BRIEF REPORT

Dermal Real-Time Quaking-Induced Conversion Is a Sensitive Marker to Confirm Isolated Rapid Eye Movement Sleep Behavior Disorder as an Early α-Synucleinopathy

Anastasia Kuzkina, MD,[1] Celine Panzer,[1] Aline Seger, MD,[2] Daniela Schmitt,[1] Jonas Rößle, MD,[1] Sebastian R. Schreglmann, MD, PhD,[1] Henrike Knacke, MD,[3] Elena Salabasidou,[1] Antonia Kohl,[1] Elisabeth Sittig,[4] Michael Barbe, MD,[2] Daniela Berg, MD,[3] Jens Volkmann, MD,[1] Claudia Sommer, MD,[1] Wolfgang H. Oertel, MD,[4,5] Eva Schaeffer, MD,[3] Michael Sommerauer, MD,[2,6] Annette Janzen, MD,[4] and Kathrin Doppler, MD[1*]

[1]*Department of Neurology, University Hospital Würzburg, Würzburg, Germany* [2]*Department of Neurology, University Hospital Cologne, University of Cologne, Köln, Germany* [3]*Department of Neurology, University Hospital Schleswig-Holstein, Campus Kiel, Kiel, Germany* [4]*Department of Neurology, Philipps University Marburg, Marburg, Germany* [5]*Helmholtz Center for Health and Environment, Institute for Neurogenomics, München-Neuherberg, Germany* [6]*Institute of Neuroscience and Medicine (INM-3), Forschungszentrum Jülich, Jülich, Germany*

ABSTRACT: Background: Skin biopsy is a potential tool for the premortem confirmation of an α-synucleinopathy.
Objective: The aim was to assess the aggregation assay real-time quaking-induced conversion (RT-QuIC) of skin biopsy lysates to confirm isolated rapid eye movement sleep behavior disorder (iRBD) as an α-synucleinopathy.
Methods: Skin biopsies of patients with iRBD, Parkinson's disease (PD), and controls were analyzed using RT-QuIC and immunohistochemical detection of phospho-α-synuclein.
Results: α-Synuclein aggregation was detected in 97.4% of iRBD patients (78.4% of iRBD biopsies), 87.2% of PD patients (70% of PD biopsies), and 13% of controls (7.9% of control biopsies), with a higher seeding activity in iRBD compared to PD. RT-QuIC was more sensitive but less specific than immunohistochemistry.
Conclusions: Dermal RT-QuIC is a sensitive method to detect α-synuclein aggregation in iRBD, and high seeding activity may indicate a strong involvement of dermal nerve fibers in these patients. © 2023 The Authors. *Movement Disorders* published by Wiley Periodicals LLC on behalf of International Parkinson and Movement Disorder Society.

Key Words: rapid eye movement sleep behavior disorder; α-synuclein; Parkinson's disease; real-time quaking-induced conversion; skin biopsy

Detection of α-synuclein aggregates in tissue biopsies and fluids that are easily accessible premortem is a promising approach for a biomarker of Parkinson's disease (PD).[1-3] Phospho-α-synuclein deposits were detected in various tissues containing peripheral nerve fibers, including skin biopsies, which reflects the knowledge that α-synuclein pathology is not restricted to the central nervous system in these diseases.[4-7] Isolated rapid eye movement sleep behavior disorder (iRBD) is supposed to represent a prodromal stage of PD, dementia with Lewy bodies, and multiple system atrophy.[8,9] Whereas many studies in the past decade focused on the histopathological detection of peripheral α-synuclein deposits using immunohistochemistry (IHC),[1] more recent studies have provided evidence that real-time quaking-induced conversion (RT-QuIC), an aggregation assay based on the seeding properties of misfolded α-synuclein, may provide a sensitive and specific method to measure α-synuclein aggregates in larger numbers of

Current address for Anastasia Kuzkina, MD Ann Romney Center for Neurologic Diseases, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, 02115, USA

***Correspondence to:** Dr. Kathrin Doppler, Department of Neurology, University Hospital Würzburg, Josef-Schneider-Str. 11, 97080 Würzburg, Germany; E-mail: doppler_k@ukw.de

Michael Sommerauer, Annette Janzen, and Kathrin Doppler contributed equally.

