# Cerebral Metabolic Patterns In Neurodegeneration

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# 7. $^{18}$F-FDG PET, Dopamine Transporter SPECT and Olfaction: Combining Biomarkers in REM Sleep Behavior Disorder

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## Abstract
**Background:** Idiopathic REM sleep behavior disorder (iRBD) is a prodromal stage of Parkinson's disease (PD) and dementia with Lewy bodies (DLB). Hyposmia, reduced dopamine transporter binding, and expression of the brain metabolic PD-related pattern (PDRP) were each associated with increased risk of conversion to PD. The objective of this study was to investigate the relationship between PDRP expression, dopamine transporter binding, and olfaction in iRBD.

**Methods:** In this cross-sectional study, twenty-one iRBD subjects underwent $^{18}$F-Fluoro-deoxyglucose PET, dopamine transporter imaging, and olfactory testing. For reference, we included $^{18}$F-Fluoro-deoxyglucose PET data of 19 controls, 20 PD patients, and 22 patients with DLB. PDRP expression *z*-scores were computed from all PET scans.
**Results:** PDRP expression was higher in iRBD compared with controls ($P$=0.048), but lower compared with PD ($P$=0.001) and DLB ($P$<0.0001). PDRP expression was higher in iRBD subjects with hyposmia and in subjects with an abnormal dopamine transporter scan (P<0.05; uncorrected).
**Conclusion:** PDRP expression, dopamine transporter binding, and olfaction may provide complementary information for predicting phenoconversion.

## Introduction

Longitudinal studies have shown that >80% of individuals with idiopathic REM sleep behavior disorder (iRBD) developed Parkinson's disease (PD) or dementia with Lewy bodies (DLB) on long-term follow-up (Postuma et al., 2009, Postuma et al., 2012, Iranzo et al., 2013b, Schenck, Boeve & Mahowald, 2013, Iranzo et al., 2014). iRBD subjects represent a suitable group to study the prodromal stage of these disorders, and may be crucial for disease-modification trials. However, such trials require biomarkers which can reliably identify at-risk individuals and predict clinical manifestation of PD/DLB.

Neurodegenerative disorders are characterized by disease-specific patterns of altered brain glucose metabolism on $^{18}$F-Fluoro-deoxyglucose Positron Emission Tomography ($^{18}$F-FDG PET) brain imaging. Such patterns can be extracted from $^{18}$F-FDG PET data with the Scaled Subprofile Model and Principal Component Analysis (SSM PCA (Eidelberg, 2009)). With SSM PCA, a PD-related pattern (PDRP) has been identified in multiple cohorts (Ma et al., 2007, Niethammer, Eidelberg, 2012, Teune et al., 2014, Meles et al., 2017a). The degree to which the PDRP is present in a new $^{18}$F-FDG PET scan can be quantified, resulting in a subject score. PDRP subject scores increase with disease progression, and decrease with effective therapy (Niethammer, Eidelberg, 2012, Huang et al., 2007).

To date, two groups have reported that iRBD subjects have higher PDRP subject scores compared with controls (Wu et al., 2014, Holtbernd et al., 2014). In a longitudinal study of 17 iRBD subjects, baseline PDRP expression was associated with a high risk of developing PD or DLB within five years (Holtbernd et al., 2014). Other markers have also been considered. Loss of striatal dopamine transporter (DAT) binding on Single Photon Emission Computed Tomography (DAT-SPECT) indicates imminent phenoconversion (Iranzo et al., 2010, Stiasny-Kolster et al., 2005). In addition, iRBD subjects with baseline hyposmia have a high risk of

developing PD/DLB within 5 years of follow-up (Mahlknecht et al., 2015, Ponsen et al., 2004).

The PDRP has potential as a disease biomarker in prodromal subjects, but further validation by an independent research group is essential. Moreover, direct comparisons between PDRP expression, DAT-binding, and olfaction in the same iRBD subjects have never been made. We therefore studied these three markers in 21 iRBD patients.

