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Alpha-synuclein seeds in olfactory mucosa of patients with isolated REM sleep behaviour disorder

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Isolated REM sleep behaviour disorder (RBD) is an early-stage α-synucleinopathy in most, if not all, affected subjects. Detection of pathological α-synuclein in peripheral tissues of patients with isolated RBD may identify those progressing to Parkinson's disease, dementia with Lewy bodies or multiple system atrophy, with the ultimate goal of testing preventive therapies. Real-time quaking-induced conversion (RT-QuIC) provided evidence of α-synuclein seeding activity in CSF and olfactory mucosa of patients with α-synucleinopathies. The aim of this study was to explore RT-QuIC detection of α-synuclein aggregates in olfactory mucosa of a large cohort of subjects with isolated RBD compared to patients with Parkinson's disease and control subjects. This cross-sectional case-control study was performed at the Medical University of Innsbruck, Austria, the Hospital Clinic de Barcelona, Spain, and the University of Verona, Italy. Olfactory mucosa samples obtained by nasal swab in 63 patients with isolated RBD, 41 matched Parkinson's disease patients and 59 matched control subjects were analysed by α-synuclein RT-QuIC in a blinded fashion at the University of Verona, Italy. Median age of patients with isolated RBD was 70 years, 85.7% were male. All participants were tested for smell, autonomic, cognitive and motor functions. Olfactory mucosa was α-synuclein RT-QuIC positive in 44.4% isolated RBD patients, 46.3% Parkinson's disease patients and 10.2% control subjects. While the sensitivity for isolated RBD plus Parkinson's disease versus controls was 45.2%, specificity was high (89.8%). Among isolated RBD patients with positive α-synuclein RT-QuIC, 78.6% had olfactory dysfunction compared to 21.4% with negative α-synuclein RT-QuIC ($P < 0.001$). The extent of olfactory dysfunction was more severe in isolated RBD patients positive than negative for olfactory mucosa a-synuclein RT-QuIC ($P < 0.001$). We provide evidence that the α-synuclein RT-QuIC assay enables the molecular detection of neuronal α-synuclein aggregates in olfactory mucosa of patients with isolated RBD and Parkinson's disease. Although the overall sensitivity was moderate in this study, nasal swabbing is attractive as a simple, non-invasive test and might be useful as part of a screening battery to identify subjects in the prodromal stages of α-synucleinopathies. Further studies are needed to enhance sensitivity, and better understand the temporal dynamics of α-synuclein seeding in the olfactory mucosa and spreading to other brain areas during the progression from isolated RBD to overt α-synucleinopathy, as well the impact of timing, disease subgroups and sampling technique on the overall sensitivity.

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Abbreviations: α-syn = α-synuclein; MDS = Movement Disorders Society; RBD = REM sleep behaviour disorder; RT-QuIC = real-time quaking induced conversion

Introduction

Isolated REM sleep behaviour disorder (RBD) is characterized by abnormal behaviours during REM sleep.[1] Long-term follow-up studies have showed that more than 80% of patients with isolated RBD may go on to develop Parkinson's disease, dementia with Lewy bodies or, less commonly, multiple system atrophy.[2,3] These disorders are characterized by pathological deposition of α-synuclein (α-syn) aggregates in different sites within the central and peripheral nervous system and thus collectively labelled as α-synucleinopathies. Therefore, isolated RBD is now commonly regarded as an early stage α-synucleinopathy.[4]

Identification of early or prodromal stages of α-synucleinopathies is a key research goal on the path to disease-modifying and neuroprotective therapies. Recently, there has been great interest in detecting α-syn deposition in peripheral tissues of subjects with isolated RBD as a potential biomarker of prodromal Lewy body disease stage. Intraneural phosphorylated α-syn (p-α-syn) deposits have been demonstrated by immunohistochemistry in tissue biopsies of colon,[5] salivary glands[6-8] and skin of patients with Parkinson's disease but also those with isolated RBD,[9,10] where they were shown to differentiate subjects with isolated RBD from control subjects with variable sensitivity (24–89%) but overall high specificity (78–100%). However, these approaches are invasive, and feasibility and patient acceptance face limits when it comes to large scale screening for prodromal Parkinson's disease or repeated prospective assessments of disease progression.[11] In addition, while colonic biopsies have tested the potential starting point of one suggested route of seeding and spreading of pathological α-syn species, i.e. from gut to brain, the olfactory system as the second proposed region of initiation of α-syn pathology in Parkinson's disease has not been studied yet in prodromal Parkinson's disease stages such as isolated RBD.[12] Real-time quaking induced conversion (RT-QuIC) is a novel assay based on the so-called 'prion replication principle' implying that pathological misfolded proteins (seeds) serve as template for imparting their conformation to normal isoform (substrate). Tissue samples such as CSF and olfactory mucosa containing α-syn aggregates initiate amyloid fibril formation by converting the recombinant α-syn which, in turn, enhances the fluorescence of thioflavin T (ThT).[13-15]

