Presynaptic Dopaminergic Imaging Characterizes Patients with REM Sleep Behavior Disorder Due to Synucleinopathy

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International REM Sleep Behavior Disorder Study Group

Objective: To apply a machine learning analysis to clinical and presynaptic dopaminergic imaging data of patients with rapid eye movement (REM) sleep behavior disorder (RBD) to predict the development of Parkinson disease (PD) and dementia with Lewy bodies (DLB).

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Methods: In this multicenter study of the International RBD study group, 173 patients (mean age 70.5 ± 6.3 years, 70.5% males) with polysomnography-confirmed RBD who eventually phenoconverted to overt alpha-synucleinopathy (RBD due to synucleinopathy) were enrolled, and underwent baseline presynaptic dopaminergic imaging and clinical assessment, including motor, cognitive, olfaction, and constipation evaluation. For comparison, 232 RBD non-phenoconvertor patients (67.6 ± 7.1 years, 78.4% males) and 160 controls (68.2 ± 7.2 years, 53.1% males) were enrolled. Imaging and clinical features were analyzed by machine learning to determine predictors of phenoconversion.
Results: Machine learning analysis showed that clinical data alone poorly predicted phenoconversion. Presynaptic dopaminergic imaging significantly improved the prediction, especially in combination with clinical data, with 77% sensitivity and 85% specificity in differentiating RBD due to synucleinopathy from non phenoconverted RBD patients, and 85% sensitivity and 86% specificity in discriminating PD-converters from DLB-converters. Quantification of presynaptic dopaminergic imaging showed that an empirical *z*-score cutoff of −1.0 at the most affected hemisphere putamen characterized RBD due to synucleinopathy patients, while a cutoff of −1.0 at the most affected hemisphere putamen/caudate ratio characterized PD-converters.
Interpretation: Clinical data alone poorly predicted phenoconversion in RBD due to synucleinopathy patients. Conversely, presynaptic dopaminergic imaging allows a good prediction of forthcoming phenoconversion diagnosis. This finding may be used in designing future disease-modifying trials.

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Patients with rapid eye movement (REM) sleep behavior disorder (RBD) are at high risk of developing parkinsonism and/or dementia over time.[1] Currently, patients with RBD without overt neurological signs or symptoms are considered to have idiopathic or isolated RBD (iRBD), despite the occasional presence of subtle or mild cognitive impairment (MCI), mild motor symptoms not fulfilling the criteria for Parkinson disease (PD), dysautonomic symptoms, or even mild nigrostriatal dopaminergic deafferentation. However, most iRBD patients have biological presence of abnormal alpha-synuclein in skin biopsy[2] and in cerebrospinal fluid.[3] Thus, considering that the vast majority of cases eventually will develop overt alpha-synucleinopathies (ie, PD, dementia with Lewy bodies (DLB) or multiple system atrophy [MSA]) if studied with a long-term follow-up,[4] most iRBD patients should be more appropriately considered patients with RBD due to alpha-synucleinopathy (RBD-syn), though in a prodromal clinical stage. This concept is in line with the recent proposals for a biological staging of alpha-synucleinopathies, suggesting that there should be no clear distinction between prodromal and overt disease.[5,6]

Putative disease-modifying treatments targeting alpha-synuclein are in development, and some of them are being tested in PD patients. However, to date clinical trials using monoclonal antibodies directed against aggregated alpha-synuclein are failing to achieve both clinical and imaging efficacy endpoints in PD cohorts.[7,8] These negative results may be due to the testing of disease-modifying therapies too late, in patients with overt neurodegenerative diseases and more advanced stage of neurodegeneration compared with those in the prodromal stages. Conversely, using them in prodromal stages of alpha-synucleinopathies, such as RBD-syn patients, may increase the likelihood of preserving both function and structure. Indeed, a recent proof-of-concept study suggested that disease-modifying trials are feasible in RBD-syn patients.[9]

Identifying RBD-syn patients, especially those at high risk of short-term phenoconversion, among the whole iRBD spectrum may be challenging. Several predictors of phenoconversion in iRBD patients have been proposed,[10] but reliable biomarkers able to predict the development of parkinsonism or dementia first are missing. Even if these 2 entities may be considered 2 clinical manifestations of the same biological disease

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(ie, neuronal alpha-synuclein disease), it may be important to predict the clinical outcome phenotype in designing clinical trials where a specific phenoconversion diagnosis would be required, for example PD-converters or DLB-converters. [^{123}I]FP-CIT-SPECT (DaT-SPECT) measures basal ganglia dopamine transporter (DaT) density, and it is a marker of nigrostriatal dopaminergic function. DaT-SPECT has been suggested to be a good predictor of short-term phenoconversion in RBD-syn patients, both in single center[11] and in multicenter studies.[12] Moreover, DaT-SPECT also provided promising preliminary results in differentiating PD-converters from DLB-converters.[12]

The aim of the present study is to apply a machine learning approach on a large dataset of RBD-syn patients collected within the International RBD study group, to evaluate the ability of DaT-SPECT in differentiating RBD-syn patients (ie, phenoconverted on a short-time) from those patients not yet phenoconverterd, as well as PD-converters from DLB-converters.

Materials and Methods

Subjects

This is a retrospective international multicenter study including 13 centers worldwide (Barcelona, Berlin, Bologna, Cagliari, Dokkyo, Genoa, Kosice, Montpellier, Oxford, Pavia, Prague, Rochester, and Rome Tor Vergata). All patients received a diagnosis of polysomnography-confirmed iRBD, according to international criteria,[13] and underwent baseline DaT-SPECT when still 'idiopathic' (ie, without overt parkinsonism and/or dementia). All centers prospectively followed patients with in-person evaluation to assess phenoconversion, and phenoconverted patients were first selected for the study. All phenoconverted patients developed PD, DLB, or MSA over time; thus, all of them had RBD due to a synucleinopathy (RBD-syn) at the time of SPECT. Parkinsonism was defined as bradykinesia plus at least one of rigidity or rest tremor,[14] and dementia was defined as functional impairment in instrumental activities of daily living with evidence of cognitive impairment on standardized testing.[15] For patients with parkinsonism as the primary disease manifestation, the diagnosis (PD/MSA) was made according to the treating neurologist, by following current criteria.[14,16] The differential diagnosis incorporated all available follow-up information (ie, any patient who was initially diagnosed as having PD at phenoconversion, but who was subsequently found to have MSA would be included as affected with multiple system atrophy). For dementia converters, all met the 2017 criteria for probable DLB.[17]

For comparisons, from the same participating centers we enrolled a group of polysomnography-confirmed iRBD patients not phenoconverted at last available follow-up (RBD-nc). Moreover, all centers also sent DaT-SPECT data in Digital Imaging and Communications in Medicine (DICOM) format and demographic data of controls, in the same age range of patients, who were judged to be free of a synucleinopathy at the end of a full diagnostic work-up and, thus, including miscellaneous conditions, such as functional or essential tremor, vascular or drug-induced parkinsonism, depression, and others. The presence of RBD in these subjects was excluded by clinical interview.

