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Reduced intraepidermal nerve fiber density in patients with REM sleep behavior disorder

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ABSTRACT

Background: Idiopathic rapid eye movement (REM) sleep behavior disorder (iRBD) has been increasingly acknowledged to be an initial specific manifestation of alpha-synucleinopathies such as Parkinson's disease (PD), multiple system atrophy (MSA) and dementia with Lewy bodies (DLB). Recent findings suggest that cutaneous abnormalities like small fiber neuropathy and alpha-synuclein deposition might reflect brain pathology and might function as early biomarkers in PD. This is the first study to elucidate whether iRBD patients already suffer from distinctive cutaneous features.

Methods: We examined skin punch biopsies from the distal leg of 18 iRBD patients and 22 age- and sex-matched controls using immunohistochemistry and microscopy. Further clinical evaluation included structured interviews, clinical motor and non-motor questionnaires and rating scales (e.g. Unified Parkinson's disease rating scale [UPDRS], non-motor symptoms questionnaire [NMS-Quest] and Beck Depression Inventory, Epworth Sleepiness Scale, evaluation of cognitive and olfactory functioning as well as blood samples.

Results: Intraepidermal nerve fiber density (IEFND) was reduced in iRBD patients compared to controls ($p = 0.037$), whereas the axon swelling ratio did not differ between groups. Patients with iRBD reported non-motor symptoms more frequently than controls (UPDRS I, NMS-Quest). Olfaction and daytime sleepiness differed between both groups, whereas there were no differences regarding cognition.

Conclusions: These *in vivo* findings demonstrate small fiber neuropathy in iRBD patients that are associated with non-motor symptoms indicating that peripheral abnormalities may occur early in iRBD. However, the prognostic value has to be further investigated in longitudinal studies.

1. Introduction

Rapid eye movement (REM) sleep behavior disorder (RBD) is a parasomnia characterized by loss of muscle atonia during REM sleep. In further consequence, this leads to dream enactment behavior with shouting, talking or laughing and exhibiting movements like kicking and boxing, which are potentially harmful to

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patients or their partners [1]. REM sleep is mainly controlled by brainstem structures in the medulla oblongata, rostral pons and the tegmentum of the midbrain. Degeneration of the pedunculopontine nucleus, the locus coeruleus/subcoeruleus complex and the laterodorsal tegmental nucleus is consistently reported in RBD patients [2].

Idiopathic RBD (iRBD) is considered to be one of the first features of neurodegenerative alpha-synucleinopathies such as Parkinson's disease (PD), dementia with Lewy bodies (DLB) and multiple system atrophy (MSA) [3–6]. Approximately 81–90% of patients with iRBD have been reported to develop one of the named disorders within the next 10–20 years [7]. Symptomatic RBD may be identified in patients with other neurodegenerative or inflammatory diseases like narcolepsy, ataxias or induced by medication [8].

The concept of iRBD as an early phenotype of evolving neurodegenerative diseases is supported by clinical findings of olfactory dysfunction, impairment of color discrimination, autonomic dysfunction or impaired cognitive function in some iRBD patients. This leads to the suggestion of already existing neuronal damage in disease-specific brain regions [3,9,10]. Moreover, radionuclide dopaminergic imaging techniques such as positron emission tomography (PET) and single photon emission computerized tomography (SPECT) revealed reduced tracer uptake in striatal regions of RBD patients compared to healthy controls [10–12] much the same as typically seen in patients with PD, MSA and DLB.

In recent years, it has become increasingly clear that neurodegenerative disorders are system disorders, involving multiple neuronal systems and that disease-related pathology is not limited to the initially affected cell populations. Instead it seems to spread within the peripheral nervous system (PNS) and the central nervous system (CNS) in a stage-dependent pattern. According to this, the brainstem is already affected in the prodromal stages of PD [13].

In PD, this results in autonomic, neuropsychiatric and sleep abnormalities. The pathologic hallmark of PD is the neuronal deposition of Lewy bodies and Lewy neurites, which mainly consist of alpha-synuclein. Several studies have also detected alpha-synuclein deposits within the peripheral nervous system of PD patients [14], which have been shown to correlate with PD severity and other measures of autonomic functioning [14], and showed signs of peripheral neuropathy such as reduced length-dependent intraepidermal nerve fiber density (IEFND) and small fiber neuropathy in PD patients [15]. Moreover, recent data provide evidence that alpha-synuclein deposition in skin nerve fibers and small nerve fiber neuropathy have diagnostic potential to differentiate PD from atypical PD (e.g. MSA) or other neurodegenerative diseases [16,17].