Olfactory dysfunction is common in patients with isolated RBD, where it represents a predictor of short-term phenoconversion to Parkinson's disease or dementia with Lewy bodies,[16] and is almost universal in established Parkinson's disease. The olfactory dysfunction in Parkinson's disease is likely related to Lewy pathology and neuronal cell loss in the olfactory bulbs, tracts and piriform cortex, and according to the Braak staging the olfactory bulbs may be an initial site of α-syn aggregation in Parkinson's disease.[17,18] The misfolded α-synuclein, which replicates and propagates with a prion-like mechanism, is believed to drive the neurodegenerative process.

In the present study, we tested a novel biomarker approach for identifying α-syn aggregates by RT-QuIC assay in olfactory mucosa samples obtained from patients with isolated RBD, established Parkinson's disease and healthy control subjects.

Materials and methods

Study design and participants

This was a cross-sectional study performed at three clinical academic centres (Department of Neurology of Innsbruck Medical

University, Austria; Hospital Clinic de Barcelona, Spain; and Neurology Clinic, Department of Neurosciences, Biomedicine and Movement Sciences of the University of Verona, Italy). The study was approved by the local ethics committees and all participants provided written informed consent according to the Declaration of Helsinki.

Sixty-three patients with polysomnography-confirmed isolated RBD were recruited at the Sleep Disorder Units of the Department of Neurology, Innsbruck Medical University, Austria, and Hospital Clinic de Barcelona, Spain. Isolated RBD was diagnosed according to the current International Classification of Sleep Disorders criteria,[1] in the absence of parkinsonism or cognitive impairment (excluded by clinical history and examination). Demographic and clinical data were collected through interview and review of medical records.

The Movement Disorder Units of Innsbruck and Barcelona clinical departments and the Neurology Clinic in Verona recruited age- and sex-matched Parkinson's disease patients ($n = 41$), diagnosed according to the Movement Disorders Society (MDS) clinical diagnostic criteria.[19] Fifty-nine age- and sex-matched controls without parkinsonism or cognitive impairment were also included. Control subjects were recruited in Innsbruck from patients of the sleep laboratory in whom isolated RBD and REM sleep without atonia were excluded by video-polysomnography (performed no longer than 6 months before olfactory mucosa sampling), and in Barcelona from non-blood relatives of isolated RBD or Parkinson's disease patients participating in the study. In all control subjects, evidence of a neurodegenerative disease was excluded by clinical interview and neurological examination. For control subjects without availability of a video-polysomnography, dream-enacting behaviours were excluded by clinical history.

Clinical markers of α-synuclein-related neurodegeneration

In all participants, smell function was assessed with the 16-item identification part of the Sniffin' Sticks[20] or with the 40-item University of Pennsylvania Smell Identification Test (UPSIT),[21] the presence and quantification of motor signs using the motor part of the MDS Unified Parkinson's Disease Rating Scale (MDS-UPDRS),[22] autonomic dysfunction with the SCOPA-AUT (Scales for Outcomes in Parkinson's Disease-Autonomic Dysfunction),[23] and cognitive function with the Montreal cognitive assessment (MoCA).[24] These evaluations were performed no longer than 3 months before olfactory mucosa sampling. Additionally, in Parkinson's disease patients a history suggestive of isolated RBD was obtained using the RBD single question screen (RBD1Q) at the day of olfactory mucosa sampling.[25]

For data analysis, UPSIT scores were converted to Sniffin' 16 scores using published equating scores.[26] Olfactory dysfunction was defined according to published updated age- and sex-adjusted normative data for the Sniffin' 16 scores, using the 10th percentile as cut-off.[20]

MDS prodromal Parkinson's disease calculation was performed for isolated RBD patients, according to the updated MDS research criteria for prodromal Parkinson's disease,[27] using the online prodromal Parkinson's disease calculator.[1] For patients aged <50 years we selected the age range 50–54 years (the lowest age range provided by the calculator).