Relevant conflicts of interest/financial disclosures/funding sources: This study was supported by a grant from the International Parkinson Fonds to K.D., W.H.O., and C.S. A.K. and K.D. are supported by grants from the Interdisciplinary Center of Clinical Research of the University Hospital Würzburg. C.P. is supported by a grant from the Graduate School of Life Sciences of the University of Würzburg. S.R.S. is a fellow of the Thiemann Foundation. M.S. was supported by the Koeln Fortune Program/Faculty of Medicine, University of Cologne (grant number: 453/2018), and the Else Kröner-Fresenius-Stiftung (grant number: 2019_EKES.02).

Received: 27 September 2022; **Revised:** 27 December 2022; **Accepted:** 19 January 2023

Published online in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/mds.29340

TABLE 1 *Overview of the detection rates and quantification of dermal α-synuclein aggregates using RT-QuIC and immunofluorescence staining*

	iRBD (n = 38)	PD (n = 39)	Controls (n = 23)	Comparison/*P*-value
Sex	**5 female, 33 male**	**6 female, 33 male**	**8 female, 15 male**	**–**
Age (median, range) (y)	65.5 (55–84)	65 (50–79)	57 (45–85)	$P = 0.01$*
Duration of disease** (median, range) (y)	5.4 (2–32)	8 (1–22)	–	–
Stage (Hoehn & Yahr)	0	I: 6, II: 19, III: 12, IV: 1, V: 1	–	–
NMSQ (median, range)	6 (0–19)	10 (0–24)	na	$P = 0.02$
MDS-UPDRS-III (median, range)	2.5 (0–8)	21 (2–47)	–	–
Olfactory function***	5 normosmic, 16 hyposmic, 15 anosmic	na	na	–
Positive using RT-QuIC	37/38 (97.4%)	34/39 (87.2%)	3/23 (13%)	iRBD/PD > controls, $P < 0.0001$
Leg	26/35 (74.3%)	22/30 (73.3%)	3/20 (15%)	
C7	31/38 (81.6%)	27/39 (69.2%)	2/22 (9.1%)	
Th10	12/15 (80%)	7/11 (63.6%)	0/21 (0%)	
Marburg	11/12 (91.7)	2/4 (50%)	1/3 (33.3%)	
Cologne	19/19 (100%)	na	na	
Kiel	3/3 (100%)	6/8 (75%)	n/a	
Würzburg	4/4 (100%)	26/27 (96.3%)	2/20 (10%)	
Maximum fluorescence (rfu, mean, all sites)	47,142 (±13,888)	37,688 (±18,298)	12,386 (±10,385)	iRBD/PD > controls, $P < 0.0001$ iRBD > PD, $P < 0.05$
Leg	44,063 (±20,869)	36,936 (±21,277)	14,883 (±19,577)	iRBD/PD > controls, $P < 0.0005$
C7	46,149 (±20,253)	37,722 (±21,828)	10,498 (±12,765)	
Th10	47,142 (±22,675)	35,160 (±23,027)	12,002 (±11,955)	
Lag phase (h, mean, all sites)	37.15 (±12.15)	44.45 (±12.37)	64.93 (±13.33)	iRBD/PD < controls, $P < 0.0001$ iRBD < PD, $P < 0.05$
Leg	42.02 (±18.27)	42.89 (±17.13)	61.23 (±22.12)	iRBD/PD < controls, $P < 0.002$
C7	35.86 (±16.63)	45.91 (±15.49)	66.34 (±16.67)	iRBD/PD < controls, $P < 0.0001$ iRBD < PD, $P < 0.006$
Th10	37.03 (±16.61)	51.14 (±13.68)	65.79 (±11.00)	iRBD/PD < controls, $P < 0.004$ iRBD < PD, $P = 0.02$
Ratio of positive replicates (mean, all sites)	0.83 (±0.21)	0.76 (±0.30)	0.27 (±0.23)	iRBD/PD > controls, $P < 0.0001$
Leg	0.76 (±0.37)	0.78 (±0.36)	0.3 (±0.35)	
C7	0.82 (±0.32)	0.76 (±0.36)	0.23 (±0.29)	
Th10	0.82 (±0.36)	0.68 (±0.40)	0.29 (±0.20)	iRBD/PD > controls, $P < 0.01$

