Association between probable REM sleep behavior disorder and increased dermal alpha-synuclein deposition in Parkinson's disease

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Abstract

Introduction: Many patients with Parkinson's disease suffer from REM sleep behavior disorder, potentially preceding the onset of motor symptoms. Phospho-alpha-synuclein is detectable in skin biopsies of patients with isolated REM sleep behavior disorder several years prior to the onset of manifest PD, but information on the association between dermal phospho-alpha-synuclein deposition and REM sleep behavior disorder in patients with manifest PD is limited. We therefore aimed to investigate the alpha-synuclein burden in dermal peripheral nerve fibers in patients with Parkinson's disease with and without REM sleep behavior disorder.

Methods: Patients with Parkinson's disease (n=43) who had undergone skin biopsy for the immunohistochemical detection of phosphorylated alpha-synuclein were screened for REM sleep behavior disorder using RBDSQ and Mayo Sleep Questionnaire. Skin biopsies from 43 patients with isolated polysomnography-confirmed REM sleep behavior disorder were used as comparators.

Results: Dermal alpha-synuclein deposition was more frequently found (81.8% vs. 52.4%, p=0.05) and was more abundant (p=0.01) in patients with Parkinson's disease suffering from probable REM sleep behavior disorder compared to patients without REM sleep behavior disorder and was similar to patients with isolated REM sleep behavior disorder (79.1%).

Conclusion: The phenotype of REM sleep behavior disorder is associated with high amounts of dermal alpha-synuclein deposition, demonstrating a strong involvement of peripheral nerves in patients with this non-motor symptom and may argue in favor of REM sleep behavior disorder as an indicator of a "body-predominant" subtype of Parkinson's disease.

Introduction

REM sleep behavior disorder (RBD) is a frequent non-motor symptom in patients with idiopathic Parkinson's disease (iPD) and multiple system atrophy (MSA) and often precedes the onset of motor symptoms [1]. In contrast to most other prodromal symptoms that are rather unspecific, it is a strong predictor of alpha-synucleinopathy, more than 80% of patients of patients with isolated RBD (iRBD) develop manifest iPD, dementia with Lewy bodies (DLB) or MSA within 14 years [1]. Identification of prodromal iPD has gained importance in the last few years as the development of disease-modifying treatments requires early diagnosis of patients prior to substantial neuronal loss. Many recent studies therefore focused on patients with iRBD as a cohort for studying prodromal PD and its course of disease . However, less than half of the patients with de-novo iPD suffer from RBD [1], and it is therefore unclear if results from studies on cohorts of patients with isolated RBD can be transferred to prodromal alpha-synucleinopathies in general. Recent studies gave evidence that phosphorylated alpha-synuclein (p-alpha-syn) can frequently be detected in skin biopsies of patients with isolated RBD and suggested that dermal p-alpha-syn might be a marker for future conversion to manifest iPD, DLB or MSA [2]. Other studies hypothesized that early iPD may be divided into two subtypes, namely a brain-first and a body-first form, with alpha-synuclein spreading from the central nervous system (CNS) to the periphery in the brain-first subtype and from the autonomic peripheral nervous system (PNS) to the brain in the body-first subtype [3], suggesting different degrees of involvement of the PNS in prodromal PD. So far, this hypothesis is mostly supported by nuclear imaging findings that gave evidence of an early reduction of cardiac sympathetic innervation and colonic parasympathetic innervation [3], but histopathological evidence is scarce. While performing several studies on the detection of dermal p-alpha-syn in iPD, we got the impression that high amounts of dermal p-alpha-

syn are most frequently seen in iPD patients with a clinical diagnosis of RBD, but studies systematically addressing a potential association between RBD and dermal p-alpha-syn-positivity are lacking.

In the present study, we therefore aimed to investigate potential differences of dermal p-alpha-syn load in patients with iPD with and without signs of RBD assessed by the RBD screening questionnaire (RBDSQ) and Mayo Sleep Questiononaire. These data might further support the hypothesis of a brain- versus body-first type of iPD on a pre-mortem histopathological level.

Methods

Patients

Forty-nine patients with a diagnosis of iPD who had undergone skin biopsy for previous studies [4, 5] were contacted and asked to complete the RBDSQ [6] and Mayo sleep questionnaire (informant version) [7] with the help of their bed partners as screening tools for RBD. Additionally, patient records were screened for a clinical diagnosis of suspected RBD. All patients gave written informed consent to participate and the study was approved by the Ethic's committee of the University of Würzburg. Out of a total number of 49 patients with iPD who were contacted, 43 finally completed the RBDSQ and were included into the study. The Mayo Sleep Questionnaire was only completed by 36 subjects because seven patients did not have a bed partner.

As a control group, skin biopsies of 43 patients with a polysomnography-confirmed diagnosis of clinically isolated RBD were included. Twelve of these patients had been recruited for another study [4].

