana Sclerosi Multipla. F.A. is associate editor of *NeuroImage: Clinical*; has received speaker honoraria from Biogen Idec, Italfarmaco, Roche, Zambon, and Ely Lilli; and receives or has received research support from the Italian Ministry of Health, the Italian Ministry of University and Research, AriSLA (Fondazione Italiana di Ricerca per la SLA), the European Research Council, the EU Joint Programme—Neurodegenerative Disease Research (JPND), and the Foundation Research on Alzheimer Disease (France).