


RESEARCH ARTICLE

Dopamine transporter imaging predicts clinically-defined α -synucleinopathy in REM sleep behavior disorder

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Abstract

Introduction: Individuals with idiopathic rapid eye movement sleep behavior disorder (iRBD) are at high risk for a clinical diagnosis of an α -synucleinopathy (aSN). They could serve as a key population for disease-modifying trials. Abnormal dopamine transporter (DAT) imaging is a strong candidate biomarker for risk of aSN diagnosis in iRBD. Our primary objective was to identify a quantitative measure of DAT imaging that predicts diagnosis of clinically-defined aSN in iRBD. **Methods:** The sample included individuals with iRBD, early Parkinson's Disease (PD), and healthy controls (HC) enrolled in the Parkinson Progression Marker Initiative, a longitudinal, observational, international, multicenter study. The iRBD cohort was enriched with individuals with abnormal DAT binding at baseline. Motor and nonmotor measures were compared across groups. DAT specific binding ratios (SBR) were used to calculate the percent of expected DAT binding for age and sex using normative data from HCs. Receiver operative characteristic analyses identified a baseline DAT binding cutoff that distinguishes iRBD participants diagnosed with an aSN in follow-up versus those not diagnosed. **Results:** The sample included 38 with iRBD, 205 with PD, and 92 HC who underwent DAT-SPECT at baseline. Over 4.7 years of mean follow-up, 14 (36.84%) with iRBD were clinically diagnosed with aSN. Risk of aSN diagnosis was significantly elevated among those with baseline putamen SBR \leq 48% of that expected for age and sex, relative to those above this cutoff

(hazard ratio = 17.8 [95%CI: 3.79–83.3], $P = 0.0003$). **Conclusion:** We demonstrate the utility of DAT SBR to identify individuals with iRBD with increased short-term risk of an aSN diagnosis.

Introduction

Rapid eye movement (REM) sleep behavior disorder (RBD) is a parasomnia defined by dream enactment behavior and the absence of atonia during REM sleep. Individuals with idiopathic RBD (iRBD), that is, in the absence of diagnosed neurologic disorder or other etiologies, are at high risk of developing α -synuclein-mediated neurodegenerative Parkinsonian disorders, or α -synucleinopathies (aSN), namely Parkinson's Disease (PD), Dementia with Lewy Bodies (DLB), and Multiple System Atrophy (MSA).^{1,2} These disorders carry significant burden on patients, families, and the economy, and their prevalence is increasing worldwide.³ It is therefore desirable to delay or halt the neurodegenerative process in the at-risk population.

Individuals with iRBD could serve as a key population for clinical trials to prevent or delay aSN. While up to 90% of individuals with iRBD will go on to develop such a disorder within 15 years from RBD diagnosis,² time to diagnosis of an aSN is variable and may occur years to even decades after the onset of RBD symptomatology. In a large multicenter study of iRBD, rate of diagnosis of an aSN was approximately 6%/year.¹ A crucial strategy to design feasible clinical trials in the iRBD population would be to enrich the sample for increased short-term risk of a clinically definable aSN diagnosis. Despite extensive progress in characterizing prodromal features of aSN in iRBD, clinical-trial-ready biomarkers that will help identify such individuals are lacking. Abnormal dopaminergic transporter (DAT) imaging has emerged as a strong candidate for predicting aSN diagnosis in iRBD,⁴ but it is unclear whether the extent of loss of DAT density is related to the risk of aSN diagnosis.

The Parkinson's Progression Markers Initiative (PPMI) study recruited an international, multicenter iRBD cohort enriched with abnormal DAT binding. The objectives of this analysis were to (1) examine differences in the iRBD cohort compared to PD and healthy controls (HC) in PPMI, (2) define the rate of diagnosis of a clinically-defined aSN in this research setting, and (3) identify a quantitative measure of DAT imaging that accurately predicts prospective diagnosis of a clinically-defined aSN in iRBD.

Methods

Data used for this analysis came from PPMI, a longitudinal, observational, international, multicenter biomarker study. Study design and methodology have been previously published^{5,6} and are available at ppmi-info.org.

Sample

Idiopathic RBD cohort: A sample of 39 individuals with iRBD were recruited between 2013 and 2015 across nine sites (one in Spain, one in Germany, one in Greece, and six in USA), as previously described.^{7,8} Inclusion criteria were: (1) age ≥ 60 , and (2) diagnosis of iRBD based on International Classification of Sleep Disorders—2 (ICSD2) criteria (clinical history consistent with iRBD, history and/or video confirmation of dream enactment behavior, and evidence of REM without atonia on polysomnography). Where available, polysomnograms (acquired as part of participants' clinical diagnostic evaluation) were submitted to the PPMI sleep core for central review. Surface EMG signal from either the mentalis muscle or limb muscles was reviewed and a determination of the presence/absence of RWA was made based on the established criteria.⁹ The study aimed to recruit an iRBD cohort enriched for deficit in DAT specific binding ratio (SBR), but approximately 10% of individuals with normal DAT binding were included to maintain investigator and participant blinding to DAT results. For this analysis, at least one annual follow-up visit was required. Therefore, one participant was excluded and the final iRBD sample included in this study was 38, three of which had normal DAT binding at baseline (defined as previously described⁸).

The PPMI iRBD cohort is compared to two other PPMI cohorts in this analysis, consisting of HC and early, untreated idiopathic PD participants. Inclusion/exclusion criteria and baseline cohort characteristics have been described in detail.^{6,10} Briefly, the PPMI PD cohort consisted of 423 individuals with PD diagnosed within the prior 2 years, untreated with PD medication at baseline, and with abnormal DAT binding on SPECT scan (as defined by visual inspection⁶). The PPMI HC cohort consisted of 196 generally healthy individuals aged ≥ 30 years. To more closely align the cohorts by age and sex, age ranges were computed separately for iRBD females and males, and only the 205 PD and 92 HC participants who fell within these sex-specific age ranges were included in this analysis.

Assessments

1. Research diagnosis of clinically-defined aSN is the primary outcome for this analysis. Information on diagnosis was acquired at each visit. The site investigator was asked to complete a diagnosis case report form to indicate the presence of an aSN or other neurologic

disorders. Possible responses on the diagnosis form include: (i) healthy (no neurologic disorder or features to suggest aSN), (ii) diagnosis of an α -synuclein-mediated Parkinsonian disorder: PD, MSA, or DLB, (iii) other neurodegenerative Parkinsonian disorders including PSP and CBS, (iv) other non-neurodegenerative movement disorders (e.g., essential tremor), and (v) “other” in which they may then specify the diagnosis. The iRBD cohort was assessed at 3-month intervals during the first year after enrollment and at 6-month intervals thereafter.