Olfactory mucosa sampling

Nasal swabbing procedure was performed in all participants using a specifically designed flocked brush (FLOQBrush®; CopanItalia Spa), as described previously.[28] Olfactory mucosa sampling was performed by otolaryngologists (L.S., J.Sc. and I.V.) and did not require local anaesthesia. The nasal swabbing procedure took <5 min and was done without the use of nasal tampons, to avoid patients' discomfort or airways obstruction (Supplementary Video 1). Coagulation disorder or anticoagulant/antiplatelet drug intake or other medical conditions were not exclusion criteria. Adverse events were immediately recorded. At the end of the procedure, 61 participants (37.4%) were asked to evaluate the degree of pain or discomfort perceived during nasal swabbing on a scale from 1 (i.e. minimal discomfort) to 10 (i.e. maximal discomfort).

One to four olfactory mucosa samples were collected from each individual, depending on nasal cavity anatomy and individual tolerability. Almost all subjects had two nasal swabbings taken from a single nostril, except for 12 subjects in whom sampling was performed bilaterally. Since two swabs were required for RT-QuIC analysis cytological quality control of samples was limited to the 12 subjects with bilateral procedures. Eight of these samples were processed for immunocytochemical analysis and from a single swab we collected 1 million total cells where 30% were olfactory marker protein positive.[29]

Olfactory mucosa sample preparation

Following nasal swabbing, the swab was immersed in a 5 ml tube containing 0.9% saline and sealed. Tubes from each patient were labelled with an anonymized code, stored at 4°C, and sent to the neuropathology laboratory at University of Verona, Italy. Cellular material was dissociated from the swab by vortexing tubes for 1 min at room temperature. The swab was then removed from the tube, the cell suspension was pelleted by centrifugation at 2000*g* at 4°C for 20 min. The supernatant was removed, and pellet frozen at –80°C until assayed.

Alpha-synuclein RT-QuIC analysis in olfactory mucosa swabs

RT-QuIC analyses were performed in the LURM (Laboratorio Universitario di Ricerca Medica) Research Center, University of Verona, Italy. Recombinant α-syn was expressed and purified from the periplasmic fraction as previously reported.[30]

The α-syn RT-QuIC test used in this study has been previously set up using brain tissue of definite cases of Parkinson's disease, dementia with Lewy bodies, and multiple system atrophy.[30] Olfactory mucosa samples were thawed and a disposable inoculating loop (Fisherbrand) was dipped into the pellet to transfer ~2 µl of the pellet into a tube containing 120 µl phosphate-buffered saline (PBS). The latter tube was sonicated at 120 W (Digital ultrasonic bath Mod.DU-32, Argo Lab) for at least 1 min until the pellet was dispersed. For each test, 2 µl of diluted olfactory mucosa sample were plated in 98 µl of reaction buffer composed of 100 mmol/l phosphate buffer (pH 8.2), 10 µmol/l ThT, and 0.05 mg/ml human recombinant full-length (1–140 aa) α-syn and 37 ± 3 mg of 0.5 mm glass beads (Sigma). The plate was sealed with a plate sealer film (Nalgene Nunc

International) and then incubated at 30°C in a BMG FLUOstar® Omega plate reader with cycles of 1 min shaking (200 rpm double orbital) and 14 min rest. ThT fluorescence measurements (450 ± 10 nm excitation and 480 ± 10 nm emission; bottom read) were taken every 45 min. Four replicate reactions were tested for each sample. ThT fluorescence threshold was calculated considering 3 standard deviations (SD) above the baseline. Because of the initial increase of fluorescence signal in all curves, the baseline was determined between 15 and 17 h.

A sample was considered positive when at least two of four replicate wells crossed this calculated threshold (100 000 rfu). If only one of the replicates was positive, the RT-QuIC was considered to be negative. Cut-off time was assessed at 80 h, based on the results from definite cases, in order to obtain the best specificity and sensitivity. The maximal fluorescence value was the highest mean fluorescent value seen during the α-syn RT-QuIC analytical run of 80 h and we considered the lag-phase as the hours required for the average fluorescence to exceed the threshold for individual cases, as reported in Fig. 1. At the end of sample analyses, RT-QuIC results were sent to the study coordinator (A.S.) who unblinded the results.