Peripheral neuropathy is induced by various causes, typically by alcohol abuse, diabetes, hypo- or hypervitaminosis and chronic or acute inflammatory diseases like vasculitis. Small fiber neuropathy typically affects autonomic and somatic nerves and may be related to diabetes, connective tissue diseases and inflammatory diseases (e.g., Hepatitis C). In PD, neuropathy is discussed to be either an intrinsic disease-related feature or to result from levodopa treatment and/or vitamin deficiencies [18]. To rule out secondary causes for neuropathy, we therefore assessed and controlled for the laboratory measures such as vitamin B12, folic acid, methylmalonic acid and creatinine.

Together these data suggest that cutaneous alterations might open an early histopathological window and can be considered as potential diagnostic markers in PD. IRBD as a specific prodromal model of synucleinopathies offers the opportunity to study premotor stages and presumably also to identify clinical subtypes. Therefore, we aimed to determine whether cutaneous abnormalities are already detectable in iRBD patients.

2. Subjects and methods

2.1. Participants, neurological and neuropsychological assessment

This study was approved by the institutional review boards at the Technische Universität Dresden (EK 242072013, EK 393122012) and the RWTH Aachen University (EK 231/09), and conducted in accordance with the declaration of Helsinki. All participants gave their written informed consent.

Patients with RBD and healthy age- and sex-matched individuals were recruited at the in- and outpatients clinics of the Department of Neurology of the Technische University (TU) Dresden and of the Department of Neurology of the Rheinisch-Westfälische Technische Hochschule (RWTH) Aachen University as well as at collaborating centers and the communities from 2010 to 2014 within the framework of the German RBD study group. All patients met the diagnostic criteria for RBD according to the American Academy of Sleep Medicine (AASM) and International Classification of Sleep Disorders (ICSD-2), requiring video polysomnographic confirmation of increased electromyographic activity associated with abnormal behaviors during REM sleep and a history of dream-enactment behaviors. The latter was also assessed using the RBD Screening Questionnaire (RBDSQ) with a cut-off score of $\geq$5 points as an indicator for possible RBD [19]. In consequence, we excluded healthy control subjects scoring above this value.

All participants were interviewed in order to obtain comprehensive data on medical history, disease duration, family history, medication and demographic characteristics including age, gender, education and handedness.

Furthermore, subjects underwent a detailed neurological examination to identify any neurodegenerative diseases, such as PD or dementia. In this context, we performed the Unified Parkinson's disease rating scale (UPDRS), to screen for non-motor and motor functioning and the Montreal Cognitive Assessment (MoCA) for evaluation of cognitive impairment. Scores in the UPDRS-III were classified as normal (0–2 points), borderline (3–5 points) or pathological (>5 points) [20]. Subjects who met criteria for neurodegenerative diseases (e.g. UPDRS-III scores >5 points) were excluded from the analysis. If signs of cognitive impairment were found in the MoCA [cut-off $\leq$ 25 points], cognitive abilities were further tested using more detailed neuropsychiatric assessments e.g. the battery of the Consortium to Establish a Registry for Alzheimer's disease (CERAD) and subjects were excluded in case of signs for mild cognitive impairment or dementia.

Other exclusion criteria were presence of peripheral neuropathy (demonstrated by clinical findings and assessment of nerve conduction velocity) and common risk factors of peripheral nerve dysfunction such as diabetes, alcohol abuse, liver or renal failure, chemotherapy, history of chronic inflammatory disease, vitamin B12- or folic acid deficiency or vasculitis. Subjects were excluded if creatinine levels exceeded 150% of the cut-off-values.

We used the Beck Depression Inventory version II (BDI-II) to screen for depression and the Epworth Sleepiness Scale (ESS) to screen for daytime sleepiness. The Non-motor symptoms questionnaire (NMS-Quest) was used to assess the frequency of non-motor symptoms [21]. Olfactory testing was assessed using the Sniffin Sticks test kit. 16 common odors (orange, leather, cinnamon, menthol, banana, lemon, licorice, garlic, coffee, apple, pineapple, rose, fish, anise, clove, and turpentine) were used to assess odor identification. This test was developed on a multiple forced choice design, which means that subjects have to identify odors by selecting from a list of four descriptors. We used the tenth percentile of the age- and sex-based cut-off-values to separate hyposmic from normosmic subjects [22].

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2.2. Laboratory measures

Laboratory tests were conducted including whole blood and differential blood counts, vitamin B12-, methylmalonic acid- and folic acid levels, electrolytes, renal and liver function tests, HbA1c, erythrocyte sedimentation rate and C-reactive protein.

