Neuropathology of idiopathic rapid-eye-movement sleep behaviour disorder: a post-mortem case series

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Summary

Background. Idiopathic REM sleep behaviour disorder (IRBD) is thought to be an early α-synucleinopathy. Nevertheless, the definitive identification of its biological substrate can only be determined by postmortem neuropathology, which might help to design disease-modifying trials in IRBD patients using compounds against this substrate. Our aim was to describe the post-mortem neuropathology of subjects with IRBD who developed or not a neurological disease before death.

Methods. We examined post-mortem brain tissue from individuals diagnosed during life with IRBD by video-polysomnography who were brain donors at the Hospital Clinic Barcelona, Spain. Post-mortem neuropathology assessed the presence and distribution of neuronal loss, gliosis, and protein aggregates using antibodies against α-synuclein, β-amyloid, phosphorylated tau, 3R and 4R tau isoforms, and TDP-43.

Results. The study comprises brains of 20 IRBD patients examined between May 28th, 2005, and March 23rd, 2023. Their clinical antemortem diagnoses were of disease-free IRBD in three(15%), Parkinson disease without dementia in two(10%), Parkinson disease dementia (PDD) in three(15%), and dementia with Lewy bodies (DLB) in 12(60%). Post-mortem neuropathological diagnoses were Lewy body disease in 19(95%) patients and multiple system atrophy (MSA) in one(5%). All patients with Lewy body disease and MSA showed neuronal loss, gliosis and α-synuclein deposits in neurons but also in astrocytes.

In all patients, α-synuclein was found in the structures that regulate REM sleep atonia (eg, subcoeruleus nucleus, gigantocellular reticular nucleus, laterodorsal tegmentum, amygdala).

Coexistent pathologies were found in all patients, including Alzheimer's disease pathology (β-amyloid plaques, neurofibrillary tangles) in 14(70%), ageing-related tau astrogliopathy in 12(60%), cerebral amyloid angiopathy in 11(55%), argyrophilic grain disease in four(20%), limbic-predominant age-related TDP-43 encephalopathy in four(20%), and early changes of progressive supranuclear palsy in three(15%).

In disease-free IRBD patients and in those who developed Parkinson disease without dementia, α-synuclein was found in the brainstem and limbic system and rarely in the cortex, while

coexisting proteinopathies were few showing low pathologic burden. In patients who developed PDD or DLB, α-synuclein had diffuse distribution in the brainstem, limbic system, and cortex, whereas multiple comorbid pathologies were common, particularly Alzheimer's disease changes.

Interpretation. Although limited by a relatively small sample size, our observations provide strong neuropathological evidence that IRBD represents an early α-synucleinopathy. Concomitant pathologies are frequent, and their role remains to be clarified: some might have contributed for the development of dementia, but some might be age-related changes. Our findings may serve for the design of disease-modifying trials in IRBD patients using compounds against specific pathological proteins (eg, α-synuclein, β-amyloid).

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Introduction

REM sleep behaviour disorder (RBD) is characterized by abnormal sleep behaviours and increased muscular activity detected by video-polysomnography in REM sleep.[1] RBD is related to the impairment of the brainstem structures that modulate muscle paralysis during REM sleep, and their anatomic connections in the limbic system and other regions.[2]

Patients with idiopathic RBD (IRBD) have no overt motor or cognitive symptoms.[1] It is thought that IRBD represents an early α-synucleinopathy since 1) most patients are eventually clinically diagnosed with the α-synucleinopathies Parkinson disease, Parkinson disease dementia (PDD), dementia with Lewy bodies (DLB), and multiple system atrophy (MSA),[3] and 2) pathologic α-synuclein can be detected in tissues and CSF in about 75% of living patients.[4] Nevertheless, RBD has also been reported in subjects with other neurological diseases not linked to α-synuclein pathology, such as Alzheimer's disease, progressive supranuclear palsy (PSP), narcolepsy, and anti-IgLON5 disease.[1]

Definitive evidence that IRBD is an early α-synucleinopathy or has a coexistent or an alternate biological origin can be currently determined only by post-mortem brain neuropathological assessment of individuals who were correctly diagnosed during life using video-polysomnography. Identification of the exact neuropathological substrate of IRBD might have implications for the design of future disease-modifying trials, using single or multiple compounds against specific proteins that impair the neuronal function. The aim of our study was to describe the post-mortem neuropathological substrate of individuals who were diagnosed with IRBD during life, whether or not they were eventually clinically diagnosed with a neurodegenerative disease before death.

Methods

Study design and participants

We examined the post-mortem brain and spinal cord of all patients who were diagnosed with IRBD at the Hospital Clinic de Barcelona, Spain, and became donors of the Neurological Tissue Bank of the Biobanc-FCRB/IDIBAPS-Hospital Clinic de Barcelona, Spain, between May 28, 2005, and March 23, 2023. The ethical committee of the Hospital Clínic de Barcelona approved this study (HCB/2023/1278), and all donors gave written informed consent for the use of nervous system tissue for diagnostic and research purposes.

Diagnosis of IRBD was based on accepted criteria which included demonstration of REM sleep without atonia by video-polysomnography, and no evidence of associated neurological diseases.[1] During follow-up visits, when neurological symptoms were detected, Parkinson disease,[5] PDD[6] and DLB[7] were diagnosed based on accepted clinical criteria. At the time of death, we reviewed whether they had developed coexistent neurological symptomatology (eg, hyposmia, constipation), and results of ancillary investigations including smell tests, DAT-SPECT, and presence of α-synuclein, β-amyloid, and total and phosphorylated tau proteins in the CSF.