All participants signed an informed consent form in compliance with the Helsinki Declaration of 1975. Ethics approval was obtained from the local institutional boards in all participating centers, and the study was also approved by the institutional board of the coordinating center (184REG2017).

[^{123}I]-Ioflupane SPECT ([^{123}I]FP-CIT-SPECT)

All subjects underwent [^{123}I]FP-CIT-SPECT (DaT-SPECT) as a marker of nigrostriatal dopaminergic functioning. Images were acquired after i.v. administration of 156.7 ± 26.2 MBq of [^{123}I] FP-CIT (DaTSCAN, GE Healthcare, Little Chalfont, Buckinghamshire, UK) according to the European Association of Nuclear Medicine (EANM) guidelines[18,19]. The adopted uptake time between injection and images acquisition in the involved centers was between 3 to 4 h (with mean acquisition time of 33.2 ± 9.2 min). DaT-SPECT images were exported in DICOM format and sent to the coordinating center (Genoa) for analysis. DICOM raw data projection images were sent to the coordinating center, and studies were centrally reconstructed (OSEM: 10 subset, 10 iterations; 0.6 Butterworth filter, correction for attenuation based on Chang method). Given the retrospective nature of the present study, no phantom-based harmonization across different gamma cameras could be a priori performed (see the discussion for a deeper comment on this limitation). Quality of images was checked by a Nuclear Medicine specialist with specific expertise in dopaminergic imaging (S.M.).

DaTQUANT™ V2 software (GE Healthcare) was used for semi-quantification of DaT-SPECT images. The basal ganglia specific to non-displaceable binding ratios (SBRs) were computed as (nucleus uptake – background uptake)/background uptake in the bilateral striatum, putamen, anterior putamen, posterior putamen, and caudate. Moreover, putamen/caudate ratios as well as putamen and caudate asymmetries were computed. Bilateral occipital lobes were used as the background reference region. Instead of the DICOM files, the Rochester center sent the semi-quantified data using the same version of DaTQUANT™, and the same reconstruction protocol. DaTQUANT™ software was chosen because it is one of the most used software worldwide, and it was already used in a previous multicenter study of the IRBDSG.[12]

To compute the z-scores for all basal ganglia features in all subjects, we used a normal dataset based on 118 healthy volunteers (no first-degree blood relatives affected by PD; 73 men and 45 women, aged 31 to 84 years) belonging to the PPMI database (more details can be found at https://www.ppmi-info.org), already included in DaTQUANT™.

No clear guidelines are available for defining when DaT-SPECT should be considered abnormal; thus, we chose to use the most common criteria that identify DaT-SPECT as abnormal when at least one of the putamen had a z-score < −1.5,[20] even if different cutoff values have been proposed.[21] For example, a recent study showed that a z-score cutoff of −1.27 at putamen level best differentiated PD patient from essential tremor patients,

while a cutoff of −0.96 was the most accurate in supporting DLB diagnosis.[22]

For statistical analysis, images were flipped to have the most affected hemisphere (MAH) and the least affected hemisphere (LAH) (ie, the highest/lowest value between left and right hemisphere, respectively) on the same side for all patients (see discussion paragraph for a deeper comment on this choice).

Baseline Clinical Variables

Baseline clinical variables were collected within 3 months from SPECT. Available baseline clinical variables included: (1) Movement Disorder Society-sponsored revision of the Unified Parkinson's Disease Rating Scale, Motor section (MDS-UPDRS-III)[23] for standardized motor examination (scores from the 1987 version of the UPDRS-III were converted into MDS-UPDRS-III[24] scores which were used for statistical analysis); (2) Mini-Mental state examination (MMSE)[25] or Montreal Cognitive Assessment (MoCA)[26] as a marker of global cognition; MoCA scores were converted into MMSE scores[27] since the majority of subjects underwent the MMSE, and only MMSE was used for statistical analysis; (3) SCOPA-AUT,[28] constipation questionnaire,[29,30] or clinical interview to assess constipation; (4) 40-item University of Pennsylvania Smell Identification Test[31], Sniffin' Sticks 16 items odor identification test[32] or Odor Stick Identification Test for Japanese[33] to assess olfaction. For statistical analysis, constipation and hyposmia have been dichotomized as abnormal or normal according to the cutoff point of each test.

Statistical Analysis

A first descriptive analysis was performed to verify the differences between RBD-syn patients and controls and to investigate the center effect. For this step, a principal component analysis was applied to DaT-SPECT data to reduce the number of the variables and to explore the characteristics of basal ganglia features in both RBD-syn patients and controls. Between-group differences were assessed using the unpaired *t*-test for continuous variables and the chi-squared test for categorical variables. Then, a general linear model was applied to investigate whether the center effect significantly interfered in the discrimination between patients and controls. Linear discriminant analysis[34] was applied to compute specificity and sensitivity of the DaT-SPECT data in discriminating patients from controls, RBD-syn (phenoconverted) from RBD-nc (non phenoconverted) patients, and subsequently PD-converters and DLB-converters.

Three machine learning approaches[35], namely (1) Decision Trees,[36] (2) Support Vector Machine,[37] and (3) K-Nearest Neighbors[38] were used to investigate the ability of DaT-SPECT in differentiating RBD-syn patients from controls, computing specificity and sensitivity of each approach. The machine learning analysis was performed using a training set (80% of the sample), and a testing set (20%). Ten stratified random identifications of the training and the testing sets have been applied,[35] and the testing set results are shown.

To investigate whether baseline DaT-SPECT and clinical data were able to predict the phenoconversion, as well as phenoconversion trajectories (ie, PD-converters versus DLB-converters), again 3 machine learning approaches have been applied (Decision Trees, Support Vector Machine, and K-Nearest Neighbors), computing specificity and sensitivity of each approach. For the PD-converters versus DLB-converters machine learning analysis, a revised split on training set (90% of the sample), and a testing set (10%) was used, due to the set of patients having a smaller size with respect to the previous analyses. Ten stratified random identifications of the training and the testing sets have been applied, and the testing set results are shown. Machine learning analysis results have been compared using the McNamar test.[39]

Analyses were performed using Matlab (MathWorks, Natick, MA) and Stata (StataCorp. 2013. Stata Statistical Software: Release 13. College Station, TX: StataCorp LP).

