npj | parkinson's disease

Article

Published in partnership with the Parkinson's Foundation

https://doi.org/10.1038/s41531-024-00770-7

CSF markers of neurodegeneration Alzheimer's and Lewy body pathology in isolated REM sleep behavior disorder

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We investigated the biomarker profile of neurodegeneration, Alzheimer's and Lewy body pathology in the CSF of 148 polysomnography-confirmed patients with isolated REM sleep behavior disorder (IRBD), a condition that precedes Parkinson's disease (PD) and dementia with Lewy bodies (DLB). We assessed misfolded α-synuclein (AS) by RT-QuIC assay, amyloid-beta peptides ($A\beta_{42}$ and $A\beta_{40}$), phosphorylated tau (p-tau), and total tau (t-tau) by CLEIA and neurofilament light chain (NfL) by ELISA. We detected AS in 75.3% of patients, pathologically decreased $A\beta_{42}/A\beta_{40}$ ratio in 22.5%, increased p-tau in 15.5%, increased t-tau in 14.9%, and elevated NfL in 14.7%. After a mean follow-up of 2.48 ± 2.75 years, 47 (38.1%) patients developed PD ($n = 24$) or DLB ($n = 23$). At CSF collection, AS positivity [HR 4.05 (1.26–12.99), $p = 0.019$], mild cognitive impairment [3.86 (1.96–7.61), $p < 0.001$], and abnormal DAT-SPECT [2.31 (1.09–4.91), $p < 0.030$] were independent predictors of conversion to PD and DLB. Among the other CSF markers, only elevated p-tau/$A\beta_{42}$ was predictive of conversion, although only to DLB and not as an independent variable. In IRBD, CSF AS assessment by RT-QuIC provides an added value in defining the risk of short-term conversion to PD and DLB independent of clinical and instrumental investigations. Positive Alzheimer's disease (AD) pathology markers and elevated NfL occur in a subgroup of patients, but p-tau/$A\beta_{42}$ is the only marker that predicts short-term conversion to DLB. Longer follow-up is needed to assess if AD biomarkers predict the later development of PD and DLB in IRBD.

The synucleinopathies Parkinson's disease (PD), dementia with Lewy bodies (DB) and multiple system atrophy (MSA) are neurodegenerative disorders characterized by misfolded α-synuclein (AS) accumulation in the nervous system[1]. The severity and extent of AS deposits in vulnerable regions are thought to be responsible for the core clinical manifestations of these diseases, namely parkinsonism and dementia[1]. The comorbid Alzheimer's disease (AD) pathology, documented by postmortem studies in DLB and, to a lesser extent, in PD, is also thought to contribute to the development of cognitive decline[2,3].

There is strong evidence indicating that isolated REM sleep behavior disorder (IRBD) is a prodromal clinical condition often progressing to the synucleinopathies PD and DLB and, less frequently, MSA[4–6]. Thus, IRBD patients constitute a candidate population to enter in neuroprotective trials addressing the early disease stages of synucleinopathies[7].

Neuropathological studies in subjects with IRBD revealed a high burden of AS deposits in the brainstem[8–11]. We have observed that in IRBD patients who develop PD with associated dementia (PDD) and DLB, the neuropathological substrate consists of widespread AS, but comorbid AD changes are also frequently found[12].

The study of cerebrospinal fluid (CSF) in living individuals allows the evaluation of biomarkers specific to synucleinopathies, AD, and neuronal degeneration. In the CSF of most PD and DLB patients, seed amplification assays (SAAs), such as real-time quaking-induced conversion (RT-QuIC),

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Table 1 | Demographic, clinical, and CSF biomarkers data of the patients with IRBD