## Methods

Twenty-one subjects with iRBD (polysomnographically-confirmed (Schenck et al., 2013)) were evaluated with $^{18}$F-FDG PET, DAT-SPECT, and olfactory testing. Per inclusion criteria, iRBD subjects did not have parkinsonism (Hughes et al., 1992) or DLB (McKeith et al., 2005) at the time of the study. Participants with a history of psychotropic medication use before the onset of iRBD were excluded (Frauscher et al., 2014).

Nineteen age-matched healthy controls were studied with $^{18}$F-FDG PET and olfactory testing. Controls did not have iRBD (score <5 on the RBD screening questionnaire (Stiasny-Kolster et al., 2007)), and furthermore had no first-degree family members with a neurodegenerative disease.

iRBD subjects and controls were investigated with the Unified Parkinson's Disease Rating Scale (UPDRS, version 2003 (Movement Disorder Society Task Force on Rating Scales for Parkinson's Disease, 2003)), and the Montreal Cognitive Assessment (MoCA (Gagnon et al., 2010)). Olfactory function was assessed with Sniffin' Sticks (Stiasny-Kolster et al., 2005, Mahlknecht et al., 2015, Hummel et al., 1997). Total olfaction scores (TDI) were obtained by summing the threshold (T), discrimination (D), and identification (I) sub-scores. Five olfactory stages were defined as follows: anosmia (TDI ≤15), severe hyposmia (15<TDI≤20), moderate hyposmia (20<TDI≤25), mild hyposmia (25<TDI≤30), and normosmia (TDI>30). In a previous study, it was determined that a baseline TDI score <18 was associated with increased risk of phenoconversion to PD/DLB within five years of follow-up (Mahlknecht et al., 2015). We therefore divided iRBD patients into 2 groups: patients with TDI scores <18 and patients with TDI scores ≥18.

For reference, we studied the $^{18}$F-FDG PET scans of retrospectively-included patients with clinical diagnoses of "probable PD" (*n*=20, non-demented, aged 67.5±8.6 years; 16 men; median disease duration, 2 years, interquartile range, 1-7 years), and "probable DLB" (*n*=22, aged 73.7±7 years; 17 men; median disease duration, 3 years, interquartile range, 1-4 years) according to consensus criteria (Postuma et al., 2015, Hughes et al., 1992, McKeith et al., 2005).

Exclusion criteria for all subjects included a history of (other) neurological diseases, diabetes mellitus, stroke, significant head trauma, or other relevant

comorbidities. The study was approved by local institutional review boards. Voluntary written informed consent was obtained from each subject after verbal and written explanation of the study, in accordance with the Declaration of Helsinki.

All subjects underwent static $^{18}$F-FDG PET imaging on a Siemens Biograph mCT-64 PET/CT camera (Siemens, Munich, Germany) at the University Medical Center Groningen, the Netherlands. Images were reconstructed with point-spread function and time-of-flight modeling, and smoothed with a Gaussian 8 mm full-width at half-maximum filter. Central nervous system depressants were discontinued in all subjects for at least 24 hours before each scan. In iRBD patients, all RBD-related medications (e.g. melatonin or clonazepam) were discontinued for at least 48 hours pre-scan. In PD and DLB patients, dopamimetics were not withheld.

All images were spatially normalized onto an $^{18}$F-FDG PET template in Montreal Neurological Institute (MNI) brain space (Della Rosa et al., 2014) using SPM12 software (Wellcome Department of Imaging Neuroscience, Institute of Neurology, London, UK) implemented in Matlab (version 2012b; MathWorks, Natick, MA). Expression of the previously-identified PDRP (Teune et al., 2014) was calculated in the new $^{18}$F-FDG PET data as described previously (Spetsieris, Eidelberg, 2011). All PDRP subject scores were *z*-transformed to the controls ($n$=19), such that the average PDRP *z*-score in controls was 0, with a standard deviation of 1.