Results

Descriptive Analysis

We enrolled 173 RBD-syn patients (mean age 70.5 ± 6.3 years, 70.5% males), 232 RBD-nc patients (mean age 67.6 ± 7.1 years, 78.4% males), and 160 controls (mean age 68.2 ± 7.2 years, 53.1% males) from 13 centers worldwide (Table 1). After 41.1 ± 30.0 months from SPECT, 93 patients (54.3%) developed PD, 74 (42.8%) developed DLB, and 5 (2.9%) developed MSA. Main clinical, demographic, and DaT-SPECT data are summarized in Table 2. As expected, RBD-syn patients were older, had worse clinical indexes, as well as DaT-SPECT metrics compared with RBD-nc patients. All patients had motor and cognitive assessment, while 287 (70.9%) had

TABLE 1. Participating Centers

Center Name	Controls	RBD-syn	RBD-nc
Barcelona	9	79	69
Berlin	6	3	3
Bologna	4	4	6
Cagliari	10	5	29
Dokkyo	7	10	11
Genoa	81	22	24
Kosice	2	1	5
Montpellier	4	4	14
Oxford	6	3	0
Pavia	3	4	12
Prague	8	13	24
Rochester	5	12	19
Rome Tor Vergata	15	13	16

RBD = rapid eye movement sleep behavior disorder.

TABLE 2. Main Clinical and Demographic Data of REM Sleep Behavior Disorder (RBD)-syn (Phenoconverted) and RBD-nc (Not Phenoconverted) Patients

Parameter	RBD-nc	RBD-syn	*p*-Value
N	232	173	
Age, years	67.6 ± 7.1 [68]	70.5 ± 6.3 [71]	**<0.001**
Sex, males (%)	78.4%	70.5%	**<0.001**
Baseline clinical data			
MMSE score	28.0 ± 2.4 [28.7]	27.1 ± 2.5 [28]	**<0.001**
MDS-UPDRS-III score	1.8 ± 2.5 [1]	3.8 ± 3.8 [3]	**<0.001**
Hyposmia, (%)	50.0%	71.2%	**<0.001**
Constipation, (%)	34.6%	68.6%	**<0.001**
Follow-up data			
PD-converters, *n* (%)		94 (54.3%)	
DLB-converters, *n* (%)		74 (42.8%)	
MSA-converters, *n* (%)		5 (2.9%)	
Follow-up time, months	40.4 ± 30.6 [36]	41.1 ± 30.0 [36]	0.72
Baseline DaT-SPECT data			
DaT-SPECT abnormal,[a] (%)	19.4%	62.4%	**<0.001**
MAH Striatum, z-score	−0.26 ± 1.34 [−0.45]	−1.54 ± 1.25 [−1.57]	**<0.001**
LAH Striatum, z-score	0.04 ± 1.41 [−0.10]	−1.16 ± 1.27 [−1.14]	**<0.001**
MAH Putamen, z-score	−0.37 ± 1.40 [−0.48]	−1.67 ± 1.27 [−1.74]	**<0.001**
LAH Putamen, z-score	−0.04 ± 1.34 [−.26]	−1.28 ± 1.30 [−1.33]	**<0.001**
MAH Caudate, z-score	−0.14 ± 1.30 [−0.29]	−1.20 ± 1.19 [−1.25]	**<0.001**
LAH Caudate, z-score	0.24 ± 1.37 [0.06]	−0.76 ± 1.22 [−0.80]	**<0.001**
MAH Anterior Putamen, z-score	−0.38 ± 1.36 [−0.50]	−1.60 ± 1.23 [−1.65]	**<0.001**
LAH Anterior Putamen, z-score	−0.02 ± 1.34 [−0.20]	−1.16 ± 1.31 [−1.25]	**<0.001**
MAH Posterior Putamen, z-score	−0.43 ± 1.34 [−0.48]	−1.72 ± 1.29 [−1.82]	**<0.001**
LAH Posterior Putamen, z-score	−0.04 ± 1.37 [−0.04]	−1.21 ± 1.33 [−1.32]	**<0.001**
MAH Putamen/Caudate ratio, z-score	−0.56 ± 1.17 [−0.50]	−1.06 ± 1.39 [−1.04]	**<0.001**
LAH Putamen/Caudate ratio, z-score	−0.31 ± 1.25 [−0.32]	−0.38 ± 1.57 [−0.58]	0.64
Caudate asymmetry, z-score	0.12 ± 1.17 [−0.26]	0.48 ± 1.47 [0.16]	**0.007**
Putamen asymmetry, z-score	0.27 ± 1.45 [−0.17]	0.78 ± 1.69 [0.35]	**0.001**

Note: Continuous variables are reported as mean ± standard deviation [median]. In bold are highlighted the significant *p*-values.
DaT = dopamine transporter; DLB = dementia with Lewy bodies; MDS-UPDRS-III = Movement Disorder Society-sponsored revision of the Unified Parkinson's Disease Rating Scale, Motor section; MMSE = Mini-Mental State Examination; MSA = Multiple System Atrophy; PD = Parkinson disease.
[a]DaT-SPECT was considered abnormal when at least one of the putamen had a z-score < −1.5.

olfaction assessment, and 397 (98.0%) had constipation assessment.

Table 3 summarizes main demographic, clinical, and DaT-SPECT data of RBD-syn patients, according to the phenoconversion diagnosis. MSA patients are not reported in the table because of the low number of subjects. Compared with DLB-converters, PD-converters were younger, more often females, had higher baseline MDS-UPDRS-III scores, and tended to have higher MMSE scores. As for the DaT-SPECT data, PD-converters tended to have lower z-scores in all imaging features, in particular showing lower putamen/caudate ratios.