Procedures

All brains and spinal cords were processed for histopathology following the standardized workflow of our Neurological Tissue Bank based on haematoxylin and eosin staining and immunohistochemistry.[8] We examined the presence, severity, and topographical distribution of neuronal loss, gliosis and pathologic protein accumulations in multiple brain areas (Supplementary material). This evaluation was performed by GM and IA under supervision of EG.

Severity and density of neuronal loss and gliosis were categorized in absent, mild, moderate and severe (Supplementary material).[8]

Pathological protein aggregates were identified using specific antibodies against oligomeric α-synuclein, β-amyloid,

phosphorylated tau, tau 3R and 4R isoforms, TDP-43, orexin-B and melanin concentrating hormone (Supplementary material). Severity and density of protein deposits were semiquantitatively classified as absent (0), isolated (0.5), mild (1), moderate (2), and severe (3).[8]

The presence of α-synuclein was examined in 1) intraneuronal Lewy bodies and Lewy neurites (Lewy pathology) that constitute the neuropathological hallmark of Parkinson's disease, PDD, and DLB[9-11], 2) inclusions located in the soma and neurites of astrocytes, that have been described in Parkinson's disease, PDD, and DLB[12], and 3) glial cytoplasmic inclusions (GCI) in oligodendrocytes, which define MSA.[13,14]. Severity and distribution of Lewy pathology was classified according to Braak et al.[10], McKeith et al.[9] and Attems et al.[11] Occurrence and localization of α-synuclein in astrocytes was also assessed.[12] MSA neuropathological distribution and quantification of GCI was graded.[13,14]

Alzheimer's disease pathology was defined by the occurrence of β-amyloid plaques in association with hyperphosphorylated tau in neurofibrillary tangles, neuropil threads and dystrophic neurites. Localization and burden of Alzheimer's disease pathology was assessed following Thal et al.[15], Braak et al.[16], and adapted Consortium to Establish a Registry for Alzheimer's Disease criteria,[17] as suggested by the National Institute on Aging-Alzheimer's Association consortium.[18] In patients who developed dementia and had mixed Lewy pathology and Alzheimer's disease pathology, the likelihood that α-synuclein explained the cognitive decline was determined by the density and distribution of Lewy pathology and neurofibrillary tangles, and categorized as low, intermediate, and high.[7,11,16] Cerebral amyloid angiopathy (CAA) was categorized.[19]

For argyrophilic grain disease (AGD), 4R tau grain-like deposits were graded.[20] For aging-related tau astrogliopathy (ARTAG), granular fuzzy and thorn-shaped astrocytes were evaluated using tau immunohistochemistry.[21] Limbic-predominant age-related TDP-43 encephalopathy neuropathological changes (LATE-NC) was classified according to TDP-43 distribution.[22] PSP-type pathology was examined according to the presence and localization of neuronal and glial 4R-tau inclusions.[23] Microangiopathy was scored as none (0), mild (1), moderate (2) and severe (3).[24]

Post-mortem α-synuclein distribution of two patients were briefly described previously.[25,26]

Statistical analysis

We report demographic, clinical, and neuropathological data as median, range, number and percentage. Comparative statistical analyses were not done because of small sample size, but differences observed across the nuclei and brain structures were described. Analyses were done with SPSS version 25.0 for Windows (SPSS, Inc., Chicago, IL).

Role of the funding source

The BBVA foundation, funder of the study, had no role in the study design, data collection, data analysis, data interpretation, or writing of the report.

Results

We evaluated 20 individuals, 19(95%) men, with a median interval between IRBD diagnosis and death of nine (range, 1-22) years. The median age at death was 81 (range, 60-93) years. At the time of IRBD diagnosis, 11(55%) patients reported hyposmia, nine(45%) depression, and 11(55%) constipation, whereas eight patients underwent DAT-SPECT, and in all of them decreased tracer uptake was detected in the striatum. At the time of IRBD diagnosis, nine patients underwent lumbar puncture, and the CSF showed AS positivity in all(100%) of them, as determined by the RT-QuIC assay, low β-amyloid levels in five(55.6%), increased phosphorylated-tau in three(33.3%), and increased total-tau in two(22.2%)(Figure 1). At the time of death, antemortem clinical diagnoses were disease-free IRBD in three(15%) patients, Parkinson disease without dementia in two(10%), PDD in three(15%), and DLB in 12(60%).