In future clinical trials of iRBD, diagnostic tool specificity will be more important than sensitivity (i.e., RBD subjects who will not phenoconvert should be excluded). We therefore reanalyzed the PDRP identification cohort (Teune et al., 2014) and selected a cut-off *z*-score that gave 100% specificity. At PDRP $z$=1.8, there was no misclassification of controls in the identification cohort (data not shown). This threshold was applied to the PDRP *z*-scores in the current study (i.e., a *z*-score of ≥1.8 was considered indicative of PD).

iRBD subjects underwent DAT imaging with $^{123}$I-2β-carbomethoxy-3β-(4-iodophenyl)-N-(3-fluoropropyl)nortropane ($^{123}$I-FP-CIT) SPECT. DAT-binding in striatal regions was quantified with The Brain Registration & Analysis Software Suite (BRASS; HERMES Medical, Sweden). Specific to non-specific binding ratios were calculated in the caudate nucleus and putamen bilaterally, using the occipital cortex for reference (i.e., non-specific binding). DAT-binding ratios that were 2 or more standard deviations lower than age-matched expected control values were considered abnormal (see Supplementary Material). The lowest putamen DAT-binding ratio of each subject was used for further analyses. The median time interval between acquisition of the $^{123}$I-FP-CIT SPECT and $^{18}$F-FDG PET was 2.7 months (interquartile range, 1.3–4.6 months; total range, 12 days–9.6 months). Loss of striatal DAT-binding in the putamen was considered abnormal for age in 9 of 21 iRBD subjects.

*Statistical Analysis*

The normality of distribution of each variable was assessed with the Shapiro-Wilk test, Q-Q-plots, and boxplots. PDRP *z*-scores and DAT-binding ratios were parametric. PDRP *z*-scores were compared across controls, iRBD, PD, and DLB with a one-way analysis of variance (ANOVA) with post-hoc Bonferroni corrections.

PDRP *z*-scores were compared between iRBD subjects with normal and abnormal DAT scans with an independent *t* test. PDRP *z*-scores and DAT-binding ratios were also compared between the 2 olfaction categories (TDI score <18 or ≥18) with an independent *t* test. These analyses were not corrected for multiple comparisons.

In the 21 iRBD subjects, correlations between PDRP *z*-scores and DAT-binding ratios were tested for significance with a Pearson correlation coefficient. TDI, MoCA, and UPDRS-III scores were non-parametric. Correlations between these variables and the imaging metrics (PDRP *z*-scores and DAT-binding) were assessed with a Spearman's rank correlation coefficient. Correlations were considered significant at P<0.05 (uncorrected). All analyses were performed using SPSS software version 23 (SPSS Inc., Chicago, IL).

## Results

UPDRS-III scores were significantly higher in iRBD subjects compared with controls. MoCA and olfaction scores were significantly lower in iRBD patients ($P$<0.01, Supplementary Table).

PDRP subject scores were not significantly different between men ($n$=9) and women ($n$=10) in the control group ($P$=0.75, independent t test). Stepwise increases in PDRP *z*-scores were observed across groups (ANOVA F(81)=59.06, $P$<0.0001, Figure 1). In 12 of 21 iRBD subjects (57%), the PDRP *z*-score surpassed the threshold ($z$≥1.8; Table 1).

In Table 1, PDRP *z*-scores, putamen DAT-binding ratios, and TDI scores are shown for each iRBD patient. This permits identification of several iRBD subgroups. Subjects 1-3 have normal values for all three markers. Subjects 17-21 have abnormal values for all three markers: supra-threshold PDRP *z*-scores, putamen DAT-binding too low for age, and TDI scores <18. Subjects 15 and 16 have supra-threshold PDRP *z*-scores and abnormal DAT scans, but TDI scores ≥18. Of the 9 subjects with abnormal DAT scans, 7 had supra-threshold PDRP *z*-scores (Subjects 15-21). Interestingly, of the 12 subjects with normal DAT scans, 5 (42%) had supra-threshold PDRP *z*-scores (Subjects 10-14).