2.3. Nerve conduction velocity

Nerve conduction velocity was tested using antidromic assessment of the sural nerve, and orthodromic assessment of the tibialis and peroneal nerves as well as the median nerve and the ulnar nerve. We used normative data to categorize sensory nerve action potential (SNAP) amplitude (>4.5 μV) and nerve conduction velocity (>40 m/s) in all individuals [23].

2.4. Skin biopsy and immunohistochemistry

We performed skin biopsies in all patients and controls taken as an 8 mm (Dresden) and 4 mm (Aachen) diameter punch biopsy at the distal leg 10 cm above the ankle under local anesthesia. The obtained biopsies were cut in half and fixed in Zamboni solution for immunohistochemistry. The neuropathologists (IK, JW) did not have any access to clinical data at the time of examination of the skin biopsies. Six consecutive 40 μm thick cryostat sections were incubated with a rabbit polyclonal anti-PGP9.5 primary and Alexa 488 secondary antibody to stain the dermal and epidermal nerve fibers, as described previously [24]. To determine the IEFND for each subject, we counted axons crossing the epidermal basement membrane but ignored nerve fragments in the epidermis [24].

We further searched for other signs of de- and regeneration, such as axonal swelling, axons larger than 1.5 μm in diameter, axonal sprouting and "crawlers" (axons extending along the epidermal basal lamina). The swelling ratio was calculated as the number of axons with intraepidermal swellings divided by the total number of axons. Axon numbers were then normalized to the total epidermal length of the sections to obtain fibers/mm.

To categorize for the presence of small fiber neuropathy based on these parameters we used the 5th percentile of already published normative age- and gender-groups as reference cut-off-levels [25].

2.5. Statistical analysis

Statistical comparisons of data between groups were made using the Mann-Whitney-U test or two-sample *t*-test where appropriate and after testing for normal distribution (Kolmogorov-Smirnov-test). Frequency distributions were tested using Chi square or Fisher's exact tests. Analysis of covariance (ANCOVA) was performed to test for an effect of co-variables (gender, age) on IEFND. The 5th percentile of established normalized age-gender groups was defined as cut-off for the diagnosis of small fiber neuropathy as reported elsewhere [25].

Spearman-rank correlation coefficients were used to examine correlations between parameters with a rho (ρ) ≤ 0.3 considered as a weak, $\rho = 0.3–0.59$ a moderate and $\rho \geq 0.6$ a strong correlation.

Data were analyzed using the software program SPSS 23.0 (SPSS Inc., Chicago, IL). If not mentioned otherwise, all data are displayed as mean ± standard deviation (SD), significance level was set at $p \leq 0.05$ (two-tailed test). Due to the explorative character of the study, we did not adjust *p* values for multiple comparisons.

3. Results

3.1. Patients and controls

29 patients with RBD and 24 controls participated in the study. All of them agreed to donate skin biopsies. We excluded four RBD patients due to signs of neuropathy in nerve conduction studies and five patients because of presence of cognitive impairment in detailed neuropsychological testing. One further RBD patient was excluded due to questionable polysomnographic recording which did not fulfill all criteria for RBD. One RBD patient was excluded due to elevated liver enzymes suggestive for liver failure. One control subject was excluded due to signs of neuropathy in nerve conduction studies and another control scored above 5 points in the RBDSQ suggestive of RBD. Hence, in total 18 iRBD patients and 22 controls were included into the analysis. All included subjects showed no signs of cognitive impairment or dementia using MoCA [cut-off $\leq$ 25 points] and the CERAD. Demographic data did not differ significantly between the two groups (see Table 1).

Apart from one patient who took levodopa for restless-legs syndrome, patients were free of dopaminergic treatment. Three iRBD patients were taking clonazepam and three of the patients were treated with antidepressants. One of the patients with depression was treated with additional quetiapine. Other medication consisted of antihypertensive treatment, thyroid medication, statins, thrombocyte aggregation inhibitors and new oral anticoagulants.

3.2. Clinical assessments and questionnaires

Total UPDRS-I scores were significantly higher in iRBD patients than in controls ($p = 0.002$, Table 1). There were no significant differences in total UPDRS-II and UPDRS-III scores between groups (Table 1).

In detail, iRBD patients reported cognitive impairment (47%), lack of motivation/apathy (24%), vivid dreaming (47%) and thought disorder (6%) in the UPDRS I. Depression was reported in 18% of patients and not in controls (Supplement Table S1).

Table 1
Demographic data and clinical characteristics.