TABLE 3. Main Demographic, Clinical, and DaT-SPECT Data of RBD-syn Patients, According to the Phenoconversion Diagnosis

Parameter	PD converters	DLB converters	*p*-Value
N	94	74	
Age at SPECT, years	69.7 ± 5.8 [69]	72.1 ± 6.1 [73]	**0.01**
Sex, males, *n* (%)	58 (61.7%)	61 (82.4%)	**0.003**
Baseline data			
MMSE	27.4 ± 2.5 [28]	26.7 ± 2.4 [27]	0.08
MDS-UPDRS-III	4.5 ± 4.4 [4]	2.9 ± 2.9 [2]	**0.01**
Hyposmia, (%)	69.5%	78.7%	0.28
Constipation, (%)	65.2%	73.6%	0.25
DaT-SPECT abnormal,[a] (%)	62.8%	62.2%	0.94
MAH Striatum, z-score	−1.59 ± 1.22 [−1.65]	−1.47 ± 1.29 [−1.45]	0.55
LAH Striatum, z-score	−1.17 ± 1.25 [−1.13]	−1.13 ± 1.29 [−1.24]	0.86
MAH Putamen, z-score	−1.75 ± 1.26 [−1.79]	−1.57 ± 1.29 [−1.58]	0.35
LAH Putamen, z-score	−1.31 ± 1.31 [−1.4]	−1.23 ± 1.30 [−1.26]	0.66
MAH Caudate, z-score	−1.19 ± 1.15 [−1.24]	−1.20 ± 1.25 [−1.28]	0.99
LAH Caudate, z-score	−0.71 ± 1.22 [−0.66]	−0.82 ± 1.24 [−0.89]	0.57
MAH Anterior Putamen, z-score	−1.65 ± 1.21 [−1.66]	−1.53 ± 1.25 [−1.56]	0.52
LAH Anterior Putamen, z-score	−1.24 ± 1.25 [−1.24]	−1.07 ± 1.39 [−1.26]	0.43
MAH Posterior Putamen, z-score	−1.83 ± 1.31 [−1.91]	−1.58 ± 1.27 [−1.72]	0.21
LAH Posterior Putamen, z-score	−1.32 ± 1.33 [−1.42]	−1.06 ± 1.32 [−1.27]	0.22
MAH Putamen/Caudate ratio, z-score	−1.21 ± 1.40 [−1.06]	−0.87 ± 1.37 [−0.98]	0.11
LAH Putamen/Caudate ratio, z-score	−0.61 ± 1.53 [−0.7]	−0.09 ± 1.56 [−0.4]	**0.03**
Caudate asymmetry, z-score	0.52 ± 1.45 [0.19]	0.43 ± 1.51 [−0.02]	0.69
Putamen asymmetry, z-score	0.90 ± 1.78 [0.43]	0.63 ± 1.58 [0.4]	0.31
Follow-up data			
Phenoconversion time after SPECT, months	40.5 ± 28.5 [34]	42.9 ± 32.6 [36]	0.61

Note: Continuous variables are reported as mean ± standard deviation [median]. In bold are highlighted the significant *p* values.
DaT = dopamine transporter; F = female; LAH = least affected hemisphere; M = male; MAH = most affected hemisphere; MDS-UPDRS-III = Movement Disorder Society-sponsored revision of the Unified Parkinson's Disease Rating Scale, Motor section; MMSE = Mini-Mental State Examination.
[a]DaT-SPECT was considered abnormal when at least one of the putamen had a z-score < −1.5.

Principal Component Analysis and Linear Discriminant Analysis

The principal component analysis, applied to both DaT-SPECT SBRs and z-scores, identifies 3 main components (Table 4), reflecting basal ganglia measured values (first component), ratios (second component), and asymmetries (third component). As expected, all basal ganglia regions (first component) are highly correlated between each other (component loadings close to 1). The 3 components obtained from the DaT-SPECT SBRs were highly correlated with the equivalent components obtained using the z-scores ($p < 0.0001$, $r > 0.8$), as evident from the similarity of their loading pattern (the exchange between second and third component role is purely contingent given the component order is only dependent by their eigenvalue) in the whole dataset. Thus, the z-scores only were used for the subsequent analyses, because this choice allows to minimize the center differences in SPECT equipment and image reconstruction parameters. In fact, DaTQUANT™ takes into account these variables in generating z-scores.

The general linear model analysis showed that only the first component (including all basal ganglia regions) significantly differentiated RBD-syn patients from controls ($p < 0.0001$), using the center as a confounding variable. This preliminary analysis showed that the center effect did not significantly interfere with the discrimination ability of DaT-SPECT z-scores; thus, it will not be used as a confounder for the subsequent analysis. The lack of a significant center-effect might be related to a "first-order" harmonization performed computing the z-scores with regard to the use of common acquisition and reconstruction parameters.

The linear discriminant analysis showed that the DaT-SPECT data discriminated RBD-syn patients from controls with 75.1% sensitivity and 85.5% specificity. RBD-syn patients were discriminated from RBD-nc with 67.7% sensitivity and 72.6% specificity. However, the linear analysis failed in the discrimination between PD and DLB converters. Indeed, no statistically relevant difference between the 2 groups was highlighted for the main principal components. This prompted us to directly shift to a non-linear most sophisticated approach.

Machine Learning Analysis. RBD-Syn Patients Versus Controls

The machine learning analysis was applied to the DaT-SPECT z-scores to investigate their ability in differentiating RBD-syn patients from controls. Decision tree showed 0.89 ± 0.05 sensitivity, 0.84 ± 0.05 specificity, and 0.14 ± 0.03 error rate. Support vector machine showed 0.86 ± 0.07 sensitivity, 0.89 ± 0.05 specificity and 0.12 ± 0.03 error rate. K-Nearest Neighbors showed 0.88 ± 0.05 sensitivity, 0.88 ± 0.05 and 0.12 ± 0.03 error rate. Adding the clinical data did not significantly change the results ($p > 0.05$). According to the decision tree algorithm, a z-score lower than -0.92 in the MAH posterior putamen was the best value identifying RBD-syn patients. Notably, 133 (79.2%) RBD-syn patients had a baseline z-score lower than -0.92 in the MAH posterior putamen.

Machine Learning Analysis. RBD-Syn Versus RBD-Nc Patients

As a first step, the machine learning analysis was applied to the clinical data only, including age and sex, to investigate their ability in differentiating RBD-syn from RBD-nc patients (Table 5). Then, the machine learning analysis was applied to the DaT-SPECT data only, and finally to both clinical and DaT-SPECT data (Table 5).

Used alone, clinical data poorly differentiated RBD-syn from RBD-nc patients, especially showing a low specificity. Using DaT-SPECT data alone significantly improved the discrimination compared with clinical data. Using both clinical and DaT-SPECT data slightly improved the discrimination compared with DaT-SPECT data as taken alone, but without achieving statistical significance. This is especially clear for the Support Vector Machine and the K-Nearest Neighbors analyses.

The Decision Trees analysis showed the worst performance; thus, we choose to explore the Support Vector Machine analysis to provide insight of the data. Figure 1A shows the relevance of the 14 DaT-SPECT variables for the Support Vector Machine classifier, showing that the most important feature is MAH striatum, followed by MAH anterior putamen. Figure 1B shows the relevance of both clinical and DaT-SPECT variables for the Support Vector Machine classifier. The most important variables were again MAH striatum, followed by MAH putamen. All clinical data had very low coefficients, suggesting that their contribution to the model was negligible.