2. Demographics: Age, sex, and family history of PD.
3. Movement Disorder Society Unified Parkinson’s Disease Rating Scale (MDS-UPDRS), which includes MDS-UPDRS parts IA and IB, part II, and part III (physician-rated motor examination). MDS-UPDRS acquired at the baseline visit and years 1 and 2 of the PPMI study is included in this analysis.
4. Nonmotor signs and symptoms assessed at baseline in PPMI included:
 - i. Olfactory function: olfaction was assessed with the University of Pennsylvania Smell Identification Test (UPSIT-40).¹¹ Age- and sex-specific percentile values were computed based on the raw UPSIT scores of 4953 participants (2414 females and 2539 males) from the Parkinson Associated Risk Syndrome (PARS) study cohort.¹²
 - ii. Autonomic measures: Orthostatic hypotension was defined by a reduction of 20 mmHg in systolic or 10 mm in diastolic blood pressure (BP) from supine to standing. Patient-reported symptoms of autonomic dysfunction were assessed with the 23-item Scales for Outcomes of Parkinson’s Disease-Autonomic (SCOPA-AUT). Constipation was identified by a response of “regularly” or “often” on either SCOPA-AUT item 5 or 6; erectile dysfunction in male participants was defined by a response of at least “sometimes” on item 22.
 - iii. Cognitive function: Montreal Cognitive Assessment (MoCA)¹³ and a panel of neuropsychological tests as described.⁸ Participants were categorized as having abnormal cognition if they scored > 1.5 SD below the mean on ≥ 2 neuropsychological tests.¹⁴
 - iv. Psychiatric symptoms: Depression was assessed with the 15-item Geriatric Depression Scale, and anxiety with the Straight Trait Anxiety Inventory.
 - v. Insomnia was defined as a score of > 0 on MDS-UPDRS item 1.7.
5. Movement Disorder Society (MDS) prodromal criteria for PD: the MDS prodromal criteria incorporate various features suggestive of PD risk or prodromal PD.^{15,16} Based on the presence/absence of these

features, likelihood ratios are assigned to each participant and posttest probability for prodromal PD calculated. Based on the features collected in PPMI, the posttest probability was calculated for each participant.

6. Biofluid biomarkers: cerebrospinal fluid (CSF) was collected at baseline in PPMI as described.^{6,17} Levels of CSF biomarkers associated with PD and Alzheimer’s disease included in this analysis were CSF fluid amyloid- β_{1-42} , total α -synuclein, and total-tau (assayed as described¹⁸).
7. DAT-SPECT data acquired at the screening visit and years 1 and 2 of the PPMI study are included in this analysis. SPECT scans were acquired after administration of Ioflupane-¹²³I (DaTscanTM; GE Healthcare) as detailed in the PPMI SPECT Manual.¹⁰ All DAT scans were analyzed at the PPMI imaging core both with visual assessment by two expert readers and with calculation of SBR for left caudate nucleus, right caudate nucleus, left putamen, right putamen, and occipital cortex. Methods for identifying eligible participants for study enrollment have been previously described.^{5,6,19,20}

The percent of expected putamenal binding for age and sex was calculated using data from the PPMI HC cohort as normative data. Linear regression of the HC data was used to derive the following formula for estimating the “expected” lowest putamen SBR value for a given age and sex:

$$2.6002 - 0.01397X_1 + 0.2082X_2$$

where: X_1 = age of the participant in years at time of scan; and X_2 = 1 if the participant is male and 2 if the participant is female.

The percent age- and sex-expected lowest putamen SBR was computed as follows:

$$100 * \frac{\text{ObservedLowestPutamenSBR}}{\text{Age- and Sex-ExpectedLowestPutamenSBR}}$$

The study protocol was approved by the institutional review board of the University of Rochester. Institutional review board approval was also obtained at each PPMI site. Written informed consent was obtained from all study participants.

Statistical analysis

Data included in this analysis were downloaded from the PPMI database (ppmi-info.org) on 6 April 2020. Baseline characteristics were compared between cohorts (iRBD vs. PD or HCs) using two-sample *t* tests, Chi-squared tests, or Fisher’s exact tests as appropriate with the exception of baseline CSF biomarkers which were compared between cohorts using Wilcoxon rank-sum tests. Among iRBD participants, the relationship between baseline

characteristics and time to diagnosis was assessed using univariate Cox proportional hazards models. Time was calculated from the date of enrollment until the date of the first study visit at which an aSN diagnosis was specified on the diagnosis case report form; iRBD participants who did not receive diagnosis of an aSN were censored at the time of their last completed visit. Ties were handled using Efron's approximation.

To explore which cut-off value for baseline percent age- and sex-expected lowest putamen SBR best predicted diagnosis of an aSN, a receiver operator characteristic curve was used to identify the point yielding the highest Youden's index ($J = \text{sensitivity} + \text{specificity} - 1$), and a Kaplan-Meier survival plot was used to visualize the outcomes of iRBD participants above versus below this cutoff.

Longitudinal MDS-UPDRS Part III scores were compared between groups using line plots (mean \pm standard error). For iRBD participants diagnosed with aSN, the time axis was shifted so that time "0" corresponded to the diagnosis visit; for all other groups (HC, PD, and iRBD without aSN diagnosis), time 0 corresponded to the baseline study visit.

To explore short-term changes in DAT binding, a Wilcoxon rank-sum test was used to compare the 2-year percent change from baseline in mean striatum SBR among iRBD participants who were versus were not diagnosed with aSN.

Analyses were performed using SAS v9.4 (SAS Institute Inc., Cary, NC, USA). Figures were created in R v4.0.3 using the ggplot2, plotROC and survminer packages.

Results

Thirty-eight individuals with iRBD are included in this analysis, and compared with a group of 205 individuals with early untreated PD and 92 HCs with similar sex-specific age ranges.