Statistical analysis

Data distribution was tested using the Kolmogorov-Smirnov or the Shapiro-Wilk test. As data were not normally distributed, quantitative variables are reported as median (interquartile range, IQR) and qualitative variables as number (%). Quantitative variables were analysed using the Kruskall-Wallis test for overall comparisons between groups. Qualitative variables were analysed with the Fisher's exact test in case of comparison of two variables with two categories, with the chi-squared test in case of two variables with more than two categories.

Sensitivity and specificity for olfactory mucosa-positive in isolated RBD plus Parkinson's disease were calculated. Correlations were evaluated using the Spearman-Rho, Pearson correlation coefficient or φ correlation coefficient, as appropriate. All statistical analyses were performed with SPSS (IBM SPSS Statistics, Version 25) and with STATA/IC 16.0 for Windows (StataCorp LLC). *P*-values < 0.05 were considered statistically significant.

Data availability

Data are available upon request to the corresponding author.

Results

This study was performed between 2017 and 2020. A total of 163 participants were included: 63 patients with isolated RBD, 41 patients with Parkinson's disease and 59 control subjects. Demographic characteristics and results of clinical assessments are summarized in Table 1.

Olfactory function was similarly impaired in both isolated RBD and Parkinson's disease as compared to controls (Table 1).

According to the MDS research criteria for prodromal Parkinson's disease, 41 of 63 patients with isolated RBD (65.1%) met criteria for probable prodromal Parkinson's disease (median estimated probability 98.3%, IQR 69.5–99.9%). When excluding polysomnography-proven RBD from the calculation, 18 of 63 (28.6%) were still classified as probable prodromal Parkinson's disease (median estimated probability 32.1%, IQR 1.4–90.3%).

Olfactory mucosa sampling and α-synuclein RT-QuIC assay

Two nasal swabbings were performed into the most easily accessible nostril: on the left side in 102 (62.6%), on the right side in 49 (30.1%) participants, and bilaterally (two from each nostril because both were easily accessible) in 12 (7.4%). Nasal swabbing was not associated with adverse events or complications except for a short and transient local discomfort during the procedure. The latter was experienced as a sudden and brief (seconds) intense discomfort (score ⩾ 8) by 9.8%, moderate discomfort (score 5–7) by 42.6%, only mild discomfort (score < 5) by 47.6%.

Nasal swabs were RT-QuIC-positive for α-syn in 28/63 (44.4%) patients with isolated RBD, in 19/41 (46.3%) patients with Parkinson's disease and in 6 of 59 (10.2%) control subjects (Fig. 1). RT-QuIC positivity for α-syn was significantly different between patients with isolated RBD and controls ($P < 0.001$), as well as between patients with Parkinson's disease and controls ($P < 0.001$), but not between patients with isolated RBD and Parkinson's disease ($P = 0.504$). Sensitivity for isolated RBD and Parkinson's disease versus controls was 45.2% [95% confidence interval (CI) 35.4–55.3%], specificity 89.8% (95% CI 79.2–96.2%).

There was no significant difference in RT-QuIC responses, either of average final ThT fluorescence value (214 720 ± 48 471 and 199 415 ± 52 560 rfu) or lag-time phase (47 ± 14.2 and 45 ± 14 h) between olfactory mucosa samples from patients with isolated RBD and with Parkinson's disease. Six of 59 olfactory mucosa samples from control subjects were positive within 80 h of seeding reaction (Fig. 1 and Supplementary Table 1).

RT-QuIC assay results and clinical data

Twenty-two (78.6%) of the 28 olfactory mucosa-positive patients with isolated RBD had olfactory dysfunction, while among the 35 olfactory mucosa-negative patients with isolated RBD, olfactory dysfunction was present in 22.9% (8 of 35). The extent of olfactory dysfunction in isolated RBD olfactory mucosa-positive patients was more severe compared to olfactory mucosa-negative subjects ($P < 0.001$); moreover, there was a moderate correlation between RT-QuIC results and smell test score (Pearson correlation coefficient –0.490, $P < 0.001$; after adjustment for age Pearson correlation coefficient –0.429, $P < 0.002$; after adjustment for sex Pearson correlation coefficient –0.500, $P < 0.001$)