	All patients	Positive α-synuclein	Negative α-synuclein	*P* value
N (%)	148[a]	110 (75.3)	36 (24.7)	–
Female, *n* (%)	35 (23.6)	27 (24.5)	8 (22.2)	>0.99
Age at LP, yrs	69.1 ± 9.7	70.9 ± 6.5	65.4 ± 10.1	**0.0002**
Age at IRBD diagnosis, yrs	66.7 ± 7.6	67.8 ± 6.5	63.4 ± 9.7	**0.0021**
Time from IRBD diagnosis to LP, yrs	1.7 (0.5–4.2)	2.1 (0.5–4.5)	0.8 (0.4–3.4)	0.126
Prodromal PD rate at LP, %	82.1 ± 28.3	89.0 ± 22.9	59.4 ± 32.3	**<0.0001**
DAT-SPECT abnormal/tested, *n* (%)	84/145 (57.9)	73/108 (67.6)	9/35 (25.7)	**<0.0001**
UPSIT-40 score	19.0 (14.0–25.0)	17.0 (13.0–22.0)	27.0 (22.0–30.0)	**<0.0001**
Mild cognitive impairment at LP, *n* (%)	33 (22.3)	26 (23.6)	6 (16.7)	0.488
A + T + N + , *n* (%)	13 (9.3)	10 (9.4)	3 (8.8)	>0.99
A + T + , *n* (%)	16 (11.4)	12 (11.3)	4 (11.8)	>0.99
A + T-, *n* (%)	16 (11.4)	13 (12.3)	3 (8.8)	0.761
$A\beta_{42,}$ pg/mL	570 (405–808)	573 (403–821)	574 (415–822)	0.761
$A\beta_{42}/A\beta_{40}$	0.8 (0.6–0.9)	0.8 (0.6–0.9)	0.8 (0.6–0.9)	0.653
p-tau, pg/mL	38 (27–49)	39 (28–50)	35 (25–44)	0.271
p-tau/$A\beta_{42}$	0.6 (0.4–0.9)	0.6 (0.4–0.9)	0.6 (0.4–0.8)	0.473
t-tau, pg/mL	280 (217–353)	290 (219–380)	257 (212–335)	0.308
NfL, pg/mL	550 (405–761)	620 (459–802)	485 (344–665)	**0.0045**

Data are expressed as mean ± SD, median (interquartile range), number, and percentage, as appropriate. Aβ positivity (A+) was defined by CSF $A\beta_{42}/A\beta4_{40}$ ratio < 0.65; tau positivity (T+) by CSF p-tau181 > 60 pg/ml; and neurodegeneration positivity (N+) by CSF t-tau > 450 pg/ml.

p values indicating statistically significant differences between groups are highlighted in bold.

LP lumbar puncture, *IRBD* isolated REM sleep behavior disorder, *PD* Parkinson disease, *Aβ42* 42 amino acid isoform of amyloid-beta, *Aβ40* 40 amino acid isoform of amyloid-beta, *p-tau* phosphorylated tau protein, *A* amyloid-beta, *T* tau, *N* neurodegeneration, *NfL* neurofilament light chain, *t-tau* total tau protein.

[a]α-synuclein status was available in 146 patients while Alzheimer's disease core biomarkers and NfL values were available in 142 individuals.

detect AS[13]. The core CSF biomarker profile of AD includes decreased levels of $A\beta_{42,}$ the 42-amino acid isoform of amyloid-beta and of its relative ratio with $A\beta_{40,}$ the 40-amino acid isoform, and increased hyperphosphorylated tau (p-tau) and total tau (t-tau) concentrations, reflecting the presence of amyloid plaques, tau-related AD pathology and neuronal degeneration in the brain[14]. This CSF AD profile can also be detected with increasing prevalence in PD, PDD, and DLB patients[15–19]. In the CSF and blood, increased neurofilament light chain (NfL) levels are indicative of myelinated axonal injury and are considered predictors of cognitive decline and disease progression in several neurodegenerative conditions including PD[18].