Notably, taking in consideration the results obtained by comparing RBD-syn patients and controls, defining a z-score of -1.0 at MAH putamen as an empirical cutoff of DaT-SPECT positivity allowed the identification of 75.6% RBD-syn of patients (phenoconverted), while only 34.9% RBD-nc patients (non phenoconverted) had positive DaT-SPECT.

Machine Learning Analysis. PD-Converters Versus DLB-Converters

As a last step, we exploit the same 3-fold machine learning analysis to differentiate PD-converters versus DLB-converters patients. Table 6 shows the results of the 3 different classifiers

TABLE 4. Principal Component Analysis Loadings of Dopamine Transporter (DaT)-SPECT SBRs and z-Scores

Parameter	1° Component	2° Component	3° Component
DaT-SPECT SBRs			
MAH Striatum, SBRs	**0.9969**	−0.00234	−0.04859
LAH Striatum, SBRs	**0.99022**	0.13071	−0.01183
MAH Putamen, SBRs	**0.99575**	−0.03489	0.05025
LAH Putamen, SBRs	**0.99105**	0.08219	0.08087
MAH Caudate, SBRs	**0.94547**	0.07453	−0.24447
LAH Caudate, SBRs	**0.94864**	0.21022	−0.18463
MAH Anterior Putamen, SBRs	**0.99225**	−0.00938	0.01079
LAH Anterior Putamen, SBRs	**0.98716**	0.09643	0.05254
MAH Posterior Putamen, SBRs	**0.96503**	−0.06899	0.12829
LAH Posterior Putamen, SBRs	**0.97111**	0.01872	0.13424
MAH Putamen/Caudate ratio, SBRs	0.09347	−0.38754	**0.78225**
LAH Putamen/Caudate ratio, SBRs	0.10168	−0.30561	**0.82978**
Caudate asymmetry	−0.16855	**0.72193**	0.31907
Putamen asymmetry	−0.11619	**0.74322**	0.2298
DaT-SPECT z-scores			
MAH Striatum, z-score	**0.99545**	−0.05692	−0.0327
LAH Striatum, z-score	**0.98975**	−0.08991	0.08264
MAH Putamen, z-score	**0.99512**	0.06567	−0.01509
LAH Putamen, z-score	**0.99014**	0.02937	0.09567
MAH Caudate, z-score	**0.94703**	−0.28818	−0.08285
LAH Caudate, z-score	**0.93398**	−0.327	0.06682
MAH Anterior Putamen, z-score	**0.98656**	0.00279	−0.01222
LAH Anterior Putamen, z-score	**0.97524**	−0.0014	0.09263
MAH Posterior Putamen, z-score	**0.96291**	0.17465	−0.00291
LAH Posterior Putamen, z-score	**0.96647**	0.14516	0.07803
MAH Putamen/Caudate ratio, z-score	0.15354	**0.95521**	0.02182
LAH Putamen/Caudate ratio, z-score	0.1139	**0.92529**	0.2713
Caudate asymmetry, z-score	−0.15709	−0.1166	**0.84608**
Putamen asymmetry, z-score	−0.21148	−0.23326	**0.78088**

Note: In bold are highlighted the loadings >0.4.
LAH = least affected hemisphere; MAH = most affected hemisphere; SBRs = specific to non-displaceable binding ratios.

(average and standard deviation across 10 different training/testing splits) as a function of the set of predictors: clinical data, SPECT data, and joint usage of clinical and SPECT data.

Used alone, clinical data poorly differentiated PD-converters from DLB-converters, especially showing a low specificity. Using DaT-SPECT data alone significantly improved the discrimination compared with clinical data.

TABLE 5. Ability of Clinical and Dopamine Transporter (DaT)-SPECT Data in Differentiating RBD-syn (Phenoconverted) from RBD-nc (Not Phenoconverted) Patients

Parameter	Sensitivity	Specificity	Error rate
Decision Trees			
Clinical data	0.62 ± 0.09	0.77 ± 0.08	0.29 ± 0.03
SPECT data	0.72 ± 0.06	0.75 ± 0.05	0.26 ± 0.04
Clinical + SPECT data	0.71 ± 0.06	0.78 ± 0.06	0.25 ± 0.04
Support Vector Machine			
Clinical data	0.44 ± 0.09	0.87 ± 0.05	0.31 ± 0.03
SPECT data	0.73 ± 0.07	0.83 ± 0.04	0.21 ± 0.03
Clinical + SPECT data	0.77 ± 0.06	0.85 ± 0.05	0.18 ± 0.03
K-Nearest Neighbors			
Clinical data	0.37 ± 0.09	0.92 ± 0.04	0.31 ± 0.03
SPECT data	0.74 ± 0.09	0.81 ± 0.06	0.22 ± 0.03
Clinical + SPECT data	0.74 ± 0.05	0.86 ± 0.04	0.19 ± 0.03

Using both clinical and DaT-SPECT data slightly improved the discrimination compared with DaT-SPECT data as taken alone, but without achieving statistical significance. This is especially clear for the Support Vector Machine and the K-Nearest Neighbors analyses.

The Decision Trees analysis showed the worst performance; thus, we choose to explore the Support Vector Machine analysis to provide insight of the data. Figure 2A shows the relevance of the 14 DaT-SPECT variables for the Support Vector Machine classifier, showing that the most important features are MAH and LAH striatum z-scores, followed by MAH and LAH anterior and posterior putamen. Figure 2B shows the relevance of both clinical and DaT-SPECT variables for the Support Vector Machine classifier. The most important variables were MAH striatum and LAH putamen, followed by MAH caudate. All clinical data had very low coefficients, suggesting that their contribution to the model was negligible. The only clinical data achieving a fair coefficient was patients' sex.

Post-hoc Analysis: Phenoconversion Time

We performed a post-hoc analysis to investigate the relationship between the phenoconversion time and the phenoconversion trajectories. First, we calculated the Pearson linear correlation between the time to phenoconversion and the 14 DaT-SPECT variables, yielding a statistically significant correlation against the caudate asymmetry ($p < 0.05$). The MAH striatum ($p = 0.10$), MAH putamen ($p < 0.10$), and MAH anterior putamen ($p < 0.10$) tended to correlate with the phenoconversion time. It is interesting to note that MAH striatum and MAH putamen were among the most important features for discriminating RBD-syn from RBD-nc patients, as well as PD-converters from DLB-converters.