	RBD	Controls	p value
Number	18	22	
Age (years)	53.7 ± 15.0	59.8 ± 9.5	0.15[c]
Age (range)	22–71	30–74	
Ratio of females	9/18	11/22	1.0[b]
Disease duration (years)[d]	11.3 ± 11.9	–	
UPDRS I	1.8 ± 2.1	0.1 ± 0.4	0.002[a**]
UPDRS II	0.3 ± 0.6	0.3 ± 0.7	0.8[c]
UPDRS III	0.4 ± 0.9	0.1 ± 0.2	0.069[c*]
RBDSQ Score	9.0 ± 2.9	2.3 ± 1.6	0.0001[a***]
ESS Score	6.8 ± 4.3	4.4 ± 1.3	0.041[c*]
NMS Score	5.2 ± 3.3	2.3 ± 2.6	0.033[c*]
BDI-II Score	8.1 ± 9.9	4.8 ± 6.3	0.37[c]
Sniffin Sticks Identification	10.4 ± 3.7	13.2 ± 1.2	0.007[c**]

Table 1 shows demographic and clinical characteristics of idiopathic REM sleep behavior disorder (iRBD) patients and controls. Data are presented in mean and standard deviation (SD) and numbers [n]; BDI-II = Beck depression inventory; ESS = Epworth sleepiness scale; UPDRS = Unified Parkinson's disease rating scale (scale I: evaluation of mentation, behavior and mood; scale II: activities of daily life; scale III: motor examination); RBDSQ = REM sleep behavior disorder screening questionnaire; *p ≤ 0.05; **p ≤ 0.01.

[a] Mann-Whitney *U* test.
[b] Fisher's exact test.
[c] Two-sample *t*-test.
[d] Estimated RBD onset duration by clinical history.

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Non-motor symptoms (NMS-Quest) were reported more frequently in iRBD patients than in controls (Table 1). When different domains included in the NMSQuest were analyzed, iRBD patients complained about sleep disturbances (94%) as expected (Supplement Table S1). However, also cardiovascular problems (e.g., dizziness, 33%), problems with urinary function (50%), apathy/memory deficits (39%) and depression (33%) were frequently reported. Limitations of gastrointestinal function (17%), sexual dysfunction (22%) and also pain were noted (6%), although at a relatively low frequency. Hallucinations were neither reported by controls nor by iRBD patients. Significant differences between both groups on a single-item-level of the NMS-Quest and UPDRS-I could be identified for reported sleep disturbances (NMS-Quest, Chi-square test, $p = 0.01$) and vivid dreaming (UPDRS I, Chi-square test, $p = 0.037$). No other items of the UPDRS-I and NMS-Quest appeared less or more frequent in iRBD compared to healthy controls (for details please see Supplement Table S1).

IRBD patients scored significantly lower than controls in the Sniffin sticks odor identification test and significantly higher in the ESS (Table 1). There were no significant differences between patients and controls regarding depression (BDI-II, $p = 0.372$). As expected, scoring in the RBDSQ was significantly higher in the iRBD cohort ($p < 0.0001$) (Table 1).

3.3. Quantification of intraepidermal nerve fiber density and swelling ratio

IEFND was significantly reduced in iRBD patients compared to controls (Fig. 1, Table 2). IEFND correlated exclusively with UPDRS-I in iRBD patients ($\rho = -0.486$; $p = 0.048$), but not with other parameters. To analyze the clinical relevance of IEFND reduction, we compared IEFND data of our patients and controls with already published age- and gender-matched histological criteria [25]. Significantly more iRBD patients (39%) than controls (4.5%) fulfilled the defined consensus criteria for small fiber neuropathy [25] (Fisher's exact test, $p = 0.014$) (Table 2). Presence of small fiber neuropathy also differed significantly between sexes (Fisher's exact test, $p = 0.044$), therefore we included gender in ANCOVA which then showed even stronger differences between both groups (iRBD and controls) (effect of group: $F = 6{,}41$; $p = 0.016$; gender: $F = 15{,}27$; $p < 0.0001$; ANCOVA). Similarly, group effects were stronger after including age as covariate (effect of group: $F = 9.73$; $p = 0.004$; age: $F = 10{,}18$; $p = 0.003$; ANCOVA). Characterization of both iRBD patients with and without small fiber neuropathy (SFN) showed that iRBD patients with SFN were more likely to be male ($p = 0.05$) but did not show any other significant differences regarding demographic or clinical features (e.g., age, gender, UPDRS, NMSQuest; Supplement Table S2). Axon swelling ratio did not differ significantly between both groups and did not correlate with any of the relevant clinical data.