SAAs detect AS in the CSF of 75–90% of the IRBD patients, a finding supporting the notion that this parasomnia represents the prodromal stage of the synucleinopathies[20–23]. In IRBD, there is limited data on AD biomarkers and NfL levels; abnormalities in these markers could have prognostic implications[24,25]. Our study aimed to assess the prevalence and clinical significance of the CSF biomarker profile of neurodegeneration in living IRBD patients.

Results

We retrospectively evaluated 148 IRBD patients, 113 (76.4%) men, who underwent a lumbar puncture at the mean age of 69.1 ± 9.7 years. Demographic, clinical and biomarker data are reported in Tables 1–3. There was a significant association between age at lumbar puncture with AS positivity, increased levels of NfL ($r = 0.396$, $p < 0.0001$), t-tau ($r = 0.280$, $p = 0.0007$), and p-tau ($r = 0.344$, $p < 0.0001$), and reduced $A\beta_{42}/A\beta_{40}$ ratio ($r = -0.207$, $p = 0.014$).

We detected AS positivity in 75.3% of the patients, decreased $A\beta_{42}/A\beta_{40}$ ratio (A+) in 22.5%, increased p-tau levels (T+) in 15.5%, high t-tau levels (N+) in 14.9% and increased NfL in 14.7%. The profile A+T− was found in 11.4% of the patients, A+T+ in 11.4%, and A+T+N+ in 9.3% (Table 1 and Fig. 1). One hundred four (73.2%) IRBD patients were A-T-.

When compared with patients with AS negativity, those with AS positivity were older, showed reduced DAT-SPECT uptake and hyposmia more frequently, greater likelihood of prodromal PD, and increased levels of NfL (Table 1). There were no significant differences in the percentage of patients with the A+T+ or A+T− profile between the AS positive and AS negative groups (Table 1). Seven patients with AS negativity had positive AD biomarkers in the CSF (four were A+T+, and three were A+T−).

The AD biomarker status A+T+ was positively associated with age, female sex, and NfL levels (Table 2).

NfL levels were associated with those of t-tau ($r = 0.492$, $p < 0.001$) and showed higher values in patients with positive AD biomarkers (A+T+) compared to patients with negative AD biomarkers (A−T−) and, to a lesser extent, in those with AS positivity compared to those with AS negativity (Table 3). However, these differences were no longer statistically significant after correcting for age.

Two IRBD patients had very high NfL levels above the range usually found in PD[18]. One patient with a very high NfL level (3900 pg/mL) was AS positive and had low $A\beta_{42}/A\beta_{40}$ and a normal p-tau level. The other patient (NfL of 1484 pg/mL) had AS negativity, and the AD biomarkers were within the normal ranges.

After a mean follow-up of 2.48 ± 2.75 (range, 0.6–12.7) years from the time of lumbar puncture and 5.3 years from IRBD diagnosis, 47 (31.8%) patients developed PD ($n = 24$) or DLB ($n = 23$).

At the time of the lumbar puncture, 33 (22.3%) IRBD patients had coexistent mild cognitive impairment. Of these 33 patients, six converted to PD (out of 24 total PD converters), 15 converted to DLB (out of 23 total DLB converters), and 12 remained disease-free at the end of the current study (out of 100 total non-converters) (Table 4).

There was a trend toward a higher prevalence of A+ status in patients who converted to DLB than in those converting to PD and in those who remained disease-free ($p = 0.055$). However, the CSF AD profile A+T+ distribution did not differ significantly among the above-mentioned groups (Table 4). As compared with non-converters, patients who converted to DLB were older and had a higher mean prodromal PD rate, a lower UPSIT-40 score, and a greater