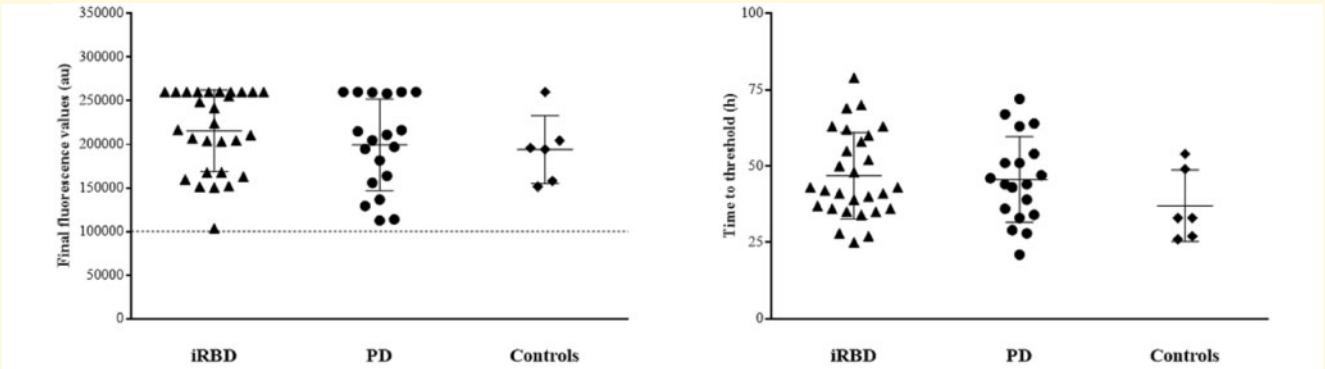

Figure 1 RT-QuIC analysis of olfactory mucosa samples from patients with isolated RBD, Parkinson's disease and control subjects. Final fluorescence values (**A**) and lag-phase (**B**) of positive α-syn RT-QuIC subjects in olfactory mucosa samples from patients with isolated RBD (iRBD), Parkinson's disease (PD) and control subjects. Data-points in **A** represent the average fluorescence value obtained for each individual case at 80 h; samples are grouped in three different classes (isolated RBD, Parkinson's disease, Controls) and bars show the average ± SD for type of case. Data-points in **B** show hours required from the average fluorescence value to exceed the threshold of 100 000 rfu for individual cases and bars show the average ± SD for type of pathology. au = arbitrary unit.

Table 1 Demographic and clinical characteristics of the study population

	Isolated RBD (*n* = 63)	Parkinson's disease (*n* = 41)	Controls (*n* = 59)	*P*-value
Age, years	70 (64–74)	70 (65–76)	70 (64–73)	0.690
Sex, *n* (%)				0.735
Female	9 (14.3)	8 (19.5)	11 (18.6)	
Male	54 (85.7)	33 (80.5)	48 (81.4)	
Disease duration (from diagnosis), y	5 (2–9)	6.5 (2–9.8)	n.a.	n.a.
MDS-UPDRS III score	4 (2–7)	21 (13–30)	1 (0–3)	**<0.001** **iRBD versus PD <0.001** **iRBD versus Ctr <0.001** **PD versus Ctr <0.001**
SCOPA-AUT score	14 (9–27)	16 (9–23)	11 (6–19)	0.052 iRBD versus PD 0.724 **iRBD versus Ctr 0.023** PD versus Ctr 0.075
MoCA score	27 (24–28)	27 (24–28)	27 (25–29)	0.833 iRBD versus PD 0.793 iRBD versus Ctr 0.526 PD versus Ctr 0.846
Sniffin' Sticks score	8 (7–12)	8 (6–10)	12 (11–13)	**<0.001** iRBD versus PD 0.310 **iRBD versus Ctr <0.001** **PD versus Ctr <0.001**
Olfactory dysfunction, *n* (%)	30 (47.6)	25 (61)	8 (13.6)	**<0.001** iRBD versus PD 0.229 **iRBD versus Ctr <0.001** **PD versus Ctr <0.001**

Data are shown as median (IQR) or *n* (%). Values in bold indicate significance. Ctr = controls; iRBD = isolated RBD; MDS-UPDRS III = Movement Disorders Society Unified Parkinson's Disease Rating Scale, part III; MoCA = Montreal Cognitive Assessment; n.a. = not applicable/available; PD = Parkinson's disease; SCOPA-AUT = Scales for Outcomes in Parkinson's Disease-Autonomic Dysfunction.

and a strong relationship with olfactory dysfunction (φ coefficient 0.554, $P < 0.001$).