At baseline, the iRBD cohort had a mean age of 69.5 (SD 5.5) years, and 84% were male (Table 1). The % age/sex-expected lowest putamen SBR, MDS-UPDRS total score, and parts II and III subscores were higher compared to the HC but lower than the PD group. SCOPA-AUT was higher in the iRBD cohort compared to the PD and HC groups, and olfactory function was lower in the iRBD group compared to the HC. The iRBD group had higher GDS-15 scores and lower MoCA scores than the HC group. A greater proportion of the iRBD group had abnormal cognitive testing compared to both the HC and PD groups. Eighty-seven percent of iRBD participants met MDS criteria for prodromal PD. Median CSF amyloid- β_{1-42} was lower in the iRBD group compared to the HC group; other CSF biomarkers did not differ between the groups including total α -synuclein levels.

In the iRBD cohort, mean estimated duration of RBD symptoms at enrollment was 10.1 (SD 7.4; range 0.4–30.4) years. Mean duration of follow-up from study enrollment was 4.7 (SD 0.8; range 2.2–6.2) years; 92% of the iRBD cohort were assessed at the year-4 annual visit. Fourteen individuals (36.8%) received a research diagnosis of aSN during follow-up; final diagnosis was PD in nine, DLB in three, and MSA in two. Median time to diagnosis was 2.5 (range 0.2–5.0) years from enrollment.

Baseline predictors of aSN diagnosis are shown in Table 2. The strongest clinical predictors of an incident aSN diagnosis were olfaction in \leq 5th percentile for age and sex (HR = 7.35, 95%CI: 1.63–33.1; $P = 0.00093$), orthostatic hypotension (HR = 3.22, 95%CI: 1.10–9.43; $P = 0.0333$), and higher MDS-UPDRS part III scores (HR = 1.18, 95%CI: 1.03, 1.36; $P = 0.0152$).

As for DAT SBR as a predictor of incident aSN diagnosis, mean %-expected DAT SBR for age and sex in the lowest putamen was associated with increased hazards of aSN diagnosis (HR = 2.53, 95%CI 1.58, 4.06, $P = 0.0001$). In ROC analyses, 48% was identified as the cutoff for percent age/sex-expected lowest putamen SBR that best discriminated between those who were diagnosed with an aSN versus those who were not (Fig. 1). At baseline, 12 of 14 iRBD participants diagnosed with an aSN were *below* this cutoff (sensitivity: 86%), whereas 20 of 24 not diagnosed were *above* this cutoff (specificity: 83%). Time to diagnosis was significantly shorter in those who had DAT binding below this cutoff versus those who did not (Fig. 2). Lowest putamen DAT SBR \leq 48% of that expected for age and sex was the strongest predictor of diagnosis of an aSN diagnosis (hazard ratio = 17.8 [95% CI: 3.79–83.3], $P = 0.0003$; Table 2).

In iRBD participants diagnosed with aSN, motor abnormalities as measured with the MDS-UPDRS III increased and approached those of the PD group within 2 years of diagnosis (Fig. 3). In contrast, MDS-UPDRS III values among iRBD participants without an aSN diagnosis remained similar to the HC group. We also explored the change in DAT SBR in those who were and were not diagnosed with aSN (Fig. 4). In the first 2 years of study enrollment, mean striatal DAT binding decreased significantly more in those who were diagnosed versus those who were not (mean percent decrease 19.6 vs. 5.8; $P = 0.0061$). In comparison, mean striatal DAT SBR in the PD group decreased over 2 years by an average of 17.5%.

Discussion

We demonstrate the utility of DAT SBR to accurately identify individuals at short-term risk of being diagnosed with a clinically-defined aSN in a multicenter cohort of individuals with iRBD. We show that the extent of DAT

Table 1. Baseline characteristics of the cohorts.

Variable ²	Group			P values ¹	
	iRBD (N = 38)	HC (N = 92)	PD (N = 205)	iRBD versus HC	iRBD versus PD
Age, mean (SD)	69.5 (5.5)	68.4 (5.3)	68.0 (4.7)	0.2840	0.0775
Sex (male), n (%)	32 (84%)	75 (82%)	172 (84%)	0.7148	0.9621
Family history of PD (any), n (%)	3 (8%)	3 (3%)	47 (23%)	0.3572	0.0472
MDS-UPDRS total score, mean (SD)	14.0 (7.6)	5.2 (4.3)	34.3 (13.6)	<0.0001	<0.0001
MDS-UPDRS part I, mean (SD)	7.3 (4.0)	3.1 (2.8)	5.5 (4.1)	<0.0001	0.0128
MDS-UPDRS part II, mean (SD)	2.2 (2.6)	0.6 (1.2)	6.1 (4.3)	0.0010	<0.0001
MDS-UPDRS part III, mean (SD)	4.5 (3.8)	1.5 (2.4)	22.7 (9.3)	<0.0001	<0.0001
SCOPA-AUT total score, mean (SD)	14.9 (8.2)	6.7 (3.7)	10.2 (6.3)	<0.0001	0.0017
GDS-15 total score, mean (SD)	2.8 (2.6)	1.3 (2.2)	2.2 (2.2)	0.0013	0.1839
STAI total score, mean (SD)	68.4 (18.4)	55.7 (12.2)	62.8 (17.1)	0.0003	0.0676
MoCA score, mean (SD)	25.5 (4.2)	28.1 (1.1)	26.7 (2.3)	0.0005	0.0812
Abnormal cognition, n (%)	14 (37%)	3 (3%)	36 (18%)	<0.0001	0.0144
MDS prodromal criteria (>80%), n (%)	33 (87%)	0 (0%)	181 (88%)	<0.0001	0.7869
Constipation, n (%)	17 (45%)	1 (1%)	28 (14%)	<0.0001	<0.0001
Orthostatic hypotension, n (%)	13 (34%)	13 (14%)	36 (18%)	0.0092	0.0197
Erectile dysfunction, n (%)	17 (53%)	25 (33%)	90 (53%)	0.0549	0.9848
Missing/not applicable	6	17	35		
Insomnia (MDS-UPDRS item 1.7), n (%)	24 (63%)	45 (49%)	109 (53%)	0.1548	0.2559
DAT binding, mean (SD, .Min, Max)					
Right caudate SBR	1.94 (0.54; 0.93, 3.53)	2.79 (0.62; 1.31, 5.09)	1.92 (0.58; 0.42, 3.42)	<0.0001	0.8222
Left caudate SBR	1.94 (0.52; 1.18, 3.59)	2.83 (0.67; 1.33, 5.30)	1.89 (0.56; 0.35, 3.72)	<0.0001	0.5623
Right putamen SBR	1.18 (0.35; 0.43, 2.07)	2.00 (0.57; 0.54, 3.54)	0.81 (0.32; 0.26, 1.89)	<0.0001	<0.0001
Left putamen SBR	1.12 (0.36; 0.49, 2.02)	1.99 (0.51; 0.74, 3.34)	0.77 (0.31; 0.12, 2.32)	<0.0001	<0.0001
% Age-/sex-expected lowest putamen	0.56 (0.18; 0.24, 1.05)	1.00 (0.26; 0.31, 1.68)	0.35 (0.13; 0.06, 0.89)	<0.0001	<0.0001
UPSIT					
Raw score, mean (SD)	18.3 (7.0)	32.6 (5.5)	20.2 (8.0)	<0.0001	0.1857
Percentile, n (%)				<0.0001	0.0197
≤5th	20 (54%)	5 (5%)	105 (51%)		
6th–10th	12 (32%)	3 (3%)	38 (19%)		
11th–20th	0 (0%)	8 (9%)	29 (14%)		
>20th	5 (14%)	76 (83%)	33 (16%)		
CSF					
amyloid-β _{1–42} , median (min, max)	772 (209, 1855)	980 (239, 3077)	823 (249, 3707)	0.0267	0.7328
α-Syn, median (min, max)	1394 (399, 4326)	1816 (672, 4683)	1411 (472, 5257)	0.1050	0.6777
α-Syn—Hb < 200 ³ , median (min, max)	1359 (446, 4326)	1742 (672, 4271)	1417 (472, 5257)	0.1906	0.8226
Phospho-tau, median (min, max)	15.4 (8.4, 38.0)	18.1 (8.3, 60.0)	14.1 (8.0, 40.1)	0.0741	0.5955
Total-tau, median (min, max)	198.9 (91.7, 436.3)	205.7 (95.9, 554.5)	162.3 (80.9, 467.0)	0.2837	0.1436