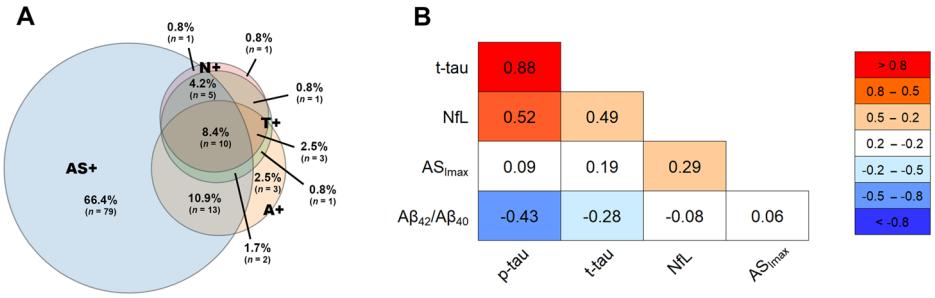

Fig. 1 | Overlap and correlations between abnormal biomarker results in IRBD. Schematic representation of concomitant pathological profiles (**A**) and correlations among biomarkers (**B**). Biomarker positivity was defined as follows: A+ = Aβ positivity ($Aβ_{42}/Aβ_{40}$ < 0.65), T+ = tau positivity (p-tau181 > 60 pg/ml), N+ = neurodegeneration positivity (t-tau > 450 pg/ml), AS+ = misfolded α-synuclein positivity by SAA. For correlations, t-tau, p-tau, and NfL values were log-transformed. Pearson's *r* values are shown. AS misfolded α-synuclein, AS_{Imax} the maximum peak of SAA fluorescence curve, $Aβ_{42}$ 42 amino acid isoform of amyloid-beta, $Aβ_{40}$ 40 amino acid isoform of amyloid-beta, p-tau tau protein phosphorylated at residue 181, NfL neurofilament light chain, t-tau total tau protein, SAA seed amplification assay.

Table 2 | IRBD cohort stratified according to AD core biomarker results tested in 142 patients

	Normal Aβ- (A-)	Abnormal Aβ (A+)	*P* value	Abnormal Aβ and p-tau (A+/T+)	*P* value
N (%)	110 (77.5)	32 (22.5)	–	16 (11.3)	–
Female, *n* (%)	23 (20.9)	12 (37.5)	0.055	8 (50.0)	**0.025**
Age at LP, yrs	68.5 ± 8.2	72.9 ± 6.1	**0.0046**	73.9 ± 6.2	**0.0120**
Age at IRBD diagnosis, yrs	65.5 ± 7.5	70.8 ± 6.8	**0.0006**	72.3 ± 7.0	**0.0009**
Time IRBD diagnosis to LP, yrs	1.5 (0.5–2.3)	1.7 (0.4–3.5)	0.391	0.6 (0.3–1.9)	0.080
Prodromal PD rate at LP, %	80.6 ± 29.9	91.2 ± 15.9	0.254	89.7 ± 15.0	0.969
Abnormal DAT-SPECT, *n* (%)[a]	62 (57.9)	20 (62.5)	0.646	10 (62.5)	0.792
UPSIT-40 score	19.0 (14.0–26.0)	16.5 (14.0–21.0)	0.128	17.0 (14.5–22.0)	0.327
Mild cognitive impairment at LP, *n* (%)	21 (19.1)	11 (34.4)	0.069	3 (18.8)	0.974
AS positivity, *n* (%)[a]	81 (75.0)	25 (78.1)	0.817	12 (75.0)	>0.99
$Aβ_{42}$, pg/mL	638 (474–899)	387 (318–449)	**<0.0001**	409 (332–541)	**0.0001**
$Aβ_{40}/Aβ_{42}$	0.9 (0.8–0.9)	0.5 (0.4–0.5)	**<0.0001**	0.4 (0.4–0.5)	**<0.0001**
p-tau, pg/mL	35 (26–44)	57 (46–85)	**<0.0001**	85 (71–100)	**<0.0001**
p-tau/$Aβ_{42}$	0.55 (0.4–0.7)	1.5 (1.2–2.3)	**<0.0001**	2.1 (1.4–2.7)	**<0.0001**
t-tau, pg/mL	254 (201–331)	365 (298–559)	**<0.0001**	545 (453–545)	**<0.0001**
NfL, pg/mL	524 (393–743)	664 (501–784)	0.096	749 (667–879)	**0.019**