These associations were not found in patients with Parkinson's disease (Table 2). Smell test results for the different study groups in relation to RT-QuIC results are shown in Fig. 2.

The median estimated probability for prodromal Parkinson's disease based on the MDS criteria (excluding

polysomnographic proven RBD from the calculator) was numerically higher in the olfactory mucosa-positive versus olfactory mucosa-negative subgroup (median estimated probability 54.4% versus 14.8%) but the ranges were large and the difference was not statistically significant (IQRs 1.5–72.2% and 1.2–89.9%, $P = 0.782$). Likewise, the percentages of patients meeting the threshold for a diagnosis of probable prodromal Parkinson's disease were not statistically different between the two groups (17.9% versus 34.3%, $P = 0.166$).

Among patients with Parkinson's disease, 28 of 41 (68.3%) screened positively for RBD with the RBD1Q. Positive response to the RBD1Q did not differ between olfactory mucosa-positive and olfactory mucosa-negative Parkinson's disease patients (12/19, 63.2% versus 16/22, 72.7%, $P = 0.737$). We did not find any difference between Parkinson's disease with and without probable RBD regarding olfactory mucosa-positivity and Sniffin' Stick score (Pearson correlation 0.129, $P = 0.676$ in Parkinson's disease without probable RBD; Pearson correlation 0.007, $P = 0.972$ in Parkinson's disease with probable RBD). The same was true for the correlation between olfactory dysfunction and olfactory mucosa-positivity (φ coefficient = –0.141, $P = 0.612$ in Parkinson's disease without probable RBD, φ coefficient = –0.062, $P = 0.743$ in Parkinson's disease with probable RBD).

Further demographic and clinical characteristics of the isolated RBD and Parkinson's disease patients did not differ between subjects with or without olfactory mucosa α-syn seeding activity (Table 2).

Among healthy controls, the six (10.2%) olfactory mucosa-positive individuals as a group did not differ from olfactory mucosa-negative controls in any of the assessed clinical parameters.

Discussion

To the best of our knowledge this is the first study evaluating α-syn RT-QuIC in the olfactory mucosa of a large cohort of patients with isolated RBD versus those with Parkinson's disease or healthy control subjects. We studied a total of 163 subjects and found a positive α-syn RT-QuIC seeding reaction in the olfactory mucosa in 44.4% of patients with isolated RBD, in 46.3% of those with Parkinson's disease and in 10.2% of controls. From the perspective of diagnostic testing this would result in an overall specificity of 89.8%, sensitivity of 45.2% and accuracy of 61.3%. Such figures, although impressive in terms of specificity, do not seem superior to what has been reported for immunohistochemical assays for p-α-syn in salivary glands and skin biopsies.[5-10] However, there are distinctive and unique features in the method used in the present study.

Olfactory mucosa sampling provides direct access to olfactory neurons and thus to one of the sites that is currently regarded as one of the potential initiation points of seeding and spread of pathological α-syn assemblies in Parkinson's disease. RT-QuIC for α-syn detects disease-associated α-syn aggregates in billion-fold diluted brain tissue preparations from different α-synucleinopathies[31] with a higher sensitivity than conventional immunohistochemistry, as shown in prion diseases.[32] RT-QuIC results are classified as negative or positive based on the extent of α-syn aggregates seeding and do not depend on observer evaluation, as occurs for α-syn immunohistochemistry in skin biopsies of patients with isolated RBD.[33]

Furthermore, immunohistochemistry is too insensitive to detect the early misfolded forms of α-syn occurring in olfactory neurons, as suggested by the low yield of Lewy pathology in olfactory neuroepithelium of subjects with α-synucleinopathies.[34] In addition, RT-QuIC positivity for α-syn has been found in olfactory mucosa of 10% of control subjects, suggesting that α-syn aggregation occurs as an incidental event in the olfactory neuroepithelium.[35] However, this finding was not unexpected since the occurrence of incidental Lewy bodies is ∼10% in individuals over 60 years,[36,37] which corresponds to the mean age of controls recruited in the present study. It is intriguing to speculate if these individuals might be at increased risk to develop Parkinson's disease or other α-synucleinopathies, but numbers here were too small for cross-sectional analysis.