DAT, Dopamine transporter. GDS-15, Geriatric Depression Scale—15 item. MDS-UPDRS, Movement Disorder Society Unified Parkinson's Disease Rating Scale. MoCA, Montreal Cognitive Assessment. SBR, Specific Binding Ratio. SCOPA-AUT, Scales for Outcomes in Parkinson's Disease-Autonomic. STAI, State Trait Anxiety Inventory, UPSIT, University of Pennsylvania Smell Identification Test.

¹P values were found using Fisher's exact (family history of PD, UPSIT percentile, abnormal cognition, and MDS prodromal criteria), Chi-square, Wilcoxon rank-sum (CSF), and t-tests. Bold values denote statistical significance at the $P < 0.05$ level.

²Family history of PD missing for one PD participant; DAT binding missing for one HC and two PD participants; UPSIT missing for one RBD participant; MDS-UPDRS missing for one HC participant; SCOPA-AUT missing for five PD subjects; orthostatic hypotension missing for one PD subject. CSF abeta missing for four iRBD participants, four HC participants, and six PD participants. CSF asyn missing for three iRBD participants, three HC participants, and six PD participants. CSF p-tau missing for six iRBD participants, five HC participants, and 23 PD participants. CSF t-tau missing for five iRBD participants, three HC participants, and 10 PD participants.

³Given the effect red blood cell contamination has on total α-synuclein levels, in sensitivity analyses, comparisons of α-synuclein among groups were repeated excluding CSF samples with hemoglobin > 200 ng/mL. CSF Hb was > 200 ng/mL for 7 iRBD participants, 16 HC participants, and 45 PD participants.

Table 2. Comparison of individuals with iRBD who were diagnosed with an α -synucleinopathy (aSN) vs. those who did not receive diagnosis of an aSN.

Variable	iRBD Subgroup		Cox Regression ¹	
	aSN Diagnosis (N = 14)	No aSN Diagnosis (N = 24)	Univariate HR (95% CI)	Univariate P value
Age, mean (SD)	67.7 (3.3)	70.6 (6.3)	0.93 (0.84, 1.03)	0.1859
Sex (female)	5 (36%)	1 (4%)	4.24 (1.41, 12.8)	0.0102
Family history of PD (any)	1 (7%)	2 (8%)	0.96 (0.13, 7.33)	0.9670
MDS-UPDRS total score, mean (SD)	16.6 (9.1)	12.5 (6.3)	1.14 (1.05, 1.23)	0.0021
MDS-UPDRS part I, mean (SD)	8.4 (4.6)	6.7 (3.5)	1.17 (1.02, 1.36)	0.0273
MDS-UPDRS part II, mean (SD)	2.4 (3.2)	2.1 (2.3)	1.15 (0.94, 1.40)	0.1747
MDS-UPDRS part III, mean (SD)	5.9 (4.6)	3.7 (3.2)	1.18 (1.03, 1.36)	0.0152
SCOPA-AUT total score, mean (SD)	16.0 (9.4)	14.2 (7.5)	1.04 (0.98, 1.11)	0.2253
GDS total score, mean (SD)	2.7 (2.4)	2.8 (2.8)	1.01 (0.84, 1.22)	0.9167
STAI total score, mean (SD)	70.1 (19.4)	67.4 (18.1)	1.00 (0.98, 1.03)	0.7460
MoCA score, mean (SD)	25.6 (3.6)	25.5 (4.5)	0.99 (0.86, 1.14)	0.8896
Abnormal cognition, n (%)	7 (50%)	7 (29%)	2.09 (0.73, 6.02)	0.1702
MDS prodromal criteria (>80%), n (%) ²	14 (100%)	19 (79%)	—	0.1365
Constipation, n (%)	7 (50%)	10 (42%)	1.16 (0.41, 3.33)	0.7765
Orthostatic hypotension, n (%)	7 (50%)	6 (25%)	3.22 (1.10, 9.43)	0.0333
Erectile dysfunction, n (%)	4 (44%)	13 (57%)	0.66 (0.18, 2.48)	0.5407
Insomnia (MDS-UPDRS item 1.7), n (%)	8 (57%)	16 (67%)	0.91 (0.31, 2.63)	0.8574
% Age-/sex-expected low putamen DAT SBR ³ , mean (SD)	0.43 (0.13)	0.64 (0.16)	2.53 (1.58, 4.06)	0.0001
DAT binding \leq 48% age/sex-expected value, n (%)	12 (86%)	4 (17%)	17.8 (3.79, 83.3)	0.0003
Olfaction \leq 5 th percentile for age and sex, n (%)	12 (86%)	8 (35%)	7.35 (1.63, 33.1)	0.0093

¹P values were found using Fisher's exact tests (MDS prodromal criteria) and univariate Cox regression models. Bold values denote statistical significance at the $P < 0.05$ level.