Data are expressed as mean ± SD, median (interquartile range), number, and percentage, as appropriate. Aβ positivity (A+) was defined by CSF $Aβ_{42}/Aβ_{40}$ ratio < 0.65; and tau positivity (T+) by CSF p-tau181 > 60 pg/ml. For group comparisons, the normal Aβ (A-) group was used as reference.
p values indicating statistically significant differences between groups are highlighted in bold.
LP lumbar puncture, *IRBD* isolated REM sleep behavior disorder, *PD* Parkinson disease, *AS* α-synuclein, *$Aβ_{42}$* 42 amino acid isoform of amyloid-beta, *$Aβ_{40}$* 40 amino acid isoform of amyloid-beta, *p-tau* phosphorylated tau protein, *NfL* neurofilament light chain, *t-tau* total tau protein.
[a]DAT imaging and α-synuclein status in the normal Aβ group were available in 107 and 108 cases, respectively.

prevalence of mild cognitive impairment status and AS positivity. Patients who converted to PD had a higher mean prodromal PD rate and abnormal DAT-SPECT uptake than non-converters and also a lower prevalence of mild cognitive impairment than those who converted to DLB (Table 4).

On univariable Cox regression, the variables significantly associated with a greater likelihood of developing DLB or PD (i.e., either one of the two) at the time of lumbar puncture were older age, the presence of mild cognitive impairment, abnormal DAT-SPECT, high prodromal PD rate, and AS positivity (Table 5). Besides the above-mentioned variables, increased p-tau/$Aβ_{42}$ ratio and abnormal UPSIT-40 score (but not DAT-SPECT) predicted phenoconversion in the analysis considering only the conversion to DLB (Table 6). Differently, only abnormal DAT-SPECT and a high prodromal PD rate predicted phenoconversion in the analysis limited to PD converters (Table 7).

Multivariable analyses confirmed AS positivity, mild cognitive impairment, and reduced DAT-SPECT uptake as independent predictors of conversion to DLB or PD (i.e., both conversions considered) (Table 5). Even after excluding the patients with mild cognitive impairment, AS positivity remained an independent predictor of conversion (HR 4.33, 95% CI 1.02–18.42, $p = 0.047$). Finally, mild cognitive impairment remained the only independent predictor of conversion in the multivariable analysis limited to DLB converters (Table 6), whereas there was no independent predictor in the multivariable analysis limited to PD converters (Table 7).

Kaplan-Meier curve analysis assessing the effect of time from the lumbar puncture to conversion in IRBD patients stratified for the AS status demonstrated a significant rise ($p = 0.004$) in the slopes of conversion over time in the AS positive group (41/45, 91.1% AS positive converters with a median time to conversion of five years; 4/45, 8.9%, AS negative converters with a median time to conversion of nine years) independent of AD status