4. Discussion

This study demonstrates for the first time peripheral nerve fiber pathology in patients with iRBD, a prodromal disease stage for synucleinopathies. In PD cutaneous changes such as small fiber neuropathy [14,15] and alpha-synuclein deposition in nerve fibers [16] have been described, suggesting that cutaneous neuropathy occurs early in the course of the disease and might be used as a biomarker.

We conducted immunohistochemical and light microscopic analyses of skin biopsies to assess cutaneous abnormalities and combined these data with comprehensive clinical assessments and nerve conduction studies in iRBD patients and controls. We found a significantly reduced IEFND in iRBD patients compared to age- and sex-matched healthy controls with manifest small fiber neuropathy in 39% of the investigated iRBD patients and 4.5% of the controls. A comparable study of Doppler et al. revealed length dependent small fiber neuropathy in 36.7% of the studied PD sample, suggesting that small fiber neuropathy is an early feature in the neurodegenerative process [14].

IEFND has been also studied in MSA and DLB. Sofar rather differentiated findings have been reported in MSA [16,17] – in comparison to PD, for somatosensory fibers seem to be more affected in MSA, whereas autonomic fibers are more involved in PD [17]. Thus, a diagnostic potential to differentiate PD from MSA remains to be further investigated.

Peripheral neuropathy is induced by various causes. In our

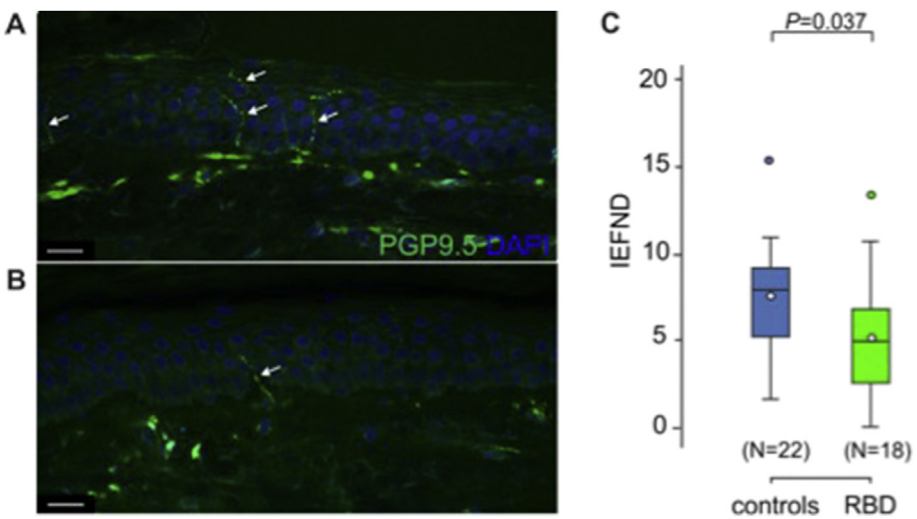

Fig. 1. **(A–B)** Representative confocal microscopy images of immunohistochemical stainings of skin biopsies using PGP9.5 (green) antibody and nuclear staining (DAPI, blue) in **(A)** A healthy control showing dense subepidermal innervation and multiple intraepidermal nerve fibers (arrows). **(B)** A representative iRBD patient biopsy with small fiber neuropathy showing reduced subepidermal innervation and a single intraepidermal axon (arrow). Scale bar = 20 μm. **(C)** Box plots of IEFND in controls and iRBD patients. The plots show the minimum, the lower quartile, the median, the upper quartile and the maximum for each parameter. Closed circles represent outliers, open circles the mean. Numbers in parentheses indicate number of analyzed patients. P value derived from *t*-test for independent samples. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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Table 2
Histological findings and nerve conduction studies.

	RBD (n = 18)	Controls (n = 22)	p value
IEFND	5.2 ± 3.4	7.4 ± 3.1	0.037[a]*
Swelling ratio	0.1 ± 0.1	0.1 ± 0.08	0.612[a]
Small fiber neuropathy (in %)	39	4.5	0.014[b]*

Table 2 shows histological findings and data of nerve conduction studies in idiopathic REM sleep behavior disorder (iRBD) patients and controls. Data are presented in mean and standard deviation (SD). IEFND = intraepidermal nerve fiber density; small fiber neuropathy: percentage of subjects fulfilling the criteria for small fiber neuropathy. *$p \leq 0.05$.

[a] Group comparisons performed with two-sample *t*-test.