(Continues)

TABLE 1 *Continued*

Sex	iRBD (n = 38) 5 female, 33 male	PD (n = 39) 6 female, 33 male	Controls (n = 23) 8 female, 15 male	Comparison/*P*-value –
Positive using IHC	23/35 (65.7%)	11/27 (40.7%)	0/23 (0%)	iRBD/PD > controls, $P < 0.0005$
Leg	14/35 (40%)	5/27 (18.5%)	0	iRBD > controls, $P < 0.0001$
C7	13/35 (37.1%)	7/27 (25.9%)	0	iRBD/PD > controls, $P < 0.05$
Th10	6/15 (40%)	2/11 (18.2%)	0	iRBD > controls, $P < 0.05$
Marburg	8/12 (66.7%)	0/4 (0%)	0/3 (0%)	na
Cologne	12/19 (63.2%)	na	na	
Würzburg	3/4 (75%)	11/23 (47.8%)	0/20 (0%)	
Ratio of positive slides (mean, all sites)	0.1 (±0.15)	0.07 (±0.11)	0	iRBD/PD > controls, $P < 0.01$

Note: *Kruskal–Wallis test, **from onset of symptoms, and ***TDI (Würzburg, Marburg), 16-item screening (Kiel), 12-item screening (Cologne).
Abbreviations: RT-QuIC, real-time quaking-induced conversion; iRBD, isolated rapid eye movement sleep behavior disorder; PD, Parkinson's disease; na, not available; MDS-UPDRS, Movement Disorder Society-Sponsored Revision of the Unified Parkinson's Disease Rating Scale; rfu, relative fluorescence units; IHC, immunohistochemistry.

samples,[10-12] not only in cerebrospinal fluid (CSF)[13,14] but also in skin biopsy lysates of PD patients.[10-12,15]

In the present study, we aimed to assess the use of dermal RT-QuIC as an early diagnostic marker of α-synucleinopathy in patients with iRBD. For direct comparison, dermal RT-QuIC was analyzed in parallel with histopathological detection of phospho-α-synuclein in skin biopsies.

Patients and Methods

Subjects

Thirty-eight patients with a video-polysomnography-confirmed diagnosis of iRBD according to the consensus criteria of the International RBD Study Group[16] were included at the University Hospitals Marburg (n = 12), Cologne (n = 19), Kiel (n = 3), and Würzburg (n = 4). Thirty-nine patients with a diagnosis of PD based on the Movement Disorder Society Clinical Diagnostic Criteria for Parkinson's Disease[17] and 23 healthy controls were recruited at the University Hospitals Marburg (PD: n = 4, controls: n = 3), Kiel (PD: n = 8), and Würzburg (PD: n = 27, controls: n = 20). Details on the clinical testing of patients are given in the Supplemental Methods. Demographic data of the participants are summarized in Table 1 and provided in detail in Supplementary Table S1.

Skin Biopsy Procedure, IHC, and RT-QuIC

Skin biopsies (5-mm diameter) were taken at C7 (all centers), Th10 (Marburg, partly Würzburg), and proximal leg (Marburg, Cologne, and Würzburg) as previously described.[4] Samples were shipped to Würzburg for testing using IHC and RT-QuIC. One half was used for IHC and the other half for RT-QuIC. In 15 patients (3 iRBD and 12 PD), no material was taken for immunofluorescence staining.

IHC and RT-QuIC were performed as previously described[11,18] (see Fig. S1 and Supplemental Methods for details).

Statistical Analysis

Statistical analysis was performed using GraphPad Prism, version 9.4.0 (GraphPad Software, San Diego, CA, USA). To compare the numbers of positive cases between groups, Fisher's exact test was used. For comparison of quantitative measurements, Mann-Whitney *U* test was applied. Spearman's correlation was used to test if the quantification of positivity as measured using RT-QuIC and IHC (both ratio data) correlates, and the Phi coefficient was used for correlation analysis of binary data (positive/negative). For comparing the rate of positivity between RT-QuIC and IHC, McNemar's test was used. A significance level of 0.05 was applied for all tests.