Table 3 | IRBD cohort stratified according to NfL values tested in 142 patients

	NfL low	NfL intermediate	NfL high	*P* value
N (%)	47 (33.1)	48 (33.8)	48 (33.1)	–
Female, *n* (%)	16 (34.0)	8 (16.7)	10 (20.8)	0.116
Age at LP, years	65.7 ± 9.0[a]	69.7 ± 5.9	72.8 ± 7.3	**<0.0001**
Age at IRBD diagnosis, yrs	63.2 ± 8.1[b]	66.2 ± 6.1[c]	70.3 ± 6.9	**<0.0001**
Prodromal PD rate at LP, %	75.2 ± 30.0[c]	77.4 ± 33.0	91.5 ± 17.4	**0.0076**
DAT-SPECT tested/abnormal, *n* (%)	23/44 (52.3)	24/47 (51.1)	32/48 (66.7)	0.234
UPSIT-40 score	22 (16.0–27.5)	19 (13.8–24.5)	17.5 (14.0–23.0)	0.182
Converted during follow-up, *n* (%)	12 (25.5)	17 (35.4)	15 (31.3)	0.578
Converted to DLB/PD (%)	4/8 (33.3/66.7)	10/7 (58.8/41.2)	8/7 (53.3/46.7)	0.381
Time LP to conversion, yrs	1.4 (0.5–4.1)	2.3 (0.6–5.3)	1.3 (0.3–3.7)	0.143
Mild cognitive impairment, *n* (%)	10 (21.3)	9 (18.8)	13 (27.1)	0.604
AS positivity, *n* (%)	29 (63.0)	37 (77.1)	40 (83.3)	0.069
A+T+, *n* (%)	0 (0.0)[a]	5 (10.9)	11 (24.4)	**0.0008**
A+T-, *n* (%)	6 (13.0)	7 (15.2)	3 (6.7)	0.420
$A\beta_{42}$, pg/mL	544 (381–703)	604 (429–812)	733 (429–921)	0.156
$A\beta_{40}/A\beta_{42}$	0.8 (0.7–0.9)	0.8 (0.6–0.9)	0.8 (0.5–0.9)	0.627
p-tau, pg/mL	31 (25–39)[b,c]	44 (30–50)	46 (35–82)	**<0.0001**
p-tau/$A\beta_{42}$	0.6 (0.4–0.7)	0.6 (0.4–0.9)	0.7 (0.4–1.2)	0.145
t-tau, pg/mL	238 (191–290)[b,d]	305 (212–368)	341 (249–550)	**<0.0001**

Data are expressed as mean ± SD, median (interquartile range), number, and percentage, as appropriate. NfL tertiles are defined as follow: low = 129–465 pg/ml; intermediate = 466–677 pg/ml; high = 678–1469 pg/ml. Aβ positivity (A+) was defined by CSF $A\beta_{42}/A\beta_{40}$ ratio < 0.65; and tau positivity (T+) by CSF p-tau181 > 60 pg/ml.
p values indicating statistically significant differences between groups are highlighted in bold.
LP lumbar puncture, *IRBD* isolated REM sleep behavior disorder, *PD* Parkinson disease, *DLB* dementia with Lewy bodies, *AS* α-synuclein; *$A\beta_{42}$* 42 amino acid isoform of amyloid-beta; *$A\beta_{40}$* 40 amino acid isoform of amyloid-beta, *p-tau* phosphorylated tau protein, *A* amyloid-beta, *T* tau, *NfL* neurofilament light chain, *t-tau* total tau protein.
[a]vs. high $p < 0.001$.
[b]vs. high $p < 0.0001$.
[c]vs. high $p < 0.01$.
[d] vs. intermediate $p < 0.05$.

(Fig. 2). Specifically, the analysis showed that after five years of follow-up from lumbar puncture, the estimated rate of phenoconversion to PD and DLB was 50% in the AS+ patients and 15% in the AS- patients.

Kaplan-Meier curves showed that patients with abnormal $A\beta_{42}/A\beta_{40}$ ratio ($p = 0.207$), p-tau ($p = 0.373$), t-total ($p = 0.283$) and NfL levels ($p = 0.179$) had no short-term increased risk of developing PD and DLB (Fig. 3).

Discussion

In the present study, we confirmed and expanded previous findings on CSF biomarkers in IRBD and their predictive value on the short-term conversion to PD or DLB. We extended the AS RT-QuIC analysis to a larger cohort and added the evaluation of AD core markers and NfL in the CSF. Moreover, we studied the predictive values of biomarkers and associated demographic and clinical findings to estimate the risk of conversion using the whole cohort and a longer longitudinal observation compared to a previous study[20].