Post-mortem primary diagnoses were Lewy body disease in 19(95%) patients, and MSA in one(5%). In the 19 patients with Lewy body disease, α-synuclein was detected in neurons as Lewy bodies and Lewy neurites, and in astrocytes as fine processes in the soma and also as unspecific fine granular positivity surrounding the nucleus. α-synuclein aggregates, in both neurons and astrocytes, were found in spinal cord, brainstem, limbic system and cortex with similar distribution in each of the patients with Lewy body disease. In the MSA patient, oligodendroglia showed GCI. In all 20 patients, neuronal loss and gliosis were widespread in the brain and spinal cord, showing maximal involvement in the brainstem **(Table 1, Figures 1-3).**

Three patients had the antemortem diagnosis of disease-free IRBD (Supplementary material). Post-mortem neuropathological diagnoses were MSA in participant 1, and Lewy body disease in participants 2 and 3. In these two patients with Lewy body disease, Lewy pathology showed limbic transitional McKeith type and followed a caudo-rostral gradient that was severe in brainstem, moderate in limbic system, and absent or mild in cortex. Disease-free IRBD patients showed Alzheimer's disease pathology with low burden, and other changes (eg, AGD, ARTAG, CAA) with mild severity. Neuronal loss and gliosis were mild to

moderate in the brainstem and limbic system, and absent or mild in the cortex. Participant 1 showed typical MSA features with mild to moderate olivopontocerebellar atrophy and mild striatonigral degeneration in whom α-synuclein deposits were found mainly as GCI in the oligodendrocytes and also as diffuse neuronal cytoplasmic aggregates.

Similar to participants 2 and 3 with disease-free IRBD before death, participants 4 and 5 with Parkinson disease without dementia, showed 1) postmortem Lewy body disease with limbic transitional McKeith type and caudo-rostral gradient, albeit more severe, 2) few copathologies with mild burden, and 3) mild to moderate neuronal loss and gliosis in brainstem and limbic system, and absent or minimal in cortex.

Participants 6, 7, and 8 were diagnosed in life with PDD showing postmortem diffuse neocortical Lewy pathology McKeith type, and coexistent Alzheimer's disease pathology, AGD and ARTAG. Participant 8 showed minimal changes suggestive of early PSP-type pathology in brainstem and basal ganglia due to the presence of few 4-repeat tau positive tufted astrocytes, coiled bodies, and tangles.[23]

In the 12 patients with antemortem diagnosis of DLB (participants 9-20), Lewy pathology was diffuse neocortical McKeith type in ten and limbic transitional in two, showing severe Lewy pathological burden in brainstem and limbic system, and less prominent in cortex. Coexisting changes were invariably seen in all 12 DLB patients including Alzheimer's disease pathology, ARTAG, CAA, AGD, LATE-NEC and minimal changes of early PSP-type.

In the intermediolateral column of the spinal cord, α-synuclein showed a decreasing rostro-caudal gradient, with deposits more abundant in the cervical region than in the thoracic and lumbar regions. This gradient was more evident in disease-free IRBD patients than in those who developed Parkinson disease, PDD or DLB.

In the brainstem, α-synuclein, neuronal loss and gliosis were marked in all 20 patients always involving the dorsal nucleus of the vagus nerve and its nerve emergence **(Figure 4).**

In all patients, α-synuclein and neuronal loss were found in the structures that regulate REM sleep atonia (eg,

subcoeruleus nucleus, gigantocellular reticular nucleus, laterodorsal tegmentum, lateral posterior hypothalamus, amygdala) (Figure 4). Alzheimer's disease pathology was more frequent in the coeruleus-subcoeruleus complex than in the gigantocellular reticular nucleus, particularly in patients with dementia. Substantia nigra pars compacta showed marked α-synuclein and neuronal loss in all participants, including those three with disease-free IRBD in whom parkinsonism was absent. Substantia nigra contained neurofibrillary tangles in 13 of the 15(87%) patients with dementia.

In the limbic system, α-synuclein was observed in all 20 participants, and its burden was mild to moderate in disease-free IRBD and PD, and high in PDD and DLB. In the limbic structures, Lewy pathology was more abundant than Alzheimer's disease pathology.

In the cortex, the five participants who did not develop dementia showed mild or absent α-synuclein deposits, and isolated tau deposits and β-amyloid plaques. In the 15 patients with dementia, the cortex showed invariably widespread moderate Lewy pathology plus AD neuropathologic changes, a pattern that was more severe in DLB than in PDD. In patients with dementia, the hippocampus and the nucleus basalis of Meynert had neuronal loss and mixed Lewy and Alzheimer's disease pathologies. The likelihood that Lewy pathology explained dementia was high in 13(77%) patients and intermediate in two(13%, one had high Alzheimer's disease neuropathology, and the other moderate ARTAG). Among patients with dementia, Alzheimer's disease pathology was observed in 11(73%) patients, ARTAG in 10(67%), CAA in nine(60%), AGD in four(27%), LATE-NC in four(27%), and PSP changes in three(15%).

Concomitant pathologies consisted in microangiopathy in all participants, low to high Alzheimer's disease pathology burden in 14(70%), ARTAG in 12(60%), CAA in 11(55%), AGD in four(20%), LATE-NC in four(20%), and PSP-type pathology in three(15%). Eleven different combinations of pathologies were found among the 20 patients. The most frequent combination was Lewy pathology plus Alzheimer's disease, ARTAG and microangiopathy **(Figure 5).**

Discussion

In this clinicopathological study, we evaluated 20 video-polysomnography-confirmed IRBD patients who were clinically followed-up until death. Antemortem diagnoses were IRBD who remained disease-free, and IRBD that evolved into PD, PDD and DLB. Post-mortem assessment found that all brains had widespread and severe α-synuclein pathology, and primary diagnoses were Lewy body disease in 19 patients and MSA in one patient. In the patients without dementia, neuropathology showed α-synuclein pathology in the brainstem and limbic system with minimal or absent involvement of the cortex. Patients who developed dementia had more extensive Lewy pathology in the brainstem, limbic system, and cortex. Copathologies were found in all participants, particularly in those who developed dementia.