On average, subjects with abnormal DAT scans ($n$=9) had higher PDRP *z*-scores compared with subjects with normal DAT scans ($P$=0.044, uncorrected).

Subjects with olfaction scores <18 (*n*=9) had higher PDRP *z*-scores compared with subjects with olfaction scores of ≥18 (*P*=0.032, uncorrected). Putamen DAT-binding ratios were not significantly different between the 2 olfaction groups (*P*=0.117). PDRP *z*-scores, DAT-binding, and olfaction were not significantly correlated, but trends were observed (*n*=21; supplementary Figure 1).

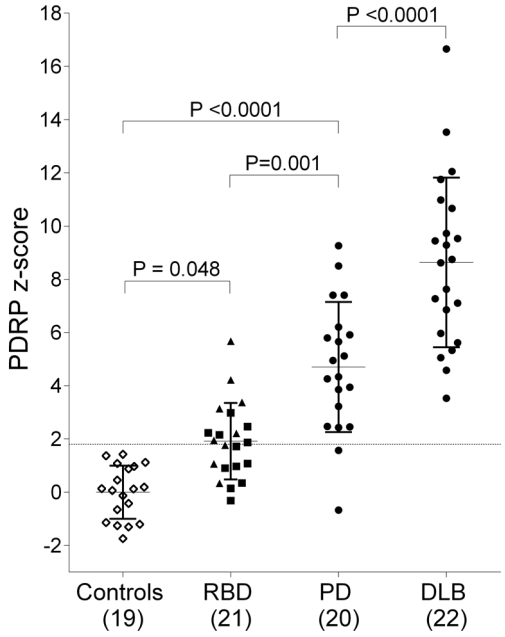



**Figure 1.** *PDRP z-scores across groups. PDRP expression was calculated in all groups and z-transformed to the healthy controls. PDRP expression z-scores were compared across groups with a one-way analysis of variance. Post-hoc comparisons were Bonferroni-corrected. The dashed line (z=1.8) indicates the cutoff for PDRP expression. Triangles indicate iRBD subjects with abnormal DAT scans. Squares indicate subjects with normal DAT scans*