Results

In 37 of 38 (97.4%) of the patients with iRBD, comprising 34 of 39 (87.2%) patients with PD and 3 of 23 (13%) controls, α-synuclein aggregates were detected using RT-QuIC ([$P < 0.0001$] iRBD/PD

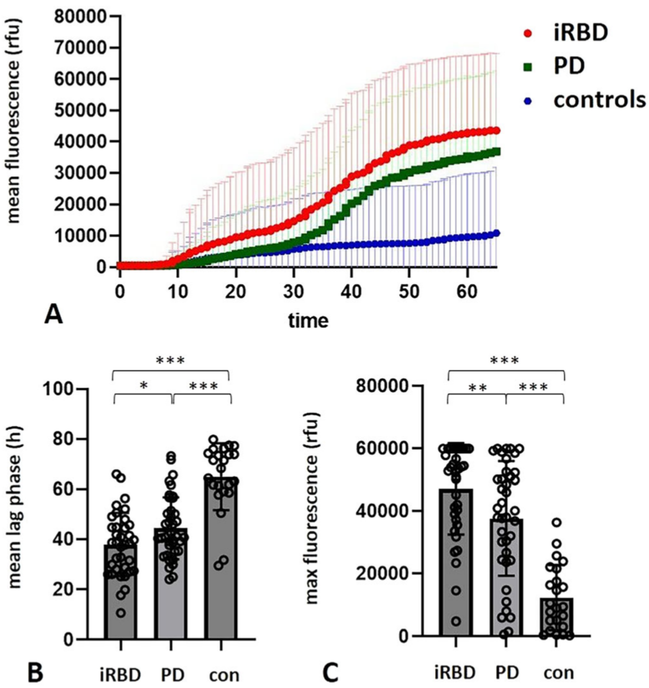

FIG. 1. Mean fluorescence (including all biopsy sites) during incubation time; (**A**) error bars mark standard deviations: the curve of the patients with iRBD (isolated rapid eye movement sleep behavior disorder) is slightly above those of patients with PD (Parkinson's disease), and both curves representing patients are clearly above the control curve, demonstrating slightly higher fluorescence intensity in iRBD patients compared to PD and higher intensity in patients compared to controls. (**B**) The mean lag phase of patients (iRBD and PD) was shorter, and the (**C**) mean maximum fluorescence intensity was higher than in controls. The mean lag phase was shorter in iRBD than in PD, and the mean maximum fluorescence was higher (***$P < 0.0001$, **$P < 0.01$, and *$P < 0.05$) (max = maximum, con = controls, PD = Parkinson's disease, iRBD = isolated rapid eye movement sleep behavior disorder). [Color figure can be viewed at wileyonlinelibrary.com]

against controls; Table 1). The detection rate per single biopsy was 78.4% in iRBD, 70% in PD, and 7.9% in controls. Using IHC, dermal phospho-α-synuclein was detectable in 23 of 35 (65.7%) patients with iRBD, 11 of 27 (40.7%) patients with PD, and in none of the 23 controls. The detection rate per single biopsy was 38.8% in iRBD and 21.5% in PD. The detection rates across biopsy sites did not differ significantly between groups and within groups (Table 1).

One of the RT-QuIC-positive healthy controls had a probability of prodromal PD of 16% according to the MDS criteria, and the other two had <1%.[19,20] One reported hyposmia, 1 had a positive family history of PD (father affected), and 1 had mild motor symptoms (hypophonia, unsteady gait).

The 5 RT-QuIC-negative patients with PD presented with a tremor-dominant phenotype and were at an early stage of disease (1 patient H&Y 1, 4 patients H&Y 2, average disease duration: 1.8 years). None of them suffered from RBD based on the REM Sleep Behavior Disorder Screening Questionnaire (RBDSQ) or anamnestic exploration.