A second analysis regards patients' sex which, as discussed above, is the only clinical feature privileged by the support vector machines (SVM) in differentiating phenoconversion trajectories. In this case, we conducted a 1-way analysis of variance (ANOVA) test on the time-to-conversion grouped by sex which yielded a non-statistically significant difference between the 2 groups. Similarly, an additional 1-way ANOVA tested the difference between the time to phenoconversion grouped by outcome (PD vs DLB). Also in this case, we observed no statistically significant differences between the 2 groups. Finally, we carried a log-rank test between the time to phenoconversion of PD and DLB patients, showing no significant difference between the 2 groups. In summary, these results suggest that phenoconversion time is not different between PD-converters and DLB-converters.

Discussion

In the present multicenter study of the International RBD study group, we demonstrated that DaT-SPECT can

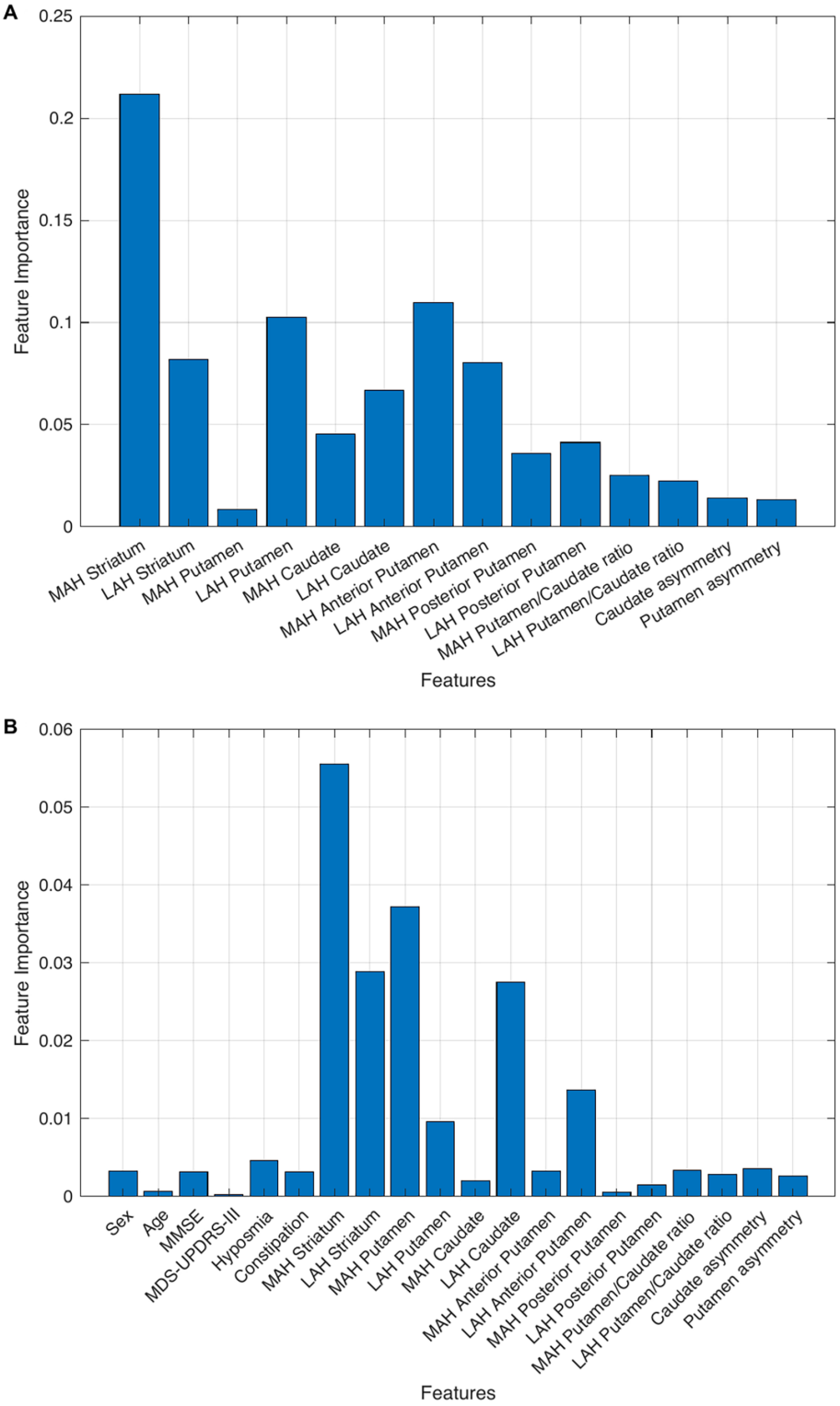

FIGURE 1: (A) Relevance of the 14 dopamine transporter (DaT)-SPECT variables in the support vector machine classification between rapid eye movement (REM) sleep behavior disorder (RBD) due synucleinopathy (ie, phenoconverted on a short term) from RBD patients not yet phenoconverted at last follow-up. (B) Relevance of the 6 clinical variables and of the 14 DaT-SPECT variables in the support vector machine classification between RBD due synucleinopathy from RBD patients not yet phenoconverted at last follow-up.

predict phenoconversion in RBD-syn patients, with high sensitivity. By means of machine learning analysis, we highlighted that the prediction ability of DaT-SPECT is significantly higher than routine clinical data alone (which in turn was characterized by poor specificity). Using the combination of clinical and DaT-SPECT data, the

TABLE 6. Ability of Clinical and Dopamine Transporter (DaT)-SPECT Data in Differentiating Parkinson Disease (PD) Converters from Dementia with Lewy Bodies (DLB) Converters

Parameter	Sensitivity	Specificity	Error Rate
Decision Trees			
Clinical data	0.77 ± 0.13	0.66 ± 0.24	0.28 ± 0.08
SPECT data	0.73 ± 0.14	0.68 ± 0.12	0.29 ± 0.06
Clinical + SPECT data	0.65 ± 0.16	0.76 ± 0.16	0.30 ± 0.09
Support Vector Machine			
Clinical data	0.93 ± 0.06	0.50 ± 0.20	0.26 ± 0.07
SPECT data	0.84 ± 0.13	0.78 ± 0.14	0.19 ± 0.04
Clinical + SPECT data	0.85 ± 0.11	0.86 ± 0.14	0.15 ± 0.05
K-Nearest Neighbors			
Clinical data	0.98 ± 0.05	0.46 ± 0.20	0.25 ± 0.10
SPECT data	0.81 ± 0.12	0.68 ± 0.14	0.24 ± 0.04
Clinical + SPECT data	0.83 ± 0.15	0.79 ± 0.10	0.19 ± 0.05

prediction ability slightly improved, without achieving statistical significance. Nevertheless, the combination of clinical and imaging metrics granted an increase in specificity that is worth considering.