To the best of our knowledge, there are only two additional published polysomnography-confirmed disease-free IRBD patients who underwent autopsy.[27,28] In both cases, Lewy bodies were found in the brainstem and limbic system sparing the cortex. Likewise, our disease-free IRBD patients, in whom parkinsonism and dementia were absent before death, had widespread α-synuclein in the brainstem and limbic system with minimal cortical involvement. In addition, we report that our disease-free IRBD patients had coexistent copathologies, but with low burden. These observations indicate that IRBD is mainly a α-synucleinopathy, in line with a recent amyloid PET study showing that cortical β-amyloid load in disease-free IRBD patients is comparable with controls and much lower than in DLB.[29]

Like our disease-free IRBD patients, our patients with Parkinson's disease without dementia showed a similar pattern of Lewy pathology, albeit more severe, characterized by limbic predominant McKeith type, a caudo-rostral distribution in the brain, and a rostro-caudal distribution in the spinal cord. Like in our IRBD patients, these had low burden of few concomitant pathologies.

In patients with dementia (PDD and DLB), Lewy pathology was severe in the brainstem, limbic system, and less in the neocortex. All patients with dementia showed comorbid pathological changes in the brain. Alzheimer's disease changes were found in most patients with dementia involving the hippocampus or the cortex, but the likelihood that these changes explained the cognitive impairment was lower compared to Lewy

pathology. Neuronal loss in the cholinergic nucleus basalis of Meynert was more evident in patients with dementia than those without dementia. Alzheimer's disease pathology and other proteinopathies were more common in people with DLB than in those with PDD, as previously reported. Overall, in our cohort, the occurrence and degree of dementia might be the effect of widespread Lewy pathology, but also of multiple coexistent pathologies (in particular the Alzheimer's disease changes), and neuronal loss in the cholinergic nucleus basalis of Meynert.

α-synuclein was the main abnormally deposited protein and the defining pathological feature in our series. Parkinson disease, PDD and DLB constitute a clinical constellation of neurological symptoms linked to a pathological spectrum of α-synuclein burden from low to high in the brainstem, limbic system, and cortex.[11] Our disease-free IRBD patients represent an earlier stage of this PD-PDD-DLB continuum, in which various neurological symptoms occur such as hyposmia and dysautonomia, parkinsonism and dementia are absent, and α-synuclein pathology is less severe and more restricted to the brainstem than in Parkinson's disease, PDD and DLB. In PDD and DLB, coexisting Alzheimer's pathology was common but less severe than Lewy pathology. Our observations are in line with previous neuropathological studies analysing the frequency and distribution of α-synuclein and Alzheimer's disease pathology along the continuum PD-PDD-DLB.[30]

In addition, we found α-synuclein deposits in both the neurons and astrocytes. The relevance of α-synuclein in astrocytes is unknown, but experimental studies have shown that misfolded α-synuclein might be propagated by glial-glial transmission, similarly to the neuron-neuron transference of α-synuclein.[10,12]

Our study provides post-mortem evidence that IRBD is part of a neurodegenerative Lewy body disease continuum, that evolves with time from early to late stages following an IRBD-PD-PDD-DLB clinical pattern, in parallel with more severe and widespread deposition of α-synuclein pathology in the brain. However, one of our patients showed abundant α-synuclein-positive GCI and was neuropathologically diagnosed with MSA. The brain also had coexistent low burden of Alzheimer's disease pathology and mild CAA, in line with previous studies showing that concomitant pathologies occur in almost all patients with MSA, including tau, β-amyloid, and TDP-43.[12] The observation that one of our

patients showed MSA pathology suggests that, in a minority of patients with disease-free IRBD, Lewy pathology might be absent.

In addition to α-synuclein pathology, coexistent neuropathological changes were found in all our patients. They included Alzheimer's disease pathology, CAA, AGD, ARTAG, LATE-NC and changes suggestive of early PSP-type pathology. Patients with dementia had more coexisting proteinopathies than those without. The severity of Alzheimer's disease pathology was higher in DLB than in disease-free IRBD, Parkinson's disease and PDD. These observations agree with previous autopsy studies demonstrating that coexisting neurodegenerative and age-related pathologies are common in the Lewy body diseases, especially once dementia develops.[31,32]

In the α-synucleinopathies, severity of α-synuclein deposits and neuronal loss in vulnerable regions are thought to be responsible for the clinical expression of the disease. The clinical relevance of the mixed pathologies remains unclear. They might be either part of the neurodegenerative process or be related to ageing.[13] A primary pathologic event (e.g., misfolded α-synuclein) might induce fibrilization and aggregation of other proteins (eg, tau, β-amyloid, TDP-43) that might contribute to worsen symptoms.[13] In the Lewy body diseases, the accumulation of tau, β-amyloid and TDP-43 is higher in DLB and PDD than in Parkinson's disease and might contribute to the development of dementia.[31] Alternatively, the pathologies found in our patients might not be clinically crucial, as many cognitively intact adults who undergo autopsy after the age of 80 can show Alzheimer's disease pathology, AGD, ARTAG, LATE-NC and cerebrovascular changes.[13]