In RT-QuIC, the lag phase was shorter and the mean maximum fluorescence was higher in patients with iRBD and PD compared to controls ($P < 0.0001$) and also in patients with iRBD compared to PD (mean lag phase: $P = 0.012$, mean maximum fluorescence: $P = 0.0096$; Fig. 1), indicating higher seeding activity in iRBD.

Considering all biopsy sites of each subject, all patients who were positively tested using IHC were also positive using RT-QuIC. There were more patients positive using RT-QuIC than using IHC ($P < 0.0001$). When directly assessing biopsies that were cut in half, 33.6% of all biopsy halves tested positive using both methods (iRBD: 45.9%, PD: 21.3%, and controls: 0%) and 61.1% using RT-QuIC only (iRBD: 50.8%, PD: 70.2%, and controls: 100%), and only four biopsy halves tested positive using IHC, not using RT-QuIC (3.5% of all, iRBD: 3.3%, PD: 8.5%, and controls: 0%) (Fig. S2A/B).

A correlation between IHC (ratio of positive sections) and seeding activity of RT-QuIC (mean lag phase, mean maximum fluorescence) was found (mean maximum fluorescence: $r = 0.5$, $P < 0.0001$, mean lag phase: $r = -0.4$, $P = 0.0008$, Fig. S2C,D), and there was a moderate correlation of positivity between both methods (phi = 0.32).

We did not find a correlation between dermal α-synuclein aggregation as detected using RT-QuIC or IHC and duration of iRBD, RBDSQ, Non-Motor Symptoms Questionnaire (NMSQ), MDS-UPDRS-III (Movement Disorder Society-Sponsored Revision of the Unified Parkinson's Disease Rating Scale), or age. RT-QuIC was positive in all 6 iRBD patients with normal 123I-N-ω-fluoropropyl-2β-carbomethoxy-3β-(4-iodophenyl) tropane single photon emission computed tomography (FP-CIT-SPECT), and IHC was positive in 4 of them; all 4 normosmic iRBD patients tested positive using RT-QuIC and only 1 of them using IHC.

In patients with PD, the mean lag phase was shorter in patients with suspected RBD ($n = 16$) based on the RBDSQ or anamnestic exploration (mean lag phase: $P = 0.03$, mean maximum fluorescence: $P = 0.13$), but correlation between the mean lag phase and duration of disease failed to reach significance ($P = 0.067$, $r = -0.3$).

Discussion

Our study reveals a high detection rate of aggregated dermal α-synuclein using RT-QuIC in RBD and an even higher seeding activity compared to PD. IHC and RT-QuIC results correlated, RT-QuIC appearing to be more sensitive but less specific. Our data provide evidence that dermal RT-QuIC is a very sensitive tool to confirm iRBD as an α-synucleinopathy in individual patients.

The even higher seeding activity measured using dermal RT-QuIC in iRBD compared to PD may be explained either by a decrease in dermal α-synuclein deposition during the course of PD due to degeneration of the respective fibers or by an early involvement of peripheral nerves in patients with iRBD. When considering a recent study on dermal RT-QuIC where we could already show a higher α-synuclein seeding activity in PD patients who reported RBD symptoms[11] and previous immunohistochemical studies that also provided evidence of higher amounts of dermal α-synuclein deposits associated with iRBD,[21-23] it seems likely that the high rate of positivity of dermal RT-QuIC in iRBD is explained by high α-synuclein deposition in peripheral nerves in these patients.

The high sensitivity and acceptable specificity of dermal RT-QuIC and the possibility to process high numbers of samples support its use as a diagnostic tool. However, high sensitivity was achieved only by multiple biopsies per patient, most probably due to the patchy distribution of dermal α-synuclein,[5] and may be inconvenient. Single false-positive controls may be explained by unspecific aggregation due to interaction effects of the skin as a complex biomatrix with α-synuclein, by slight contaminations of single samples that may induce aggregation in this very sensitive assay, or by potential prodromal stages of an α-synucleinopathy in controls. Indeed, all 3 RT-QuIC-positive controls had suspicious findings of prodromal PD. All negative patients with PD had a tremor-dominant subtype and a short duration of disease.