In the only previous study[13] using α-syn RT-QuIC on olfactory mucosa samples from patients with synucleinopathies (18 Parkinson's disease, 11 multiple system atrophy versus 18 controls with non α-syn-related disorders) sensitivity and specificity were broadly similar (56% and 83%, respectively) to the present study, supporting the reproducibility of the analytical approach.

Recent studies have also explored the performance and diagnostic accuracy of the α-syn RT-QuIC assay of CSF in patients with α-synucleinopathies. In patients with Parkinson's disease, diagnostic sensitivity and specificity were 84% and 89%, respectively regarding differentiation from non-synucleinopathy parkinsonism.[38]

Since the olfactory system is believed to be one of the earliest sites of pathology in Parkinson's disease, RT-QuIC for α-syn in olfactory mucosa samples is a particularly attractive approach not only in the quest for biomarkers of α-synucleinopathies but also for understanding the dynamics of seeding and spread of α-syn pathology. Previous observations in isolated RBD showed that olfactory dysfunction can identify patients at high risk of short-term conversion to overt α-synucleinopathy.[16] Intriguingly, in our study, α-syn RT-QuIC positivity in the olfactory mucosa from patients with isolated RBD was preferentially associated with olfactory dysfunction, but this was not observed in Parkinson's disease patients. A possible explanation could be that different olfactory areas are involved in the two conditions. For instance, olfactory dysfunction in Parkinson's disease is driven by the involvement of the anterior olfactory nucleus and olfactory bulb or larger areas of the olfactory system beyond the olfactory mucosa, while patients with isolated RBD may have a more relevant involvement of peripheral sites such as olfactory mucosa. These differences might reflect rostral trans-

Table 2 Demographic and clinical characteristics of the isolated RBD group (*n* = 63) and of the Parkinson's disease group (*n* = 41)

	Isolated RBD			Parkinson's disease		
	α-syn-positive *n* = 28 (44.4%)	α-syn-negative *n* = 35 (55.6%)	*P*-value	α-syn-positive *n* = 19 (46.3%)	α-syn-negative *n* = 22 (53.7%)	*P*-value
Age, years	72 (67–74.8)	67 (60–74)	0.059	70 (65–75)	71 (63.8–76.3)	0.704
Age at diagnosis, years	67 (63–68.8)	60 (56–67)	**0.004**	64 (57–68)	64 (58–71)	0.708
Age at onset, years	62 (59–67.5)	53 (45–61)	**0.011**	64 (56–68)	63 (57–69)	0.936
Disease duration, years	4.5 (1.3–8)	6 (2–10)	0.176	7 (1–12)	5 (2–9)	0.934
Sex, *n* (%)			0.170			0.703
Female	6 (21.4)	3 (8.6)		3 (15.8)	5 (22.7)	
Male	22 (78.6)	32 (91.4)		16 (84.2)	17 (77.3)	
MDS-UPDRS III score	4 (1–7)	4 (2–7)	0.692	21 (13–30)	21 (12–28)	0.937
SCOPA-AUT score	18 (11–29)	13 (8–21)	0.133	15 (10–20)	19 (8–26)	0.432
MoCA score	27 (24–28)	27 (24–28)	0.737	27 (23–28)	27 (24–28)	0.926
Sniffin' Sticks score	7 (5–8)	11 (8–13)	**<0.001**	7 (6–11)	8 (7–9)	0.968
Olfactory dysfunction, *n* (%)	22 (78.6)	8 (22.9)	**<0.001**	11 (57.9)	8 (36.4)	0.757
Hoehn and Yahr stage	n.a.	n.a.	n.a.	2 (2–2)	2 (2–2)	n.a.
Rigidity, *n* (%)	n.a.	n.a.	n.a.	13 (100)	21 (95.5)	1.000
Rest tremor, *n* (%)	n.a.	n.a.	n.a.	7 (53.8)	12 (54.5)	0.536

Data are shown as median (IQR) or *n* (%). iRBD = isolated RBD; MDS-UPDRS III = Movement Disorders Society Unified Parkinson's Disease Rating Scale, part III; MoCA = Montreal Cognitive Assessment; n.a. = not applicable/available; SCOPA-AUT = Scales for Outcomes in Parkinson's Disease-Autonomic Dysfunction. *P*-values were calculated using the Mann-Whitney U-test.