IRBD patients constitute a target population to receive neuroprotective interventions to prevent the onset of parkinsonism and dementia. One potential approach is to interfere with pathological aggregation and spreading of abnormal proteins throughout the brain. In mice, microinjection of α-synuclein in the sublaterodorsal tegmental nucleus (analogous to the subcoeruleus nucleus in humans) induces REM sleep without atonia and propagation of α-synuclein to the substantia nigra, olfactory bulb and dorsal motor nucleus of the vagus nerve resulting in olfactory dysfunction and gastrointestinal dysmotility.[32] In living IRBD patients, RT-QuIC assay in the CSF detects misfolded α-synuclein in 75% of the patients.[4] It is uncertain whether the remaining 25% IRBD patients with α-synuclein negativity in the CSF should be

included in future neuroprotective trials using anti-α-synuclein compounds, because it is unknown if they represent false negatives for the assay (eg, brainstem-only damage) or have RBD not related to a α-synucleinopathy. Our current study shows that Alzheimer's disease pathological burden is absent or minimal in postmortem investigations of individuals with disease-free IRBD before death. Nevertheless, positive Alzheimer's disease biomarkers can be found in the CSF of 22% living disease-free IRBD patients, most of them with coexistent α-synuclein positivity.[34] Since α-synuclein seems to be an obvious protein target in IRBD patients with α-synuclein positivity in the CSF, it is unclear whether those with coexistent Alzheimer's disease biomarkers in the CSF, should also be treated with anti-amyloid medications in future clinical trials in IRBD.

This study has several limitations. First, sample size is relatively small. However, to the best of our knowledge, ours is the largest neuropathological series of IRBD patients in whom the sleep disorder was confirmed by video-polysomnography and they were followed until death. If our sample had been larger, we might have found some brains without α-synuclein deposits and with another type of proteinopathy.[35] Second, in our series there was a male predominance, although this reflects the gender distribution in IRBD.[3] Third, at the time of death, patients with DLB were older than those with IRBD and with PD. Thus, ageing may explain, in part, that DLB patients had more common copathologies when compared with those with disease-free IRBD and PD. Fourth, the study was conducted in a single centre. This in turn has the advantage of a homogeneous clinical and neuropathological work-up. Fifth, we did not include a control group (people who died without an underlying neurological disease; people with a postmortem confirmation of Lewy pathology who had no RBD during life; and RBD in the context of neurodegenerative diseases not linked to α-synuclein pathology such as PSP) to better characterize the intrinsic neuropathology of RBD. Sixth, our cohort included patients who died at an advanced age where incidental Lewy bodies and neurites, AG, ARTAG, and vascular changes are frequently found and, while they may have contributed partially to the clinical symptomatology, they might not be the final substrate. Finally, the assessment of thin histological slides, as used for standard diagnostic work-up, prevented us to perform a quantitative approach. Strengths of our work include the confirmation of RBD by video-polysomnography in all patients, a detailed clinical characterization of our patients during the antemortem period,

and a comprehensive and extensive neuropathologically assessment of multiple pathologies in multiple areas of the brain and the spinal cord, using a broad panel of antibodies for immunohistochemical evaluation.

In summary, we provide strong neuropathological evidence that IRBD can be considered an early feature of the α-synucleinopathies Parkinson's disease, PDD, DLB and rarely MSA. While IRBD is a brainstem predominant α-synucleinopathy that spares the cortex, neocortical α-synuclein pathology and multiple concomitant pathologies occurred when dementia and associated progression of the neurodegenerative process develops. Further studies are required to understand the clinical relevance of the mixed pathologies and the age-related brain changes found in our IRBD patients, particularly for the design of future neuroprotective clinical trials.

Data sharing

De-identified participant clinical and neuropathological data are available upon request to the corresponding author with publication. Data underlying this report will be available only for investigators whose proposed use of the data has been approved by an institutional review board, one of the institution to which the investigator requesting the data is affiliated, or who that agree to the terms and conditions of the data use agreement.

STARD guidelines for studies of diagnostic accuracy

We ensure that our report conforms to STARD guidelines for studies of diagnostic accuracy.

Research in context

Evidence before the study

We searched Medline for articles published in any language between January 1st 1987 and Aug 23th 2024, with the search terms "REM sleep behaviour disorder" "Parkinson disease", "dementia with Lewy bodies", "Alzheimer's disease", "multiple system atrophy", "Lewy pathology", "synuclein", "amyloid", "tau", "neuropathology" and "concomitant/coexisting/co-pathologies". There are two reports published in 1995 and 2007 of two IRBD patients who underwent autopsy. In an 88-year-old man with a 22-year history of IRBD, neuropathology showed Lewy bodies in the brainstem (eg, dorsal motor nucleus of the vagus nerve, locus coeruleus, gigantocellular reticular nucleus, substantia nigra) and diencephalon (eg, nucleus basalis of Meynert) sparing the cortex, but coexistent pathologies were not evaluated. The other case described a 72-year-old man with a 14-year history of IRBD in whom autopsy showed Lewy bodies in the brainstem (eg, dorsal motor nucleus of the vagus nerve, subcoeruleus nucleus, gigantocellular reticular nucleus, substantia nigra) and limbic system (eg, hippocampus, amygdala) preserving the cortex. Neurofibrillary tangles were found in the entorhinal cortex, and Aβ plaques were not detected. A retrospective study of 82 RBD patients with coexistent neurologic disorders before death showed that the neuropathological diagnoses were a α-synucleinopathy in 78 (95%), Alzheimer's disease in one, progressive supranuclear palsy in one, and hypothalamic lesions in two. In these 82 patients, RBD was detected after the diagnosis of the neurological disorder. In this study, only the primary neuropathological diagnoses were reported but the presence and types of coexistent neuropathological changes were not evaluated.