Skin biopsy

Five-mm skin punch biopsies were taken from the neck (C7, paravertebral), back (Th10, paravertebral), proximal and distal leg as described previously [4]. Skin biopsies were fixed with 4% paraformaldehyde for 30 min and cryopreserved. Twenty-µm serial cryosections were cut and double-immunofluorescence staining with anti-protein gene product 9.5 (axonal marker, Zytomed Systems, Berlin, Germany, 1:200) and anti-p-alpha-syn (clone P-Syn/81A, Covance, Princeton, New Jersey, USA, 1:500) was performed. Every tenth section (n=5) of each biopsy was analyzed using a fluorescence microscope (Ax10, Zeiss, Oberkochen, Germany). The number of positive biopsy sites and sections was used for quantification of p-alpha-syn deposition. Skin biopsies of normal controls (n=20 in total) were run with each batch of staining and did not show any p-alpha-syn positive nerve fibers.

Statistical analysis

SPSS Statistics version 26.0 (IBM, Armonk, NY) was used for statistical analysis. The number of p-alpha-syn-positive patients was compared using two-sided Fisher's exact test. Ratios of positive biopsy sites and sections were compared using two-sided Mann-Whitney-U test. A significance level of $p<0.05$ was applied in all tests.

Results

Half of the iPD patients can be classified as pRBD-iPD

Twenty-four of 43 patients with iPD had an RBDSQ score of less than six points and were considered not to suffer from RBD (median 3, range 1-5). An RBDSQ score of at least six points was found in 19 patients who were considered to suffer from probable RBD (median 8, range 6-11, further referred to as pRBD-iPD). According to the Mayo sleep questionnaire, 18 patients were classified as pRBD-iPD, and 18 patients were classified as probably RBD negative cases. Eight patients each were assessed to be pRBD-positive by one of the screening questionnaires but not by the other, resulting in a concordant diagnosis in 28 of 36 patients. The classification of pRBD/non-pRBD was solely based on the RBDSQ as no polysomnography was performed in patients with iPD. A tremor was found in 7/19 patients classified as pRBD-iPD and in 14/24 patients with non-pRBD-iPD. Demographic data are summarized in Table 1.

Higher dermal phospho-alpha-synuclein deposition in iPD patients with pRBD

P-alpha-syn deposits were detectable in 16 patients with pRBD-iPD (84.2%) and in 13 patients without pRBD (54.2%) (p=0.05, table 1). The percentage of p-alpha-syn-positive biopsy sites as well as the ratio of positive sections was higher in patients with six or more points in the RBDSQ (p=0.013 and p=0.010, Figure 1). If patients with discordant results between RBD-SQ and Mayo sleep questionnaire were excluded, p-alpha-syn deposition was still higher in the pRBD-positive patients (p=0.020 for the ratio of positive biopsy sites and sections each). The Hoehn & Yahr stage and duration of disease did not differ between pRBD-positive and –negative patients and did also not differ between p-alpha-syn-positive and –negative patients.

Comparison of p-alpha-syn deposition in patients with clinically isolated RBD and PD patients

Dermal p-alpha-syn deposition of iPD patients with and without pRBD was compared to a cohort of patients with isolated RBD (iRBD), the vast majority of whom represent prodromal Lewy body disease patients. P-alpha-syn was detectable in 34 (79.1%) patients of the iRBD cohort (table 1), and the ratio of positive biopsy sites and sections was similar to iPD with pRBD and higher compared to iPD without pRBD (positive biopsy sites: p=0.007, positive sections: 0.008, Figure 1).

Distribution of p-alpha-syn and affected dermal structures

In patients with pRBD-iPD, p-alpha-syn was detectable in 9/19 (47.4%) biopsies of the lower leg and back each and in 11/19 (57.9%) biopsies of the upper leg and 7/19 (36.8%) biopsies of the neck. Patients with non-pRBD-iPD showed a similar distribution of affected biopsy sites, namely 6/24 (25%) of the lower leg, 5/24 (20.8%) of the upper leg, 3/24 (12.5%) biopsies of the back and 7/24 (29.2%) biopsies of the neck. Thus, the number of positive biopsies did not significantly differ between biopsy sites in pRBD- and non-pRBD-iPD. In iRBD, 12/34 (35.3%) skin biopsies of the lower leg were p-alpha-syn-positive, 16/43 (37.2%), 20/43 (46.5%) and 21/43 (48.8%) of the upper leg, back and neck respectively. Thus, there was a trend to a distal-to proximal increase of p-alpha-syn, but the differences were not significant.

Regarding affected dermal structures, p-alpha-syn was found in vasomotor nerve fibers in 14/16 (87.5%) positive patients with pRBD-iPD, in 9/13 (69.2%) positive patients with non-pRBD-iPD and in 25/34 (73.5%) of positive RBD patients. Sudomotor fibers were affected in 5/16 (31.3%) of positive pRBD-iPD subjects and 2/13 (15.4%) of positive non-pRBD-iPD patients and 12/34 (35.3%) of positive iRBD patients, pilomotor fibers in 6/16 (37.5%), 1/13 (7.7%) and 8/34 (23.5%) respectively. Thus,

autonomic nerve fibers tended to be more often involved in pRBD-iPD and iRBD, however the difference was not significant. Subepidermal somatosensory nerve fibers and dermal nerve bundles appeared to be equally involved (subepidermal: 3/16 (18.8%), 3/13 (23.1%) and 7/34 (20.6%), dermal nerve bundles: 9/16 (56.3%), 7/13 (53.8%), 26/34 (76.5%)).