**Table 1.** *Clinical and Imaging Characteristics of the 21 iRBD Subjects*

| PDRP z-score category | DAT scan category | iRBD subject | PDRP z-score | Lowest putamen DAT-binding ratio | Total olfaction score (TDI) [b] | Sex | Age | RBD duration (years) | Age at onset iRBD | MoCA | UPDRS-III |
|---|---|---|---|---|---|---|---|---|---|---|---|
| <1.8 | normal | 1 | 1.7 | 2.0[c] | 33.8 | Male | 57.4 | 5.0 | 52.4 | 30.0 | 4.0 |
| | | 2 | 1.0 | 2.3 | 33.5 | Female | 58.9 | 7.0 | 51.9 | 27.0 | 0.0 |
| | | 3 | -0.3 | 2.5 | 33.5 | Female | 68.3 | 6.0 | 62.3 | 23.0 | 2.0 |
| | | 4 | 1.1 | 2.5 | 29.5 | Male | 54.0 | 6.0 | 48.0 | 26.0 | 4.0 |
| | | 5 | 0.9 | 2.4 | 28.0 | Male | 56.4 | 6.0 | 50.4 | 27.0 | 1.0 |
| | | 6 | 0.2 | 2.2 | 19.5 | Male | 67.1 | 25.0 | 42.1 | 28.0 | 0.0 |
| | | 7 | 0.4 | 2.9 | 0.0 | Male | 56.0 | 9.0 | 47.0 | 25.0 | 1.0 |
| | abnormal | 8 | 0.3 | 1.2 | 19.0 | Male | 65.9 | 12.0 | 53.9 | 26.0 | 2.0 |
| | | 9 | 1.1 | 1.0 | 13.0 | Male | 66.4 | 6.0 | 60.4 | 27.0 | 3.0 |
| ≥ 1.8 | normal | 10 | 2.2 | 2.5 | 29.0 | Male | 57.8 | 5.0 | 52.8 | 28.0 | 1.0 |
| | | 11[a] | 2.2 | 2.3 | 23.5 | Male | 62.6 | 14.0 | 48.6 | 24.0 | 5.0 |
| | | 12 | 3.0 | 2.5 | 20.5 | Male | 57.5 | 2.5 | 55.0 | 27.0 | 6.0 |
| | | 13 | 1.9 | 2.3 | 16.5 | Male | 64.5 | 2.0 | 62.5 | 26.0 | 2.0 |
| | | 14[a] | 2.5 | 2.0[c] | 15.5 | Female | 70.1 | 3.0 | 67.1 | 28.0 | 4.0 |
| | abnormal | 15 | 2.2 | 1.7 | 27.5 | Male | 64.0 | 14.0 | 50.0 | 28.0 | 4.0 |
| | | 16 | 1.8 | 1.6 | 25.8 | Male | 66.9 | 3.0 | 63.9 | 27.0 | 2.0 |
| | | 17 | 3.4 | 0.9 | 17.0 | Male | 61.5 | 4.0 | 57.5 | 27.0 | 0.0 |
| | | 18 | 3.1 | 1.7 | 13.0 | Male | 65.4 | 6.0 | 59.4 | 27.0 | 6.0 |
| | | 19 | 4.2 | 2.0[c] | 2.0 | Male | 49.9 | 4.0 | 45.9 | 24.0 | 1.0 |
| | | 20 | 5.7 | 1.2 | 0.0 | Male | 63.2 | 4.0 | 59.2 | 28.0 | 1.0 |
| | | 21 | 1.9 | 1.8 | 0.0 | Male | 66.6 | 2.0 | 64.6 | 22.0 | 5.0 |

**Table 1 :**

*[a]In these 2 iRBD subjects, [18]F-FDG PET was performed respectively 3.4 and 1.5 months before DAT SPECT.*

*[b]Olfaction was measured with the Sniffin' Sticks test; total TDI scores are reported in this table (see main text). A TDI>30 indicates normal olfactory function; a TDI≤20 indicates severe hyposmia. A TDI score of <18 was previously associated with an increased risk of phenoconversion to PD/DLB (Mahlknecht et al., 2015).*

*[c]Subjects 1, 14, and 19 all have putamen DAT-binding ratios of 2.0. Subjects 1 and 14 are still in the 'normal DAT' category, and subject 19 is in the 'abnormal DAT' category. This is because DAT-binding ratios were considered abnormal if they were 2 standard deviations below the value expected for age. For subjects 1 and 14, the ratio of 2.0 is still normal for age (57 and 70 years old, respectively); however, for subject 19, this ratio is abnormal for age (50 years old). We note that subject 1 has a borderline-normal DAT-binding ratio and PDRP z-score (z=1.7).*

*iRBD, idiopathic REM sleep behavior disorder; PDRP, Parkinson's Disease-Related Pattern; DAT, dopamine transporter; MoCA, Montreal Cognitive Assessment; UPDRS-III, part 2 of the Unified Parkinson's Disease Rating Scale (2003 version).*

## Discussion

Our findings underscore the value of the PDRP as a potential disease biomarker in idiopathic RBD. In line with two previous studies, iRBD subjects significantly expressed the PDRP (Wu et al., 2014, Holtbernd et al., 2014). Although on average, PDRP *z*-scores were lower in RBD subjects compared with PD/DLB, more than half of the iRBD subjects already had a PDRP *z*-score in the range of PD patients.