Positivity of IHC was slightly lower compared to our own previous studies that could show a detection rate of 75% in iRBD and of up to 80% in PD.[4,18] One major reason might be the lower number and volume of samples. A patchy distribution of aggregates may also account for a few biopsies that were positive using IHC but not using RT-QuIC. As the immunohistochemical detection rate differs between laboratories and protocols, the direct comparison provided here cannot be generally transferred to all centers analyzing skin biopsies.

In PD patients, a recent study of Donadio and colleagues demonstrated a similar rate of positivity of 86% using dermal RT-QuIC but achieved a sensitivity of 90% using IHC, probably because eight biopsies per patient were analyzed.[12] A previous postmortem study even achieved a sensitivity of 96%.[15] Another relevant issue for future studies is the intraindividual comparison of different tissues and fluids using RT-QuIC: CSF-RT-QuIC studies of iRBD patients reported sensitivities between 64% and 100%[24-26] compared to about 90% in PD,[27] whereas for RT-QuIC of olfactory mucosa, a sensitivity of 44% was reported in RBD patients,[28] 45% to 84% depending on the exact sampling site in PD patients.[29] As tissues may be differently affected in different subtypes of patients with α-synucleinopathies, cohorts need to be carefully stratified in future studies.

Further limitations of our study include bias due to different numbers of biopsies, interrater variability in polysomnography, and clinical data between centers and pooled analysis of samples from different centers. To clearly determine the specificity of dermal RT-QuIC, future studies need to strictly exclude control subjects with a family history of PD or any other prodromal signs and symptoms and should include various body specimens for direct comparison, for example, CSF and/or serum for extracellular vesicles as well as longitudinal data.

In summary, we provide evidence that dermal RT-QuIC is an appropriate tool to confirm iRBD as an α-synucleinopathy with good sensitivity and specificity. Higher seeding activity in iRBD compared to PD and increased seeding activity in PD patients with anamnestic RBD support the notion of an involvement of the peripheral nervous system in RBD.

Acknowledgments: The study was supported by a grant from the International Parkinson Fonds to K.D., W.H.O., and C.S., A.K., S.R.S., and K.D. are supported by grants from the Interdisciplinary Center of Clinical Research of the University Hospital Würzburg. C.P. is supported by a grant from the Graduate School of Life Sciences of the University of Würzburg. S.R.S. is a fellow of the Thiemann Foundation. M.S. was supported by the Koeln Fortune Program/Faculty of Medicine, University of Cologne (grant number: 453/2018), and the Else Kröner-Fresenius-Stiftung (grant number: 2019_EKES.02). Open Access funding enabled and organized by Projekt DEAL.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

References

1. Doppler K. Detection of dermal alpha-synuclein deposits as a biomarker for Parkinson's disease. J Parkinsons Dis 2021;11(3):937–947.
2. Tsukita K, Sakamaki-Tsukita H, Tanaka K, Suenaga T, Takahashi R. Value of in vivo alpha-synuclein deposits in Parkinson's disease: a systematic review and meta-analysis. Mov Disord 2019;34:1452–1463.
3. Miglis MG, Adler CH, Antelmi E, et al. Biomarkers of conversion to alpha-synucleinopathy in isolated rapid-eye-movement sleep behaviour disorder. Lancet Neurol 2021;20:671–684.
4. Doppler K, Ebert S, Uceyler N, et al. Cutaneous neuropathy in Parkinson's disease: a window into brain pathology. Acta Neuropathol 2014;128:99–109.
5. Donadio V, Incensi A, Leta V, et al. Skin nerve alpha-synuclein deposits: a biomarker for idiopathic Parkinson disease. Neurology 2014;82:1362–1369.
6. Braak H, de Vos RA, Bohl J, Del Tredici K. Gastric alpha-synuclein immunoreactive inclusions in Meissner's and Auerbach's plexuses in cases staged for Parkinson's disease-related brain pathology. Neurosci Lett 2006;396:67–72.

7. Beach TG, Adler CH, Sue LI, et al. Multi-organ distribution of phosphorylated alpha-synuclein histopathology in subjects with Lewy body disorders. Acta Neuropathol 2010;119:689–702.