Interestingly, RBD-syn patients (ie, those who phenoconverted within 3 years from diagnosis) were efficiently discriminated, at baseline, from both controls and from RBD-nc patients (ie, those not yet phenoconverted at last available follow-up) using an empirical z-score cutoff of −1.0 at MAH putamen. Indeed, using this cutoff, 75.6% RBD-syn patients had baseline positive DaT-SPECT, while only 34.9% RBD-nc patients had positive DaT-SPECT. This result has meaningful implications both in clinical practice and in designing disease-modifying trials. Recently, new biological definitions and staging systems for the alpha-synucleinopathy continuum were proposed.[5,6] In both proposals, polysomnography-confirmed RBD is recognized as a clinical feature attributable to alpha-synucleinopathy, and the presence of abnormal DaT-SPECT allows biological staging of patients with alpha-synucleinopathy related neurodegeneration. However, it is not clear when and how a DaT-SPECT would be defined abnormal (or "positive"). The present data suggest that a z-score cutoff of −1.0 at most affected hemisphere putamen may be used for this purpose. This threshold is very close to that from a recent study showing that patients with post-mortem confirmation of Lewy body disease had ante-mortem DaT-SPECT z-scores of −0.82[21] (using DatQUANT version 1) or − 0.91[40] (using version 2 as in the present work) in at least one putamen. Interestingly, a recent study showed that a z-score cutoff of −1.27 at putamen level best differentiate PD patients from essential tremor patients, while a cutoff of −0.96 was the most accurate in supporting DLB diagnosis.[22]

Among DaT-SPECT data able to differentiate PD-converters from DLB-converters, the putamen z-score of the LAH was the most relevant one to the classifier, followed by the caudate z-score of the most affected hemisphere. This result is intriguing, suggesting that both putamen and caudate nuclei significantly contributed to the discrimination. Nigroputaminal dopaminergic dysfunction is usually associated with motor impairment in PD patients,[41] and is related to disease progression in PD patients,[42] but also in iRBD.[43] Conversely, nigrocaudate dopaminergic dysfunction has been associated with cognitive impairment in PD patients,[44] and in iRBD patients.[45] Interestingly, DLB patients have been reported as characterized by lower [^{123}I]FP-CIT SBR in the caudate nucleus than PD patients.[46] Moreover, the putamen/caudate ratio is significantly lower in PD patients, compared with DLB patients.[46] In agreement with those results, our sample showed lower putamen/caudate ratio in PD-converters than in DLB-converters. This result is in keeping with the well-known abnormal features of DaT SPECT in DLB patients, often described as weak comma or "balanced loss" due to a more diffuse (but often milder) uptake reduction with respect to PD

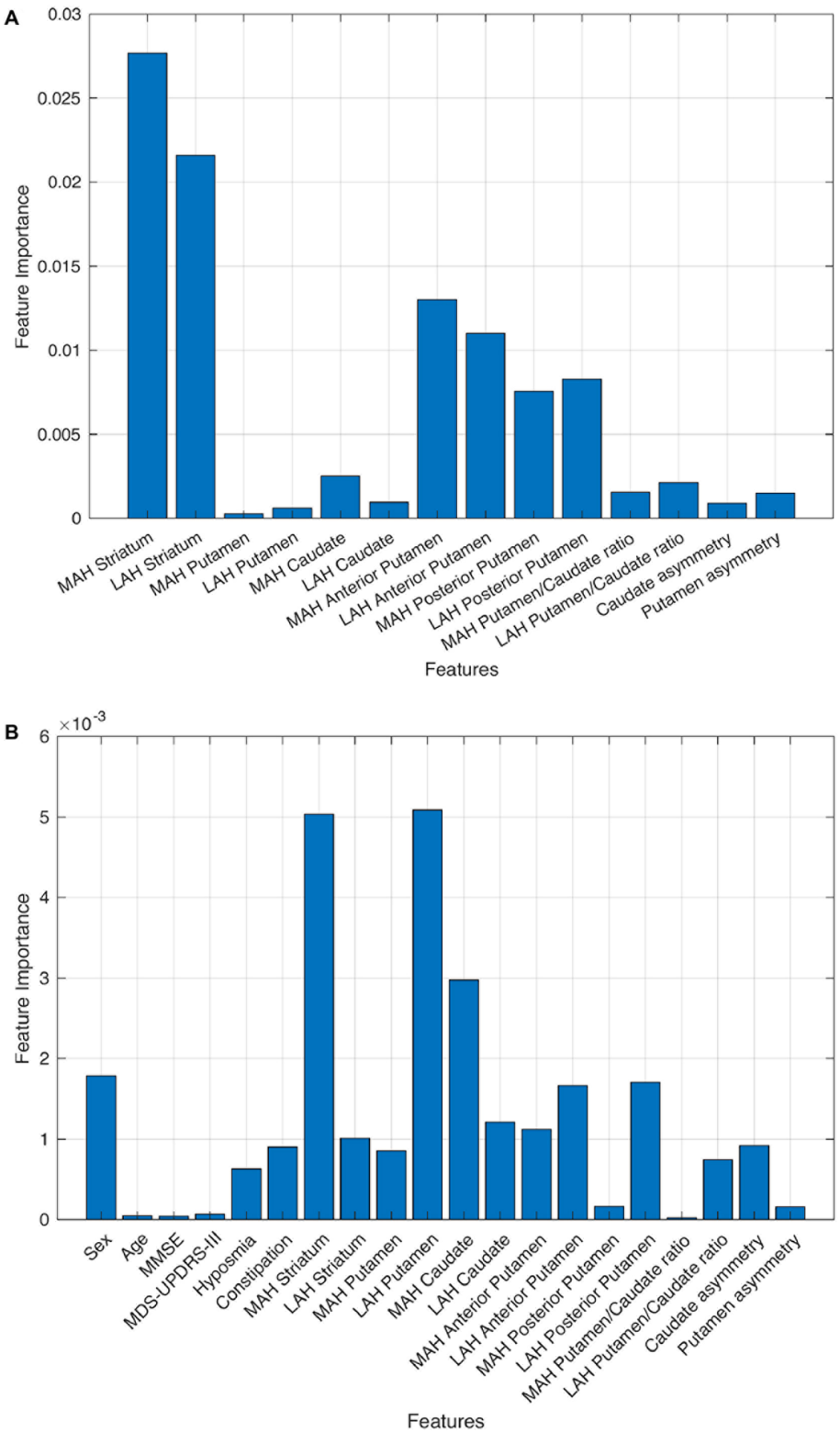

FIGURE 2: (A) Relevance of the 14 dopamine transporter (DaT)-SPECT variables in the support vector machine classification between Parkinson disease (PD)-converters and dementia with Lewy bodies (DLB)-converters. (B) Relevance of the 6 clinical variables and of the 14 DaT-SPECT variables in the support vector machine classification between PD-converters and DLB-converters.

patients.[47] Finally, the whole striatum significantly contributed to the machine learning classifier. Indeed, in our sample PD-converters tended to have lower values in all DaT-SPECT variables. In summary, these results suggest that RBD-syn patients eventually phenoconverting to PD have a more severe nigrostriatal dopaminergic impairment

in all substriatal regions, in particular at putamen level, and that a reduced putamen/caudate ratio may be a predictor of PD phenoconversion. DLB-converters had a mean baseline MAH Putamen/Caudate ratio of −0.87 z-score, while PD-converters had a mean baseline MAH Putamen/Caudate ratio of −1.21 z-score. Thus, it may be speculated that, in iRBD patients, z-scores lower than −1.0 in the MAH Putamen/Caudate ratio may predict future PD phenoconversion.