This study is the first to directly compare PDRP expression, striatal DAT-binding, and olfaction in iRBD. Although a trend was observed, PDRP and striatal DAT-binding were not significantly correlated. Previous studies in PD have shown that PDRP expression shows only modest correlation to DAT-binding (Niethammer, Eidelberg, 2012, Holtbernd et al., 2015, Tang et al., 2010a). This may indicate a partly nondopaminergic genesis of the PDRP. Remarkably, 5 of 12 iRBD patients with normal striatal DAT-binding had suprathreshold PDRP *z*-scores. In 2 of these cases, $^{18}$F-FDG PET was performed before DAT-SPECT. It has been shown that some DLB patients may initially have unremarkable DAT scans (van der Zande et al., 2016). It is possible that iRBD subjects with significant PDRP expression but normal DAT-binding will eventually develop DLB. Longitudinal imaging studies of iRBD subjects are needed to further investigate the relationship between PDRP expression and loss of DAT-binding in relation to the final clinical diagnosis.

The fact that there was no direct significant correlation between PDRP *z*-scores, DAT-binding, and olfaction could indicate that these three markers provide complementary information. For example, two cases had supra-threshold PDRP *z*-scores and abnormal DAT scans, but TDI scores ≥18. These subjects would have

been considered at low risk of phenoconversion if the olfaction scores alone had been considered (Mahlknecht et al., 2015). We also identified three subjects with normal values for all three markers. These individuals may have a low risk of converting to PD/DLB. In contrast, five subjects had supra-threshold PDRP *z*-scores, putamen DAT-binding too low for age, and TDI scores <18; these subjects may be considered to have a particularly high risk of conversion within the next five years.

The data presented in this report are cross-sectional. A longitudinal study of our iRBD cohort is ongoing. Follow-up data will be essential to elucidate if DAT SPECT-negative DLB cases, and perhaps subjects who later developed multiple system atrophy, contributed to the aforementioned findings. We expect that the PDRP will be especially informative, because in contrast to olfaction (Iranzo et al., 2013a), the PDRP is a progression marker (Huang et al., 2007). Moreover, PDRP expression is useful in the differential diagnosis of parkinsonian disorders (Tripathi et al., 2015), whereas DAT imaging is not (Stoffers et al., 2005).

## Supplemental Material for Chapter 7

*DAT SPECT Imaging and Analysis*

iRBD subjects (n=21) underwent DAT imaging with $^{123}$I-2β-carbomethoxy-3β-(4-iodophenyl)-N-(3-fluoropropyl)nortropane ($^{123}$I-FP-CIT) SPECT in Marburg, Germany as described previously (Stiasny-Kolster et al., 2005). $^{123}$I-FP-CIT binding in striatal regions was quantified with The Brain Registration & Analysis Software Suite (BRASS, HERMES Medical, Sweden). Specific to non-specific binding ratios were calculated in the caudate nucleus and putamen bilaterally, using the occipital cortex as reference (i.e. non-specific binding).

To determine whether binding ratios were abnormal for age, 24 healthy controls were used for reference (age range 18-74 years). Because these controls were scanned on a different camera (NeuroFocus system (software upgrade of the Strichman Medical Equipment system; Massachusetts, USA)), phantom measurements were performed to calculate a correction factor for our system (Siemens Symbia S, Low Energy High Resolution) to match the reference dataset. A striatal phantom (RS-901T; GE) was used for direct, quantitative comparison between the two SPECT systems. Binding ratios which were 2 or more standard deviations lower than age-matched expected control values were considered abnormal. Before analysis, all scans were anonymized to the reader.