8. Iranzo A, Fernandez-Arcos A, Tolosa E, et al. Neurodegenerative disorder risk in idiopathic REM sleep behavior disorder: study in 174 patients. PLoS One 2014;9:e89741.

9. Postuma RB, Iranzo A, Hu M, et al. Risk and predictors of dementia and parkinsonism in idiopathic REM sleep behaviour disorder: a multicentre study. Brain 2019;142:744–759.

10. Wang Z, Becker K, Donadio V, et al. Skin alpha-synuclein aggregation seeding activity as a novel biomarker for Parkinson disease. JAMA Neurol 2020;78(1):1–11.

11. Kuzkina A, Bargar C, Schmitt D, et al. Diagnostic value of skin RT-QuIC in Parkinson's disease: a two-laboratory study. npj Parkinsons Dis 2021;7:99.

12. Donadio V, Wang Z, Incensi A, et al. In vivo diagnosis of synucleinopathies: a comparative study of skin biopsy and RT-QuIC. Neurology 2021;96:e2513–e2524.

13. Fairfoul G, McGuire LI, Pal S, et al. Alpha-synuclein RT-QuIC in the CSF of patients with alpha-synucleinopathies. Ann Clin Transl Neurol 2016;3:812–818.

14. Shahnawaz M, Tokuda T, Waragai M, et al. Development of a biochemical diagnosis of Parkinson disease by detection of alpha-synuclein misfolded aggregates in cerebrospinal fluid. JAMA Neurol 2017;74:163–172.

15. Manne S, Kondru N, Jin H, et al. Blinded RT-QuIC analysis of alpha-synuclein biomarker in skin tissue from Parkinson's disease patients. Mov Disord 2020;35(12):2230–2239.

16. Schenck CH, Montplaisir JY, Frauscher B, et al. Rapid eye movement sleep behavior disorder: devising controlled active treatment studies for symptomatic and neuroprotective therapy—a consensus statement from the international rapid eye movement sleep behavior disorder study group. Sleep Med 2013;14:795–806.

17. Postuma RB, Berg D, Stern M, et al. MDS clinical diagnostic criteria for Parkinson's disease. Mov Disord 2015;30:1591–1601.

18. Doppler K, Jentschke HM, Schulmeyer L, et al. Dermal phospho-alpha-synuclein deposits confirm REM sleep behaviour disorder as prodromal Parkinson's disease. Acta Neuropathol 2017;133: 535–545.

19. Berg D, Postuma RB, Adler CH, et al. MDS research criteria for prodromal Parkinson's disease. Mov Disord 2015;30:1600–1611.

20. Heinzel S, Berg D, Gasser T, Chen H, Yao C, Postuma RB. Update of the MDS research criteria for prodromal Parkinson's disease. Mov Disord 2019;34:1464–1470.

21. Donadio V, Incensi A, Del Sorbo F, et al. Skin nerve phosphorylated alpha-synuclein deposits in Parkinson disease with orthostatic hypotension. J Neuropathol Exp Neurol 2018;77:942–949.

22. Al-Qassabi A, Tsao TS, Racolta A, et al. Immunohistochemical detection of synuclein pathology in skin in idiopathic rapid eye movement sleep behavior disorder and parkinsonism. Mov Disord 2020;36(4):895–904.

23. Doppler K, Mammadova S, Kuzkina A, et al. Association between probable REM sleep behavior disorder and increased dermal alpha-synuclein deposition in Parkinson's disease. Parkinsonism Relat Disord 2022;99:58–61.

24. Iranzo A, Fairfoul G, Ayudhaya ACN, et al. Detection of alpha-synuclein in CSF by RT-QuIC in patients with isolated rapid-eye-movement sleep behaviour disorder: a longitudinal observational study. Lancet Neurol 2021;20:203–212.

25. Poggiolini I, Gupta V, Lawton M, et al. Diagnostic value of cerebrospinal fluid alpha-synuclein seed quantification in synucleinopathies. Brain 2022;145:584–595.

26. Rossi M, Candelise N, Baiardi S, et al. Ultrasensitive RT-QuIC assay with high sensitivity and specificity for Lewy body-associated synucleinopathies. Acta Neuropathol 2020;140:49–62.