[b] Group comparisons performed with Fisher's exact test.

cohort we did not find relevant abnormalities in vitamin B12 and folic acid levels as well as other confounding laboratory measures (e.g. HbA1c); therefore small fiber neuropathy cannot be explained by any of these causes. We only found a mild correlation between IEFND and UPDRS-I in iRBD patients.

In line with these findings, significant differences in non-motor symptoms between iRBD patients and controls were detected in the UPDRS-I and NMS-Quest. Besides sleep disturbances and vivid dreaming patients frequently reported apathy/memory deficits and autonomic involvement (e.g. dysfunction of the urinary and cardiovascular system). In addition we found significantly impaired olfaction in iRBD patients compared to controls. Patients also complained about more severe daytime sleepiness than controls. We did not find significant differences between the two groups regarding depression or cognition.

Our findings are in agreement with previously published data on non-motor symptoms such as autonomic dysfunction in iRBD [3,9,10]. In a large study comparing iRBD patients and controls regarding autonomic involvement using the SCOPA-AUT-questionnaire, which was designed to assess autonomic symptoms in PD patients [26] and comprises several autonomic domains (gastrointestinal, urinary, cardiovascular, thermoregulatory, pupillomotor, and sexual dysfunction), patients reported gastrointestinal symptoms more frequently. Similar to our results, urinary and cardiovascular symptoms were as well reported more often than in controls [27]. Also in line with our results, another recent study detected hyposmia to appear more frequently in iRBD patients than in controls, while no significant differences regarding depression between both groups was found [28]. Additionally hyposmia has been reported to predict transition towards DLB in iRBD [29] and is a common prodromal symptom of PD. Still, a link between non-motor-symptoms and small fiber neuropathy has not been described yet.

In this context, it is interesting to note that it is becoming evident that patients with PD, who suffer from additional RBD might be more adversely affected by non-motor symptoms and belong to a "malignant" subtype with faster progression rate and higher likelihood of cognitive impairment [30].

Due to our findings of disproportional high percentage of small fiber neuropathy in our iRBD cohort and the absence of other causes for peripheral neuropathy, we assume that small fiber abnormalities measured in skin biopsies are disease related and may represent an early biomarker for progression to PD or maybe also other alpha-synucleopathies. However, results regarding cutaneous abnormalities in neurodegenerative diseases are still controversial and need to be further elucidated [16,31].

A limitation of the present study is the small sample size of only 18 iRBD patients with a quite large age range. Although this cross-sectional study is based on a small sample – as this is a rare and less acknowledged disease –, we found first evidence for peripheral nerve fiber pathology in iRBD patients. However, a larger sample of iRBD patients needs to be assessed to confirm our results and assessments of phosphorylated alpha-synuclein need to be added in more proximal skin biopsy samples. Also, further studies are needed to show whether cutaneous abnormalities might accompany or reflect pathology within the central nervous system [14]. In particular, longitudinal assessments are required to assess the predictive value of skin biopsy measures regarding time to conversion or type of evolving neurodegenerative disease (especially dementia or PD) and progression type. Also additional studies on non-motor symptoms in iRBD patients and alpha-synucleinopathies will further elucidate their impact on disease progression.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.parkreldis.2016.06.003.