In agreement with previous studies, we found that routine clinical data poorly differentiated PD-converters from DLB-converters. Indeed, in previous multicenter studies, both clinical and lifestyle factors did not strongly differentiate RBD-syn patients eventually phenoconverting to either PD or DLB.[1,48] In agreement with literature data, in our sample, compared with PD-converters, DLB-converters were older, more likely males, had lower motor impairment, and had higher cognitive impairment.[1,48] However, this clinical phenotype alone is not enough to predict the phenoconversion diagnosis in RBD-syn patients. In fact, the only clinical feature achieving a fair coefficient, in the present machine learning analysis is patients' sex. This finding has a practical relevance, as it was demonstrated that sex also has an effect on SBR in the caudate and putamen, but this aspect is indeed not considered by all software for DaT-SPECT semi-quantification.[19,47]

It is not surprising that motor and cognitive assessment poorly differentiates RBD-syn patients eventually phenoconverting to PD from those phenoconverting to DLB. Indeed, parkinsonism is one of the core clinical features for the diagnosis of DLB,[17] while PD patients often have MCI already at diagnosis.[49] On the other hand, even if both PD and DLB incidence increases with age, DLB patients are usually older than PD patients at the time of diagnosis. Therefore, it may be reasonable to assume that older RBD-syn patients may be at higher risk of developing DLB instead of PD, if the putamen/caudate ratio is not reduced. Notably, a long-lasting debate has been devoted to the discussion of PD and DLB as different diseases or as the 2 opposite side of the same disease spectrum. Regardless of the final conclusion of this debate, DaT-SPECT features in DLB patients have been described as partially different with respect to PD patients.[50] Our results support this concept, by highlighting a biological difference since the earliest stages.

Other biomarkers, such as brain [18F]-fluorodeoxyglucose positron emission tomography (PET), may be effective tools to predict the phenoconversion diagnosis of iRBD patients.[40] However, DaT-SPECT is a highly suitable candidate to be used as a stratification tool for disease-modifying trials in prodromal stages of synucleinopathy. Thus, if the same technique is also able to predict the phenoconversion diagnosis, it would significantly ease the clinical trials design.

The main limitation of the present study is its retrospective nature. Therefore, no phantom-based harmonization across different gamma cameras was a priori performed. However, all centers followed international acquisition guidelines and SPECT data analyses have been centralized with the aim to apply reconstruction parameters fitting as much as possible the parameters used for the acquisition of the normal dataset. In the present study, we preliminarily demonstrated the lack of significant center effect and, notably, this approach has been successfully used in a previous DaT-SPECT multicenter study.[12] It should also be noted that we flipped images to have the MAH and the LAH (ie, the highest/lowest value between left and right hemisphere, respectively) on the same side for all patients. This choice was aimed at maximizing the difference between RBD-syn which will phenoconvert to either PD or DLB and, although performed a priori (ie, in prior to statistical analysis), can be translated in a clinical trial setting as it is not based on the final phenoconversion but rather on the results of semiquantification of the baseline DaT-SPECT.

Similarly, the z-score thresholds may be influenced by both the semi-quantification software employed and certain attributes of the normal subjects' dataset. These attributes include the proportion of male and female controls, which may affect the SBR and consequently impact the resulting z-scores. The absence of any of the clinical features predicting phenoconversion in this analysis could be due to several factors, including the possibility that no single feature or combination of clinical features will ever reliably predict phenoconversion to PD versus DLB versus MSA. However, some of the clinical parameters used in this analysis were admittedly crude (ie, screening mental status examination scores are not as sensitive for cognitive impairment as a multidomain neuropsychological assessment, and total UPDRS scores may not be as predictive as the qualitative profile of extrapyramidal signs or instrumental motor assessment), and other features were considered abnormal based on cutoff scores whereas data analyzed as continuous rather than dichotomous variables may be more predictive. Moreover, only the presence of constipation was used, instead of more detailed assessment of autonomic dysfunction. Future analyses using a comprehensive battery of clinical measures performed in a standardized and longitudinal manner may provide insights on clinical data that may be predictive.

In conclusion, this study suggests that DaT-SPECT may be a valuable tool to predict phenoconversion in RBD-syn patients. In detail, we suggest using a z-score

cutoff of −1.0 at MAH putamen to identify patients at high risk of short-term phenoconversion. Moreover, a z-score cutoff of −1.0 at MAH Putamen/Caudate ratio may identify those patients with higher likelihood of developing parkinsonism first, especially in the absence of cognitive impairment. These findings are relevant for the present and future design of disease-modifying trials.

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Author Contributions

D.A., A.M., A.G., M.P., F.N., and S.M. contributed to the conception and design of the study. D.A., P.M., S.R., M.P., F.M., A.I., A.P., A.N.B., C.G., M.S., A.M.L., G.M., C.L., M.F., F.P., A.C., K.S., P.D., D.Z., J.T., B.F.B., T.M., V.J.L., T.M., M.M., M.P., M.F., A.S., M.H., C.G.C., F.B., D.K., V.C.D.C., D.D.V., G.P., E.A., M.T., I.B., K.K., A.M., A.G., M.P., F.N., and S.M. contributed to the acquisition and analysis of data. D.A., P.M., S.R., M.P., F.M., A.I., A.P., A.N.B., C.G., M.S., A.M.L., G.M., C.L., M.F., F.P., A.C., K.S., P.D., D.Z., J.T., B.F.B., T.M., V.J.L., T.M., M.M., M.P., M.F., A.S., M.H., C.G.C., F.B., D.K., V.C.D.C., D.D.V., G.P., E.A., M.T., I.B., K.K., A.M., A.G., M.P., F.N., and S.M. contributed to drafting the text or preparing the figures.

Potential Conflicts of Interest

Nothing to report.

Data availability

Anonymized data used in this study are available upon reasonable request from the corresponding author (D.A.).

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