27. Yoo D, Bang JI, Ahn C, et al. Diagnostic value of alpha-synuclein seeding amplification assays in alpha-synucleinopathies: a systematic review and meta-analysis. Parkinsonism Relat Disord 2022;104:99–109.

28. Stefani A, Iranzo A, Holzknecht E, et al. Alpha-synuclein seeds in olfactory mucosa of patients with isolated REM sleep behaviour disorder. Brain 2021;144:1118–1126.

29. Bongianni M, Catalan M, Perra D, et al. Olfactory swab sampling optimization for alpha-synuclein aggregate detection in patients with Parkinson's disease. Transl Neurodegener 2022;11:37.

Supporting Data

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.

SGML and CITI Use Only
DO NOT PRINT

Author Roles

Anastasia Kuzkina: study design and conception, acquisition and analysis of data, and writing of the manuscript
C.P.: acquisition and analysis of data
A.S.: acquisition of data
D.S.: acquisition of data
J.R.: acquisition of data
S.R.S.: acquisition of data and revision of the manuscript
H.K.: acquisition of data
Elena Salabasidou: acquisition of data
Antonia Kohl: acquisition of data
Elisabeth Sittig: acquisition of data
M.B.: acquisition of data
D.B.: acquisition of data and revision of the manuscript
J.V.: acquisition of data and revision of the manuscript
C.S.: acquisition of data and revision of the manuscript
W.H.O.: acquisition of data and revision of the manuscript.
Eva Schäffer: acquisition of data and revision of the manuscript
M.S.: acquisition of data and revision of the manuscript
A.J.: acquisition of data and revision of the manuscript
K.D.: study design and conception, acquisition and analysis of data, and writing of the manuscript

Full financial disclosures for the previous 12 months

Anastasia Kuzkina: none.
C.P.: none.
A.S.: none.
D.S.: none.
J.R.: none.
S.R.S.: S.R.S. is a fellow of the Thiemann Foundation and declares personal compensation or scientific advisory board for Elemind Inc. outside of the submitted work.
H.K.: none.
Elena Salabasidou: none.
Antonia Kohl: none.
Elisabeth Sittig: none.
M.B.: none.
D.B.: D.B. received grants from the Deutsche Forschungsgemeinschaft (DFG), German Parkinson's Disease Association (dPV), BMBF, Parkinson Fonds Deutschland gGmbH, UCB Pharma GmbH, EU, Novartis Pharma GmbH, Lundbeck, Damp Foundation, The Michael J. Fox Foundation; honoraria for presentations from AbbVie, Biogen, BIAL, UCB Pharma GmbH Zambon, Desitin, and Novartis; and honoraria for consultancies from Biogen, BIAL, UCB Pharma GmbH, and AC Immune SA all outside the submitted work.
J.V.: none.
C.S.: none.
W.H.O.: W.H.O. is Hertie senior research professor supported by the Charitable Hertie Foundation, Frankfurt/Main, Germany. During the period of the study he received study-unrelated grants from The Michael J. Fox Foundation, the German Research Foundation, and Stichting ParkinsonFonds, The Netherlands. Similarly, he received study-unrelated honoraria as a speaker or consultant/advisor for the AbbVie, Adamas, Modag, Roche, and Stada-Pharma.

Eva Schaeffer: E.S. received intramural research funding from the University of Kiel; speaker's honoraria from Bayer Vital GmbH, BIAL GmbH, Novartis, German General Medical Council, and Society for Information and Organisation mbH; and grants from the German Society for Parkinson's Disease and Movement Disorders (DPG e. V.) outside the submitted work.

M.S.: M.S. was supported by the Koeln Fortune Program/Faculty of Medicine, University of Cologne (grant number: 453/2018), and the Else Kröner-Fresenius-Stiftung (grant number: 2019_EKES.02).

A.J.: A.J. reports grants from ParkinsonFonds Deutschland outside the submitted work.

K.D.: K.D. received grants from Stichting ParkinsonFonds and the Deutsche Forschungsgemeinschaft (DFG).