²Cox regression model was not fit because all phenoconverters met criteria.

³Hazard ratio computed in terms of a 10% decrease in % age-/sex-expected lowest putamen SBR.

SBR has a strong relationship with the risk of and time to diagnosis. In this cohort enriched for abnormal DAT binding, 37% of the cohort received an aSN diagnosis during an average follow-up of 4 years. The rate of diagnosis was 9%/year, in contrast to 6%/year in other iRBD cohorts that were not enriched for abnormal DAT binding.¹

While the majority of individuals with iRBD will go on to develop a clinically-defined aSN,² this may occur only several years, or even decades, after the onset of RBD symptomatology.^{2,21} Idiopathic RBD could serve as a key population to assess individuals prior to the onset of aSN diagnosis and therefore to enable clinical trials to prevent disability from aSN. However, for such clinical trials to be feasible, it must be possible to accurately identify a group of iRBD patients near to diagnosis. DAT imaging has emerged as a biomarker predictive of PD risk among individuals with iRBD²² as well as other at-risk groups including those with olfactory loss²³ and genetic mutations.²⁴ We have herein demonstrated the utility of quantitative measures of DAT binding, namely DAT SBR, to predict future short-term risk of clinically-defined aSN diagnosis.

Individuals with iRBD who had putamen DAT binding \leq 48% of that expected for age and sex received a research diagnosis of an aSN significantly earlier than those who did not. In addition, of those who went on to receive a diagnosis of an aSN, 86% had putamen DAT binding \leq 48% of that expected for age and sex at baseline. Reduced DAT SBR reflects loss of substantia nigra dopaminergic neurons.²⁵ Available evidence based on neuropathological and imaging studies indicates that this neuronal loss begins years before motor manifestations of PD are fully manifest.²⁶

As in other studies,^{1,23,27,28} olfactory dysfunction was greater in those who received an aSN diagnosis, and orthostatic hypotension more prevalent. These features are consistent with the Braak hypothesis for progression of PD neuropathology,²⁹ with olfactory bulb, basal forebrain, and lower brainstem regions that mediate olfaction and autonomic function being involved as the pathology reaches more rostral regions that mediate RBD.³⁰ However, olfactory loss, orthostatic hypotension, and even mild motor abnormalities are nonspecific and may be seen with aging and several other neurologic and non-neurologic disorders. In addition, a larger proportion of

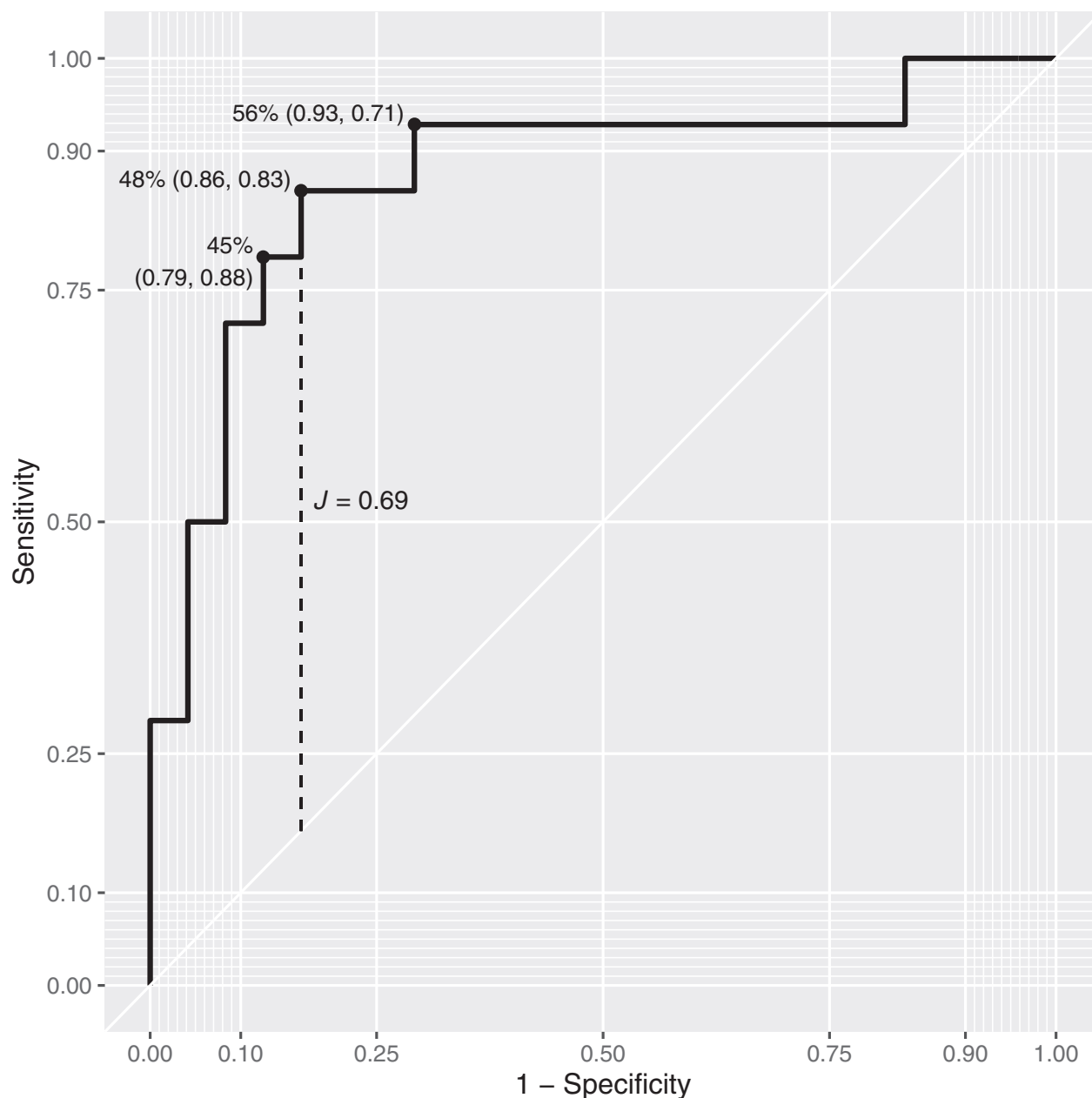


Figure 1. ROC curve analysis of optimal % age/sex-expected lowest putamen SBR cut-point for predicting diagnosis of clinically-defined α -synucleinopathy (aSN) in the iRBD cohort. Points labeled by cut-off value (i.e., % age/sex-expected lowest putamen SBR) and sensitivity versus specificity of said cutoff. Vertical dashed line indicates “optimal” cut-point (48%) as defined by point maximizing the Youden’s index (sensitivity = 86%, specificity = 83%, Youden’s $J = 0.69$).

those diagnosed were female, but the small number of females in the cohort make conclusions about this uncertain.