Added value of this study

This study provides definitive neuropathological evidence that IRBD represents an early manifestation of a α-synucleinopathy. IRBD evolves with time to functional impairment due to the presence of parkinsonism and dementia caused by severe widespread Lewy pathology and neuronal loss in the brain. In addition, we found the frequent coexistence of age-related changes and multiple copathologies, particularly Alzheimer's disease, that may have contributed, in part, to the development

of cognitive impairment. α-synuclein deposits were found not only in neurons but also in glia.

Implications of all the available evidence

Our finding of prominent α-synuclein pathology and coexistent proteinopathies in the brains of the people with IRBD might be useful in the design of disease-modifying trials, particularly in the choice of a drug directed against the neuropathological substrate, in this case against α-synuclein and opening the window to also interfere against comorbid proteins such as β-amyloid.

Figure 1. Clinical and neuropathological findings

M: male; F: female; C: Caucasian. IRBD: idiopathic REM sleep behaviour disorder; PD: Parkinson disease; PDD: Parkinson disease dementia; DLB: dementia with Lewy bodies; MSA: multiple system atrophy; LBD: Lewy body disease; *: brain weight includes a massive frontotemporal haemorrhage; L: limbic; N: neocortical; Int: intermediate; GCI: glial cytoplasmic inclusions; OPCA: olivo-ponto-cerebellar atrophy; SND: striatonigral degeneration; CERAD: consortium to establish a registry for Alzheimer's disease; AGD: argyrophilic grain disease; ARTAG: aging-related tau astrogliopathy; BG: basal ganglia; NA: non-available; NCIs: when other 4R tauopathy rather than ARTAG could explain the findings, ARTAG scoring was scored as non-classifiable; LATE-NC: limbic-predominant age-related TDP-43 encephalopathy neuropathological changes; PSP: progressive supranuclear palsy (** participant 16 had overlapping features of argyrophilic grain disease and progressive supranuclear palsy); SVD: small vessel disease (arteriolosclerosis) in cerebral and cerebellar white matter and basal ganglia, and lacunar infarcts; DAT- SPECT: dopamine transporter single photon emission computed tomography; CSF: cerebrospinal fluid; AS: α-synuclein; RT-QuIC: Real-time quaking induced conversion; β-amyloid 1-42 (normal values >600pg/ml); t-tau: total tau (normal values <385 pg/ml); p-tau: phosphorylated tau (normal values <65 pg/ml); Results are expressed as median and range or number and percentage. ***A summary of each neuropathological disease criteria is presented in the supplementary material.

Figure 2. Quantitative assessment of neuronal α-synuclein deposits (A), glial α-synuclein deposits (B), and neuronal loss plus gliosis (C) in patients with Lewy pathology

IRBD-LP: idiopathic REM sleep behaviour disorder with Lewy pathology: Parkinson disease; PDD: Parkinson disease dementia; DLB: dementia with Lewy bodies; LSC: intermediolateral column of the lumbar spinal cord; AH: anterior horn. THSC: intermediolateral column of the thoracic spinal cord; CSC: intermediolateral column of the cervical spinal cord; GRN: gigantocellular reticular nucleus; Xn: margin of the vagus nerve; DMV: dorsal motor nucleus of the vagus nerve; LC-SC: locus coeruleus-subcoeruleus complex; LPT/PPN: lateral pontine tegmentum/pedunculopontine nucleus; SNpc: substantia nigra pars compacta; LDT: Laterodorsal tegmentum; RN: red nucleus; DRN: dorsal raphe nucleus; NBM: nucleus basalis of Meynert; Olf: olfactory bulb: Ag: amygdala; HC-CA2: hippocampus CA2 region; aC: anterior cingulate; Tcx: temporal cortex; Fcx: frontal cortex. Results are represented by medians obtained by statistical analysis.

Figure 3. Quantification of neuronal loss, gliosis, and proteinopathies in key anatomic areas

Each column represents a single patient. NL+G: neuronal loss plus gliosis; AS: α-synuclein; Aβ: beta-amyloid; TDP-43: transactive response DNA binding protein of 43 kDa; LC-SC: locus coeruleus-subcoeruleus complex.

Figure 4. Schematic representation of the REM sleep circuits involved in REM sleep behaviour disorder and their affection in the three premortem disease-free IRBD patients by neuronal loss, gliosis, and α-synuclein deposits. The assessment of thin histological slides, as used for standard diagnostic work-up, prevented us to perform a quantitative approach.

A. REM sleep muscular tone regulation.

B. Afferents regulating the locus coeruleus-subcoeruleus complex and the gigantocellular reticular nucleus.

Below each anatomical region the degree of neuronal loss and gliosis (column 1), neuronal α-synuclein deposits (column 2), and glial α-synuclein deposits (column 3) are represented by a colour gradient.