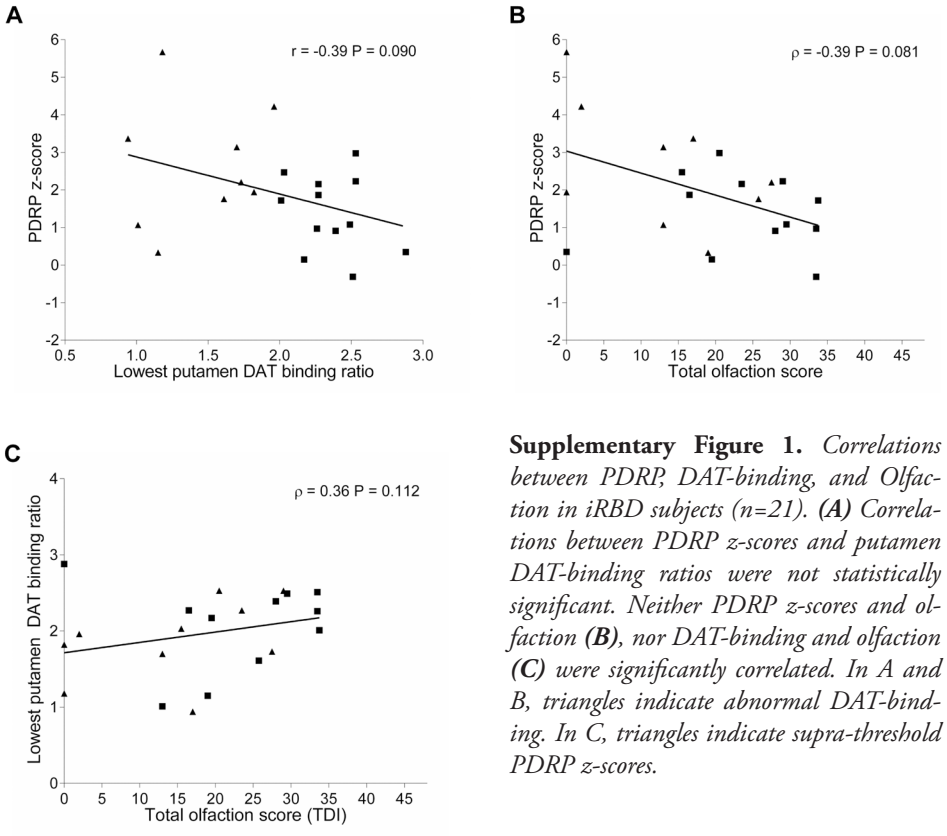
**Supplementary Table:** *Group Averages of Clinical Information and Comparison to Controls*

| | Controls (*n*=19) | iRBD (*n*=21) | *P* value[a] |
|---|---|---|---|
| Age (years) | 62.4±7.5 | 61.9±5.4 | 0.82 |
| Gender (male/female) | 9/10 | 18/3 | 0.010 |
| Age at onset of RBD | | 55.0±7.1 | |
| RBD duration (years) | | 6.9±5.4 | |
| | | | |
| MoCA | 28.3±1.6 | 26.4±1.9 | 0.003 |
| UPDRS-III | 0.8±1.25 | 2.6±2.0 | 0.002 |
| Olfaction (TDI score)[b] | 33.3±5.1 | 19.0±11.3 | <0.001 |

*Values are mean ± standard deviation unless otherwise specified.*

*[a]Independent t test for age and PDRP z-scores, Chi$^2$ test for gender, Mann-Whitney U-test for MoCA, UPDRS-III, and olfaction. Uncorrected P values are shown.*

*[b]Olfaction was measured with Sniffin' Sticks. In this test, the olfactory threshold (T), discrimination (D) and identification (I) of smells is tested. Total scores summing these three aspects (TDI) are reported.*




**Supplementary Figure 1.** *Correlations between PDRP, DAT-binding, and Olfaction in iRBD subjects (n=21).* ***(A)*** *Correlations between PDRP z-scores and putamen DAT-binding ratios were not statistically significant. Neither PDRP z-scores and olfaction* ***(B)****, nor DAT-binding and olfaction* ***(C)*** *were significantly correlated. In A and B, triangles indicate abnormal DAT-binding. In C, triangles indicate supra-threshold PDRP z-scores.*