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Author’s contributions

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References

[1] C.H. Schenck, S.R. Bundlie, M.G. Ettinger, M.W. Mahowald, Chronic behavioral disorders of human REM sleep: a new category of parasomnia, Sleep 9 (1986) 293–308.
[2] B.F. Boeve, M.H. Silber, C.B. Saper, T.J. Ferman, D.W. Dickson, J.E. Parisi, E.E. Benarroch, J.E. Ahlskog, G.E. Smith, R.C. Caselli, M. Tippman-Peikert, E.J. Olson, S.C. Lin, T. Young, Z. Wszolek, C.H. Schenck, M.W. Mahowald, P.R. Castillo, K. Del Tredici, H. Braak, Pathophysiology of REM sleep behaviour disorder and relevance to neurodegenerative disease, Brain 130 (2007) 2770–2788.
[3] R.B. Postuma, A.E. Lang, J. Massicotte-Marquez, J. Montplaisir, Potential early markers of Parkinson disease in idiopathic REM sleep behavior disorder, Neurology 66 (2006) 845–851.
[4] R.B. Postuma, J.F. Gagnon, M. Vendette, M.L. Fantini, J. Massicotte-Marquez, J. Montplaisir, Quantifying the risk of neurodegenerative disease in idiopathic REM sleep behavior disorder, Neurology 72 (2009) 1296–1300.
[5] C.H. Schenck, M.W. Mahowald, REM sleep behavior disorder: clinical, developmental, and neuroscience perspectives 16 years after its formal identification in SLEEP, Sleep 25 (2002) 120–138.
[6] A. Iranzo, J.L. Molinuevo, J. Santamaria, M. Serradell, M.J. Marti, F. Valldeoriola, E. Tolosa, Rapid-eye-movement sleep behaviour disorder as an early marker for a neurodegenerative disorder: a descriptive study, Lancet Neurol. 5 (2006) 572–577.
[7] M.J. Howell, C.H. Schenck, Rapid eye movement sleep behavior disorder and neurodegenerative disease, JAMA Neurol. 72 (2015) 707–712.
[8] B.F. Boeve, REM sleep behavior disorder: updated review of the core features, the REM sleep behavior disorder-neurodegenerative disease association, evolving concepts, controversies, and future directions, Ann. N. Y. Acad. Sci. 1184 (2010) 15–54.
[9] R.B. Postuma, J.F. Gagnon, M. Vendette, J.Y. Montplaisir, Markers of neurodegeneration in idiopathic rapid eye movement sleep behaviour disorder and Parkinson’s disease, Brain 132 (2009) 3298–3307.
[10] K. Stiasny-Kolster, Y. Doerr, J.C. Moller, H. Hoffken, T.M. Behr, W.H. Oertel, G. Mayer, Combination of 'idiopathic' REM sleep behaviour disorder and olfactory dysfunction as possible indicator for alpha-synucleinopathy demonstrated by dopamine transporter FP-CIT-SPECT, Brain 128 (2005) 126–137.
[11] I. Eisensehr, R. Linke, S. Noachtar, J. Schwarz, F.J. Gildehaus, K. Tatsch, Reduced striatal dopamine transporters in idiopathic rapid eye movement sleep behaviour disorder. Comparison with Parkinson’s disease and controls, Brain 123 (Pt 6) (2000) 1155–1160.
[12] A. Iranzo, F. Lomena, H. Stockner, F. Valldeoriola, I. Vilaseca, M. Salamero, J.L. Molinuevo, M. Serradell, J. Duch, J. Pavia, J. Gallego, K. Seppi, B. Hogl, E. Tolosa, W. Poewe, J. Santamaria, Decreased striatal dopamine transporter uptake and substantia nigra hyperechogenicity as risk markers of synucleinopathy in patients with idiopathic rapid-eye-movement sleep behaviour disorder: a prospective study [corrected], Lancet Neurol. 9 (2010) 1070–1077.
[13] H. Braak, K. Del Tredici, U. Rub, R.A. de Vos, E.N. Jansen Steur, E. Braak, Staging of brain pathology related to sporadic Parkinson’s disease, Neurobiol. Aging 24 (2003) 197–211.
[14] K. Doppler, S. Ebert, N. Uceyler, C. Trenkwalder, J. Ebentheuer, J. Volkmann, C. Sommer, Cutaneous neuropathy in Parkinson’s disease: a window into brain pathology, Acta Neuropathol. 128 (2014) 99–109.
[15] M. Nolano, V. Provitera, A. Estraneo, M.M. Selim, G. Caporaso, A. Stancanelli, A.M. Saltalamacchia, B. Lanzillo, L. Santoro, Sensory deficit in Parkinson’s disease: evidence of a cutaneous denervation, Brain 131 (2008) 1903–1911.
[16] L. Zange, C. Noack, K. Hahn, W. Stenzel, A. Lipp, Phosphorylated alpha-synuclein in skin nerve fibres differentiates Parkinson’s disease from multiple system atrophy, Brain 138 (2015) 2310–2321.
[17] K. Doppler, J. Weis, K. Karl, S. Ebert, J. Ebentheuer, C. Trenkwalder, S. Klebe, J. Volkmann, C. Sommer, Distinctive distribution of phospho-alpha-synuclein in dermal nerves in multiple system atrophy, Mov. Disord. 30 (2015) 1688–1692.
[18] F. Mancini, C. Comi, G.D. Oggioni, C. Pacchetti, D. Calandrella, M. Coletti Moja, G. Riboldazzi, S. Tunesi, M. Dal Fante, L. Manfredi, M. Lacerenza, R. Cantello, A. Antonini, Prevalence and features of peripheral neuropathy in Parkinson’s disease patients under different therapeutic regimens, Park. Relat. Disord. 20 (2014) 27–31.
[19] K. Stiasny-Kolster, G. Mayer, S. Schafer, J.C. Moller, M. Heinzel-Gutenbrunner, W.H. Oertel, The REM sleep behavior disorder screening questionnaire—a new diagnostic instrument, Mov. Disord. 22 (2007) 2386–2393.
[20] U. Sommer, T. Hummel, K. Cormann, A. Mueller, J. Frasnelli, J. Kropp, H. Reichmann, Detection of presymptomatic Parkinson's disease: combining smell tests, transcranial sonography, and SPECT, Mov. Disord. 19 (2004)