The first row represents the patient with premortem disease-free IRBD diagnosis and postmortem multiple system atrophy neuropathology (patient 1). The second and third rows represent the two patients with premortem disease-free IRBD diagnosis and Lewy pathology at postmortem investigation (patients 2 and 3). The pedunculopontine nucleus and the lateral pontine tegmentum, as well as the locus coeruleus-subcoeruleus complex were assessed as a whole region due to ill-defined limits between individual nuclei, although they are represented separately in this scheme. In the spinal cord, neuronal loss and gliosis were evaluated at the level of the anterior horn as a whole, without segmentation of subregions. α-synuclein deposits in the motoneurons (second column) were quantified separately from α-synuclein deposits in glia and interneurons (third column). Scheme modified from Iranzo A. The REM sleep circuit and how its impairment leads to REM sleep behavior disorder. Cell Tissue 2018;373:245-266.(reference 2)

Figure 5. α-synuclein and tau immunoreactivity in the brainstem

IRBD: idiopathic REM sleep behaviour disorder; PD: Parkinson disease; DLB: dementia with Lewy bodies; MSA: multiple system atrophy; AS: α-synuclein; LC-SC: locus coeruleus-subcoeruleus complex; DMV: dorsal motor nucleus of the vagus nerve; GRN: gigantocellular reticular nucleus; Xn: margin of the vagus nerve.

Each column represents a single patient.

Scale bars: A-P are 100µm while Q-T are 200µm.

First column: patient 2 with antemortem disease-free IRBD and postmortem Lewy body disease. Mild tau deposits in locus coeruleus-subcoeruleus complex (A), and abundant α-synuclein in neurons and astrocytes in locus coeruleus-subcoeruleus complex (E), dorsal motor nucleus of the vagus nerve (I), gigantocellular reticular nucleus (M), and margin of the vagus nerve (Q).

Second column: patient 4 with antemortem diagnosis of Parkinson disease and postmortem Lewy body disease. Mild tau deposits in locus coeruleus-subcoeruleus complex (B), and abundant α-synuclein deposits in neurons and astrocytes in locus coeruleus-subcoeruleus complex (F), dorsal motor nucleus of the vagus nerve (J) and gigantocellular reticular nucleus (N), and moderate in the margin of the vagus nerve (R).

Third column: patient 17 with antemortem diagnosis of DLB and postmortem Lewy body disease. Moderate tau in locus coeruleus-subcoeruleus complex (C), and abundant α-synuclein aggregates in neurons and astrocytes in locus coeruleus-subcoeruleus complex (G), and dorsal motor nucleus of the vagus nerve (K), and frequent in the neurons but moderate in the astrocytes within the gigantocellular reticular nucleus (O). The α-synuclein deposits were moderate in the margin of the vagus nerve (S).

Fourth column: patient 1 with antemortem diagnosis of disease-free IRBD and postmortem multiple system atrophy. Tau deposits in locus coeruleus-subcoeruleus complex were absent (D). Oligodendroglial cytoplasmic inclusions were abundant within the locus coeruleus-subcoeruleus complex (H), dorsal motor nucleus of the vagus nerve (L) and gigantocellular reticular nucleus (P), while they were absent in the margin of the vagus nerve (T). Neuronal α-synuclein accumulation was moderate in locus coeruleus-subcoeruleus complex (H), mild in gigantocellular reticular nucleus (P), and abundant in basis pontis and striatum (not shown).

Figure 6. Frequency and combinations of pathologies according to the antemortem diagnoses

A: Frequency of pathologies; B: Combinations of pathologies (Alzheimer disease and cerebral amyloid angiopathy are combined); LBD: Lewy body disease; AD: Alzheimer's disease; ARTAG: age-related tau astrogliopathy; CAA: cerebral amyloid angiopathy; AGD: argyrophilic grain disease; LATE-NC: limbic-predominant age-related TDP-43 encephalopathy neuropathological changes; PSP: progressive supranuclear palsy; MSA: multiple system atrophy; DLB: dementia with Lewy bodies; PDD: Parkinson disease with associated dementia; PD: Parkinson disease; IRBD: idiopathic REM sleep behaviour disorder.

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Accessed and verified the data: GM, AI, CG, MS, JS and RSV for clinical data, and GM, LMP, EG and IA for neuropathological data. These statements regarding who accessed and verified the data in our study cover all the data included in the manuscript. All the authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

References

1. American Academy of Sleep Medicine. International classification of sleep disorders, 3rd ed, test revision. Darien, IL: American Academy of Sleep Medicine, 2023.

2. Iranzo A. The REM sleep circuit and how its impairment leads to REM sleep behavior disorder. Cell Tissue 2018;373:245-266.

3. 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;142:744-748.

4. Iranzo A, Mammana A, Muñoz-Lopetegi A, et al. Misfolded α-synuclein assessment in the skin and CSF by RT-QuIC in isolated REM sleep behavior disorder. Neurology 2023;100:e1944-e1954.

5.Berg D, Adler CH, Bloem BR, et al. Movement disorder society criteria for clinically established early Parkinson's disease. Mov Disord 2018;33:1643-1646.

6.Emre M, Aarsland D, Brown R, et al. Clinical diagnostic criteria for dementia associated with Parkinson's Disease. Mov Disord 2007;22:1689-1707.

7.McKeith IG, Boeve BF, Dickson DW, et al. Diagnosis and management of dementia with Lewy bodies: Fourth consensus report of the DLB Consortium. Neurology 2017;89:88-100.

8. Borrego-Écija S, Turon-Sans J, Ximelis T, et al. Cognitive decline in amyotrophic lateral sclerosis: Neuropathological substrate and genetic determinants. Brain Pathol 2021;31:12941

9.McKeith IG, Dickson DW, Lowe J, et al. Diagnosis and management of dementia with Lewy bodies. Neurology 2005;65:1863-1872.