In the largest prospective study of iRBD,¹ significant predictors of diagnosis of an aSN included age, motor signs and symptoms, olfactory deficit, mild cognitive impairment, erectile dysfunction, degree of muscle

activity during REM sleep, and abnormal DAT imaging. Despite the large sample size, the effect sizes were low, ranging from hazard ratios of 1.54 to 3.16. In that study, DAT imaging was only performed in a subset of participants, and standardized methods for imaging were not used across sites. Therefore, the substantially smaller effect size for DAT imaging as a predictive marker in

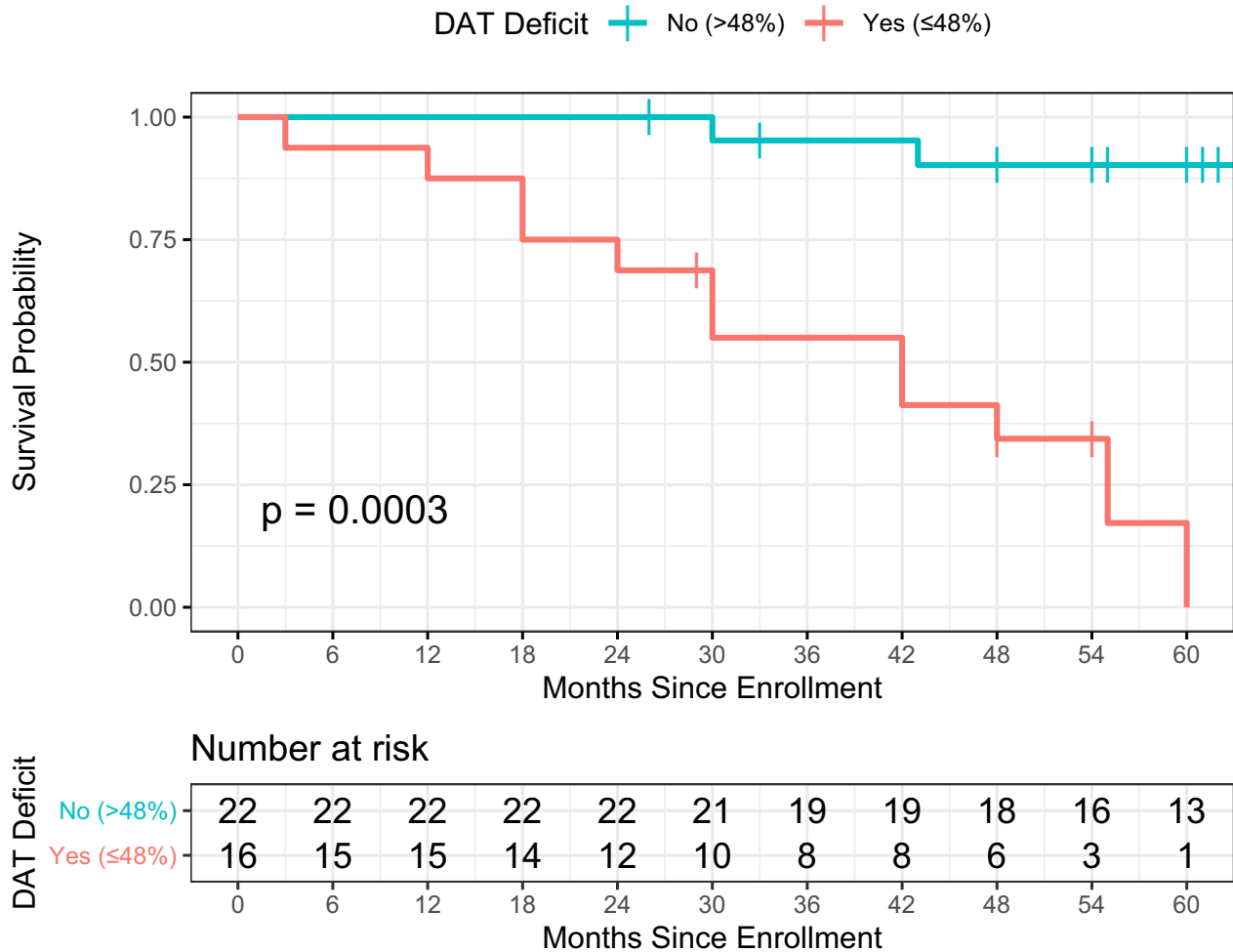


Figure 2. Kaplan-Meier survival curve for time to diagnosis of α -synucleinopathy using cutoff identified by ROC analysis.

that study is an underestimate. As for the small effect sizes for the clinically assessed markers, this further reflects that many prodromal features are nonspecific, and highlights the need for more objective quantitative measures for predicting the diagnosis of aSN in at-risk groups. In contrast, in our cohort, the hazards of aSN diagnosis were 18 times greater in those who had abnormal DAT binding (accounting for the effects of age and sex). DAT binding is specific to nigrostriatal denervation, especially when other etiologies of reduced DAT binding, such as medications, are systematically excluded, as per the PPMI protocol. Our data are further evidence that individuals with iRBD and abnormal DAT binding constitute a key population in which to institute therapeutic trials to prevent disability from aSN. iRBD individuals with DAT SBR deficit likely have neurodegenerative neuropathological changes of aSN but without the extent of neuronal loss that reaches threshold for a clinical diagnosis.