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1196–1202.
[21] K.R. Chaudhuri, P. Martinez-Martin, A.H. Schapira, F. Stocchi, K. Sethi, P. Odin, R.G. Brown, W. Koller, P. Barone, G. MacPhee, L. Kelly, M. Rabey, D. MacMahon, S. Thomas, W. Ondo, D. Rye, A. Forbes, S. Tluk, V. Dhawan, A. Bowron, A.J. Williams, C.W. Olanow, International multicenter pilot study of the first comprehensive self-completed nonmotor symptoms questionnaire for Parkinson's disease: the NMSQuest study, Mov. Disord. 21 (2006) 916–923.
[22] T. Hummel, G. Kobal, H. Gudziol, A. Mackay-Sim, Normative data for the "Sniffin' Sticks" including tests of odor identification, odor discrimination, and olfactory thresholds: an upgrade based on a group of more than 3,000 subjects, Eur. Arch. Otorhinolaryngol. 264 (2007) 237–243.
[23] C. Bischoff, W.J. Schulte-Mattler, B. Conrad, Das EMG-Buch. EMG und periphere Neurologie in Frage und Antwort, Thieme Verlag, 2011.
[24] J. Weis, I. Katona, G. Muller-Newen, C. Sommer, G. Necula, C. Hendrich, A.C. Ludolph, A.D. Sperfeld, Small-fiber neuropathy in patients with ALS, Neurology 76 (2011) 2024–2029.
[25] G. Lauria, M. Bakkers, C. Schmitz, R. Lombardi, P. Penza, G. Devigili, A.G. Smith, S.T. Hsieh, S.I. Mellgren, T. Umapathi, D. Ziegler, C.G. Faber, I.S. Merkies, Intraepidermal nerve fiber density at the distal leg: a worldwide normative reference study, J. Peripher Nerv. Syst. 15 (2010) 202–207.
[26] M. Visser, J. Marinus, A.M. Stiggelbout, J.J. Van Hilten, Assessment of autonomic dysfunction in Parkinson's disease: the SCOPA-AUT, Mov. Disord. 19 (2004) 1306–1312.
[27] L. Ferini-Strambi, W. Oertel, Y. Dauvilliers, R.B. Postuma, S. Marelli, A. Iranzo, I. Arnulf, B. Hogl, R. Manni, T. Miyamoto, M.L. Fantini, M. Puligheddu, P. Jennum, K. Sonka, J. Santamaria, M. Zucconi, P.M. Rancoita, S. Leu-Semenescu, B. Frauscher, M. Terzaghi, M. Miyamoto, M. Unger, K. Stiasny-Kolster, A. Desautels, C. Wolfson, A. Pelletier, J. Montplaisir, Autonomic symptoms in idiopathic REM behavior disorder: a multicentre case-control study, J. Neurol. 261 (2014) 1112–1118.
[28] C. Aguirre-Mardones, A. Iranzo, D. Vilas, M. Serradell, C. Gaig, J. Santamaria, E. Tolosa, Prevalence and timeline of nonmotor symptoms in idiopathic rapid eye movement sleep behavior disorder, J. Neurol. 262 (2015) 1568–1578.
[29] P. Mahlknecht, A. Iranzo, B. Hogl, B. Frauscher, C. Muller, J. Santamaria, E. Tolosa, M. Serradell, T. Mitterling, V. Gschliesser, G. Goebel, F. Brugger, C. Scherfler, W. Poewe, K. Seppi, Olfactory dysfunction predicts early transition to a Lewy body disease in idiopathic RBD, Neurology 84 (2015) 654–658.
[30] S.M. Fereshtehnejad, S.R. Romenets, J.B. Anang, V. Latreille, J.F. Gagnon, R.B. Postuma, New clinical subtypes of Parkinson disease and their longitudinal progression: a prospective cohort comparison with other phenotypes, JAMA Neurol. 72 (2015) 863–873.
[31] K. Doppler, J. Volkmann, C. Sommer, Skin biopsies in the differential diagnosis of parkinsonism: are we ready for simplified protocols? Brain 139 (2016) e5.

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