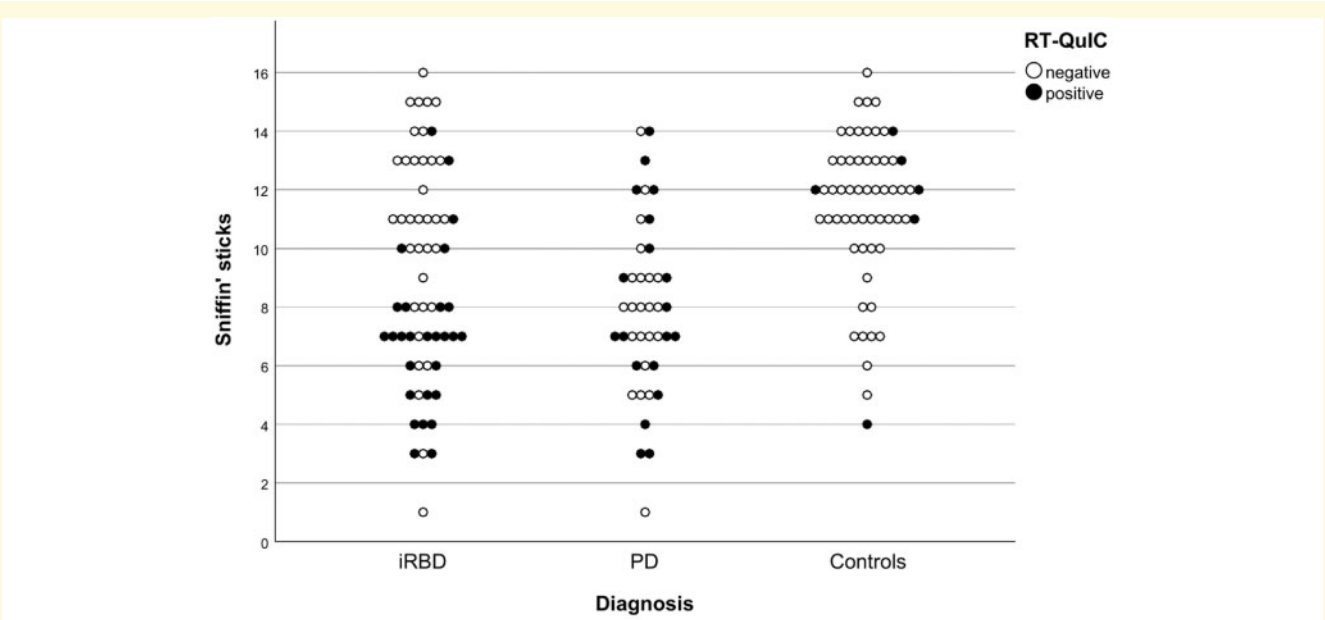

Figure 2 Olfactory mucosa RT-QuIC for α-synuclein results and smell test scores in isolated RBD, Parkinson's disease and control groups. Positive olfactory mucosa results are shown as a black circle, negative olfactory mucosa results are shown as a white circle. iRBD = isolated RBD; PD = Parkinson's disease.

synaptic propagation of α-syn pathology, as reported for the enteric neurons in Parkinson's disease.[5] The fact that the rate of α-syn RT-QuIC positivity in the olfactory mucosa of Parkinson's disease patients was present in <50% of subjects might suggest that numbers of olfactory neurons progressively decrease with disease progression,[39] a process which might occur also in isolated RBD. To investigate this hypothesis further, prospective studies including isolated RBD as well as early Parkinson's disease subjects are needed. This approach is expected to provide further insight into the temporal dynamics of α-syn seeding activity in the olfactory mucosa and its association with phenoconversion. Future studies should include larger Parkinson's disease samples in different disease stages to enable differential assessment of

olfactory mucosa RT-QuIC in Parkinson's disease subgroups based on in-depth phenotypic characterization.

In summary, we show that α-syn RT-QuIC enables detection of pathological seeding activity in olfactory neurons from patients with isolated RBD and Parkinson's disease. This reaction appears highly specific for these synucleinopathies as compared to control subjects and is associated with olfactory disturbances in isolated RBD. Probing the olfactory mucosa with α-syn RT-QuIC may provide a valuable marker to recognize patients in an early-stage of α-synuclein-related neurodegeneration and might help to select subjects for clinical disease-modification trials of interventions targeting α-syn pathological conversion and spread.

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Competing interests

The authors report no competing interests.

Supplementary material

Supplementary material is available at *Brain* online.

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