Consistent with idea that iRBD is a prodromal manifestation of aSN,^{1,2} several nonmotor and motor features of PD were present in our iRBD cohort, including olfactory loss, autonomic dysfunction, and neuropsychological abnormalities. Many of these motor and nonmotor measures were intermediate in range between HCs and a group of early, de novo untreated PD. A greater proportion of the iRBD group showed abnormal neuropsychological tests compared to the HC group. Cognitive dysfunction is seen in other PD at-risk groups with abnormal DAT binding, such as older adults with hypsmia,^{31,32} and cognitive abnormalities constitute one of the criteria for prodromal PD.¹⁵ As for the greater proportion in the iRBD group demonstrating abnormal cognition compared to the PD group, while this may reflect the older age of the iRBD group, or the cognitive changes seen in prodromal/early PD,^{31,32} it may also indicate the increased risk of DLB in individuals with iRBD. Indeed, RBD constitutes a core diagnostic criterion for DLB,³³

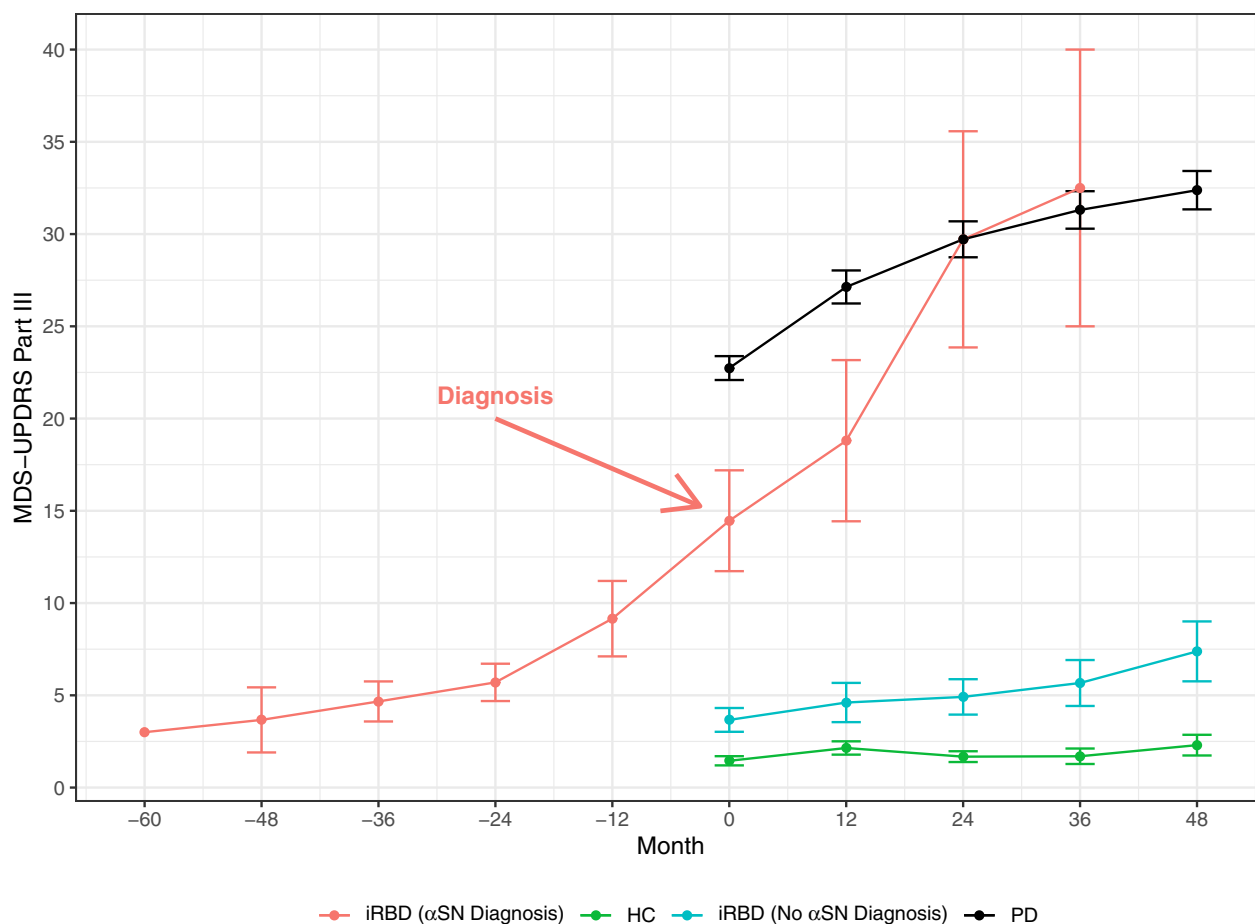


Figure 3. Mean values for MDS-UPDRS part III score are plotted over time for each group. Time 0 is the visit at which diagnosis occurred among the iRBD group that received a diagnosis of an α -synucleinopathy. Time 0 is the baseline visit for all other groups represented.

and about half of individuals with RBD go onto to manifest the parkinsonism-dementia syndrome¹ of DLB.^{2,34} DLB constituted 20% of cases of diagnoses in our cohort, and additional cases of Lewy body dementia may emerge with longer follow-up. Lower CSF amyloid- β_{1-42} in our iRBD group compared to the HC group may reflect the increased risk of Alzheimer's disease co-pathology in aSN.³⁵ Regarding other CSF biomarkers, the lack of substantial differences in CSF total α -synuclein between the iRBD group and the HC group highlights the ongoing need for more accurate CSF biomarkers in PD including assays specific to pathologic forms of α -synuclein. As for the pattern of striatal denervation indicated by the DAT binding measures, it was similar to that seen in PD^{36,37}: DAT binding in putamen < caudate, with bilateral involvement but mild asymmetry, though less severe as compared to the PD group. Similarly, clinical abnormalities at baseline were less severe than in the PD group as well. Importantly, motor abnormalities increased in the RBD group who were diagnosed with an aSN and

approached values seen in the PD group at about 2 years postdiagnosis. The clinical and imaging characteristics of our iRBD cohort indicate that the strategy of recruiting iRBD cohorts enriched with abnormal DAT imaging identifies individuals with measurable abnormalities that are, for the most part, not as severe as those seen in early, de novo PD (with the exception of cognitive and autonomic dysfunction), suggesting that putative preventive therapies could be especially meaningful in this population.