Discussion

In the present study, we demonstrate higher dermal p-alpha-syn deposition in iPD patients with pRBD compared to patients without pRBD, indicating that RBD in iPD is associated with a higher p-alpha-syn load in peripheral nerve fibers.

The major limitation of our study is the missing polysomnography assessment in the iPD patients as gold-standard to diagnose RBD. Instead, we used validated questionnaires relying on symptoms of RBD showing fairly well discriminatory value [8], an approach that corresponds to the clinical practice of many hospitals as polysomnography is not routinely performed in all iPD patients in most departments. Nevertheless, our RBD-assessment certainly leads to a relevant number of patients to be falsely categorized and lower statistical power [9]. Despite this fact, a significant difference of p-alpha-syn deposition between pRBD-iPD and non-pRBD-iPD is detectable, and p-alpha-syn deposition is also higher in polysomnographically confirmed iRBD compared to non-pRBD-iPD.

Our data are in line with a recent study that reported higher alpha-synuclein seeding activity in dermal real-time quaking-induced conversion assay in iPD patients who reported symptoms of RBD [10], an immunohistochemical study that demonstrated higher dermal p-alpha-syn load in iPD patients with orthostatic hypotension, associated with RBD [11], and with a previous study that reported even higher detection rates of p-alpha-syn deposition in skin biopsies of iRBD compared to iPD patients [12], whereas in others, the detection rate was lower in iRBD compared to iPD [4, 13]. This might be explained by large numbers of RBD-iPD patients in these cohorts; however, RBD-assessments were not reported. Together with previous studies that reported increased alpha-syn deposition in nine of ten brain regions of RBD-positive iPD patients [14] and higher detection rates of alpha-syn aggregates in colonic biopsies of RBD-iPD patients compared to non-RBD-iPD [15], our data give evidence of either a higher and more widespread p-alpha-syn deposition associated with RBD-iPD or a “body-first“ subtype in the majority of patients with RBD-iPD including increased dermal p-alpha-syn aggregates. Assuming “body-/brain-first subtypes”, iRBD patients would represent “body-first” subjects and non-RBD-iPD “brain-first” subjects. In our study, RBD-iPD cannot be definitely categorized because our cohort included patients of advanced stages of disease who may have developed RBD during the course of disease as assessment with questionnaires did not provide sufficient information if RBD symptoms preceded the onset of parkinsonism.

Lower dermal p-alpha-syn deposition in patients with non-pRBD-iPD indicates that immunofluorescence staining of skin sections might not be a good biomarker for an early diagnosis of iPD in this relatively large subgroup of patients and that biomarker data from pRBD-iPD cannot readily be transferred to non-pRBD-iPD.

In summary, our data show that increased dermal p-alpha-syn is associated with the non-motor symptom of RBD, and skin biopsy as a pre-mortem histopathological biomarker may therefore be most useful in this subgroup of iPD. The idea of a “body-/brain-first” subtype may be supported by a

higher amount of p-alpha-syn deposits in patients with pRBD, and the existence of different subtypes needs to be considered when using alpha-synuclein deposition in peripheral nerves as a diagnostic marker or in clinical trials targeting alpha-synuclein deposition.

Table 1: Demographic data and p-alpha-syn deposition in skin biopsies of iPD patients with and without pRBD and patients with isolated RBD

	non-pRBD-iPD (n=24)	**pRBD-iPD (n=19)**	**isolated RBD (n=43)**
median age (range)	67.5 (55-82)	68 (60-88)	69 (50-79)
sex	13 f, 11 m	8 f, 11 m	5 f, 38 m
median duration of disease (years)	4 (1-21)	3 (1-20)	5 (1-23)
Hoehn & Yahr stage	2.5 (1-4)	2 (1-4)	-
p-alpha-syn-positive patients	13 (54.2%)	16 (84.2%)	34 (79.1%)
median ratio of positive biopsy sites	0.25 (0-0.75)	0.5 (0-1)	0.25 (0-1)
median ratio of positive sections	0.05 (0-0.35)	0.25 (0-0.6)	0.15 (0-0.93)

Figure legends

Figure 1: Box plots illustrating the median percentage/ratio of p-alpha-syn-positive biopsy sites (A) and sections (B). The horizontal line represents the median, quartiles and ranges are represented by the box and whiskers, the cross marks the mean. P-alpha-syn-positivity is increased in pRBD-iPD and isolated RBD compared to non-pRBD-iPD. *$p<0.05$.

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