10.Braak H, Del Tredici K, Rüb U, et al. Staging of brain pathology related to sporadic Parkinson's disease. Neurobiol Aging 2003;24:197-211.

11.Attems J, Toledo JB, Walker L, et al. Neuropathological consensus criteria for the evaluation of Lewy pathology in post-mortem brains: a multi-centre study. Acta Neuropathol 2021;141:159-172.

12. Altay MF, Lun Liu AK, Holton JC, Parkkinen L, Lashuel AK. Prominent astrocytic alpha-synuclein pathology with unique post-translational modification signatures unveiled across Lewy body disorders. Acta Neuropathol Commun 2022; 12;10:163:1-18

13.Jellinger KA, Seppi K, Wenning GK. Grading of neuropathology in multiple system atrophy: proposal for a novel scale. Mov Disord 2005;20 (Suppl 12):S29-36.

14. Brettschneider J, Irwin DJ, Boluda S. Progression of alpha-synuclein pathology in multiple system atrophy of the cerebellar type. Neuropathol Appl Neurobiol 2017;43:315-329.

15.Thal DR, Rüb U, Orantes M, Braak H. Phases of Aβ-deposition in the human brain and its relevance for the development of AD. Neurology 2002;58:1791-1800.

16.Braak H, Alafuzoff I, Arzberger T, Kretzschmar H, Del Tredici K. Staging of Alzheimer disease-associated neurofibrillary pathology using paraffin sections and immunocytochemistry. Acta Neuropathol 2006;112:389-404.

17.Mirra SS, Heyman A, McKeel D, et al. The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Neurology 1991;41:479-486.

18. Montine TJ, Phelps CH, Beach TG, et al. National Institute on Aging-Alzheimer's Association guidelines for the neuropathologic assessment of Alzheimer's disease: a practical approach. Acta Neuropathol 2012;123:1-11.

19.Allen N, Robinson AC, Snowden J, Davidson YS, Mann DMA. Patterns of cerebral amyloid angiopathy define histopathological phenotypes in Alzheimer's disease. Neuropathol Appl Neurobiol 2014;40:136-148.

20.Saito Y, Ruberu NN, Sawabe M, et al. Staging of argyrophilic grains: An age-associated tauopathy. J Neuropathol Exp Neurol 2004;63:911-918.

21.Kovacs GG, Ferrer I, Grinberg LT, et al. Aging-related tau astrogliopathy (ARTAG): harmonized evaluation strategy. Acta Neuropathol 2016;131:87-102.

22. Nelson PT, Lee EB, Cykowski MD, et al. LATE-NC staging in routine neuropathologic diagnosis: An update. Acta Neuropathol 2023;145:159-173.

23.Roemer SF, Grinberg LT, Crary JF, et al. Rainwater Charitable foundation criteria for the neuropathologic diagnosis progressive supranuclear palsy. Acta Neuropathol 2022;144:603-614.

24. Deramecourt V, Slade JY, Oakley AE et al. Staging and natural history of cerebrovascular pathology in dementia. Neurology 2012;78:1043-1050.

25. Gaig C, Iranzo A, Tolosa E, Vilaseca I, Rey MJ, Santamaria J. Pathological description of a non-motor variant of multiple

system atrophy. J Neurol Neurosurg Psychiatry 2008;79:1399-1400.

26. Iranzo A, Gelpi E, Tolosa E, et al. Neuropathology of prodromal Lewy body disease. Mov Disord 2014;29:410-415.

27.Uchiyama M, Isse K, Tanaka K, et al. Incidental Lewy body disease in a patient with REM sleep behavior disorder. Neurology 1995;45:709-712.

28.Boeve BF, Dickson DW, Olson EJ, et al. Insights into REM sleep behavior disorder pathophysiology in brainstem-predominant Lewy body disease. Sleep Med 2007;8:60-64.

29. Diaz-Galvan P, Przybelski SA, Lesnick TG, et al. β-amyloid load on PET along the continuum of dementia with Lewy bodies. Neurology 2023;101:e178-e188.

30. Forrest SL, Kovacs GG. Current concepts of mixed pathologies in neurodegenerative diseases. Can J Neurol Sci 2023;50:329-345.

31. Colom-Cadena M, Grau-Rivera O, Planellas L, et al. Regional overlap of pathologies in Lewy body disorders. Neuropathol Exp Neurol 2017;76:216-224.

32. Sierra M, Gelpi E, Martí MJ, Compta Y. Lewy and Alzheimer-type pathologies in midbrain and cerebellum across the Lewy body disorders spectrum. Neuropathol Appl Neurobiol 2016;42:451-462.

33. Shen Y, Yu WB, Shen B, et al. Propagated α-synucleinopathy recapitulates REM sleep behaviour disorder followed by parkinsonian phenotypes in mice. Brain 2020;143:3374-3392.

34. Muñoz-Lopetegi A, Baiardi S, Balasa M, et al. Cerebrospinal fluid biomarker profile of neurodegeneration in patients with isolated REM sleep behavior disorder. NPJ Parkinsons Dis 2024;10:157.

35.Boeve BF, Silber MH, Ferman TJ, et al. Clinicopathologic correlations in 172 cases of rapid eye movement sleep behavior disorder with or without a coexisting neurologic disorder. Sleep Med 2013:14:754-762.