Short-term changes in DAT binding have the potential to identify longer-term outcomes in early PD.³⁸ Iranzo et al.³⁹ reported a significant reduction in DAT binding in iRBD over a 3-year follow-up period, which was greater in those who went on to be diagnosed with an aSN compared to those who were not. Similarly, among our sample which was enriched for abnormal DAT binding, there was a significant decrease in DAT SBR over a 2-year period, with declines being greater among those who were diagnosed with aSN compared to those who

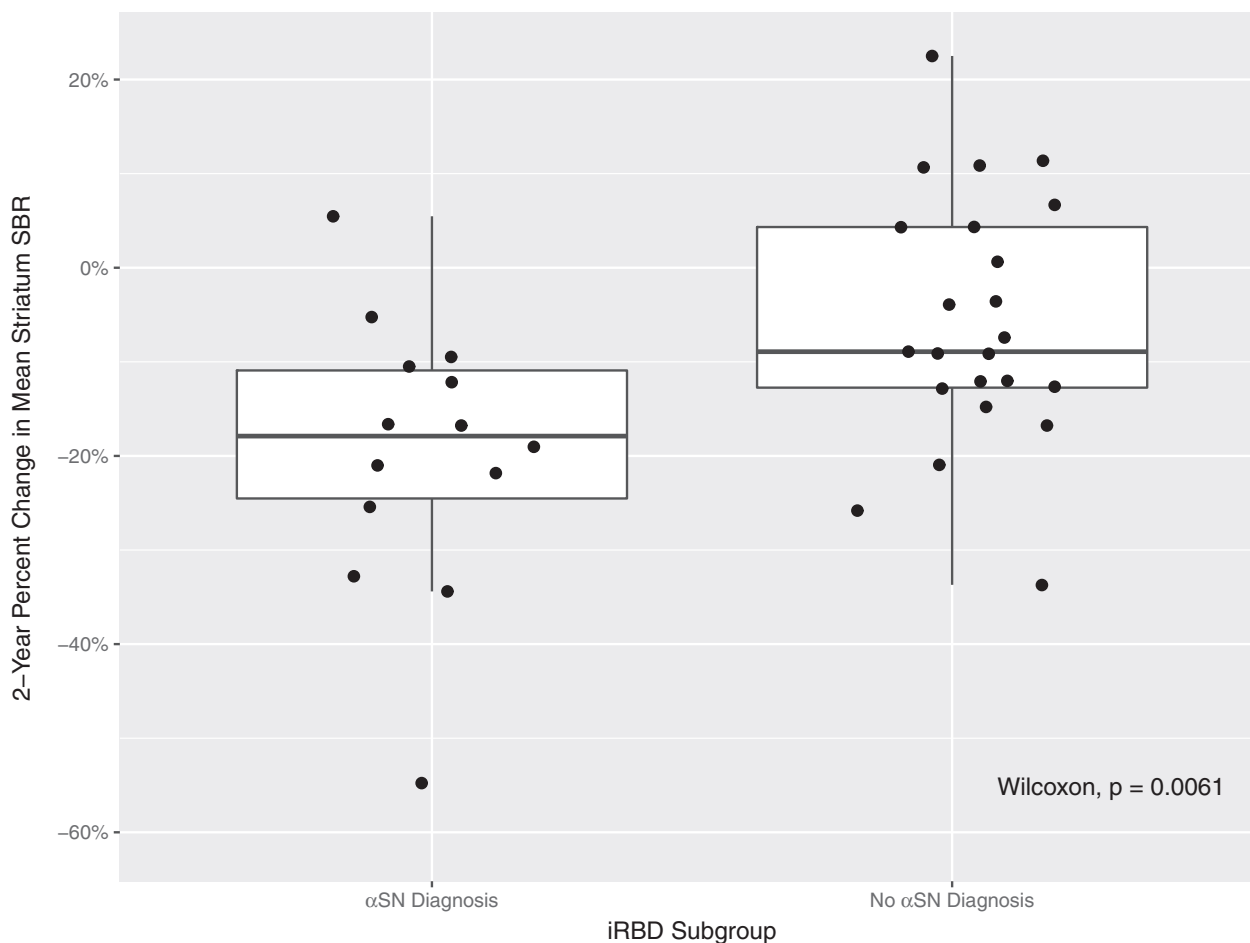


Figure 4. 2-year percent change from baseline in mean striatum SBR in iRBD participants diagnosed with α -synucleinopathy (aSN) versus iRBD participants without aSN diagnosis.

were not. There are preliminary data to indicate that DAT SBR also significantly declines in other groups at risk for aSN, such as carriers of mutations associated with PD risk.²⁴ These data provide preliminary evidence that DAT SBR may be useful not only as an enrichment biomarker in clinical trials but also potentially as an outcome measure to monitor disease (nigrostriatal deficit) progression over time even during the period prior to aSN diagnosis.

In other iRBD cohorts,^{1,2} approximately 30–50% of those who develop a clinically-defined disorder have a motor-predominant clinical syndrome and 30–50% have a cognitive syndrome. In our cohort, the site investigator indicated PD as the research diagnosis in approximately 80% of participants. This difference in profile of the clinical syndrome emerging in our iRBD cohort could be the result of enrichment of our cohort with abnormal DAT binding, and again reflects the potential utility of DAT imaging for sample selection in PD-prevention trials.

Future studies will be needed to determine if enrichment based on other criteria, including cognitive function, will modify the clinical features and diagnoses that emerge on follow-up.

Limitations of this study include the small sample size of the iRBD group that likely reduced that reliability of the effect sizes demonstrated. Due to the small sample size, we were not able to examine potential confounders in the relationship between DAT SBR and aSN risk in multivariable analysis. The cutoff of DAT SBR for age and sex must be replicated in independent studies and on larger cohorts and should be considered an estimate. In addition, our study included only a small number of individuals with iRBD with normal DAT SBR. Thus, our results may not apply to the general iRBD population. Importantly, it is possible that it is those individuals who will ultimately benefit most from disease-prevention strategies. Finally, the PPMI sample, in general, is largely white and non-Hispanic, limiting its generalizability.

In summary, we demonstrate the utility of DAT SBR measures to identify individuals with iRBD at short-term risk for aSN diagnosis in an international multicenter study. These findings provide the rationale for the next stage of PPMI,⁴⁰ which will recruit a larger and more diverse sample, including individuals with iRBD with and without abnormal DAT binding. Future work will incorporate clinical features, biological fluid biomarkers, additional imaging biomarkers, and genetic determinants to synthesize multimodal models that can identify a group of iRBD individuals most appropriate to enroll in clinical trials for disease prevention, and to serve as outcome measures for such trials.

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Conflict of Interest

None of the authors report a potential conflict of interest related to this work.

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