The AS RT-QuIC results confirmed that our assay detects the misfolded protein in about 75% of patients with IRBD and that compared to the AS negative group, patients with AS positivity were older and showed a higher likelihood of prodromal PD (e.g., hyposmia and abnormal DAT-SPECT). Questions arise regarding the nature of the IRBD patients who are AS negative and about the fact that the percentage of AS negativity between different studies varied significantly[20–22]. In a recent neuropathological case series, we demonstrated a clear association between RT-QuIC sensitivity in the CSF and the stage and burden of Lewy body pathology in the brain[26]. In patients with neocortical or limbic Lewy body stages, the sensitivity to detect AS in the CSF was 100% but was significantly lower in patients at the brainstem stage (60.8%). According to these findings, the AS negative IRBD patients may have a lower burden of synuclein pathology in the brain, probably confined to the lower brainstem structures that modulate muscle atonia in REM sleep. Alternatively, it is also plausible that some IRBD patients had a different neurobiological pathogenesis unrelated to Lewy body pathology, particularly those with AS negativity and positive AD markers. Clinical follow-up and longitudinal CSF assessments of AS may elucidate the underlying etiology in these IRBD patients.

Although the neuropathological hallmark of PD and DLB is the presence of intraneuronal Lewy bodies and neurites, where abnormal AS aggregates are the main component[1], autopsy studies of PD, PDD and DLB patients have shown coexisting AD pathology in 20–70% of the patients, particularly in those who had cognitive impairment[2,3,27]. Moreover, approximately 5% of patients with PD, 10% with PDD and 25–40% of those with DLB show the core CSF AD profile[16–18]. In PD and DLB, reduced CSF $A\beta_{42}$ levels predict cognitive impairment and a more aggressive disease course and correlate with postmortem β-amyloid plaques in the brain[16,17,19]. In our study, the proportions of IRBD patients with reduced $A\beta_{42}$ levels, increased p-tau levels, and increased t-tau levels were similar to those described in PD patients of similar age and lower than those seen in DLB patients[16]. Altogether, this observation combined with the equal rate of conversion to PD and DLB in our cohort indicate that IRBD is a prodromal feature of the full spectrum of Lewy body disease and that the relatively high conversion rate to DLB in these patients seems to be largely independent of the association with AD pathology.

NfL proteins are cytoskeletal proteins expressed in neurons. In neurodegenerative diseases such as amyotrophic lateral sclerosis, increased NfL levels in both CSF and plasma are the result of nonspecific axonal damage[28]. In PD patients, NfL levels are similar to those of healthy controls but may predict motor and cognitive decline in those patients with increased levels[25]. CSF NfL levels are elevated in DLB and MSA patients and in individuals with

Table 4 | Baseline characteristics of IRBD patients stratified according to clinical follow-up in non-converters, PD converters, and DLB converters

	Non-converters	Converted to PD	Converted to DLB	P value
N	100	24	23	
Female, *n* (%)	22 (22.0)	7 (29.2)	6 (26.1)	0.731
Age at LP, yrs	67.7 ± 10.6	71.7 ± 6.1	73.5 ± 5.8	**0.0104**[a]
Age at IRBD diagnosis, yrs	65.3 ± 10.1	68.6 ± 5.5	68.9 ± 7.2	0.126
Time from IRBD diagnosis to LP, yrs	1.4 (0.4–3.6)	1.9 (0.5–4.5)	4.3 (0.5–6.5)	**0.0218**[a]
Prodromal PD rate at LP, %	76.2 ± 31.5	92.7 ± 16.6	95.7 ± 10.5	**0.0005**[b,c]
DAT-SPECT abnormal/tested, *n* (%)	48/97 (49.5)	20/24 (83.3)	15/23 (65.2)	**0.0079**[c]
UPSIT-40 score	21.0 (15.5–27.0)	19.0 (14.5–22.5)	15.0 (12.3–17.8)	**0.0139**[a]
Mild cognitive impairment at LP, *n* (%)	12 (12.0)	6 (25.0)	15 (65.2)	**<0.0001**[d,e]
AS positivity, *n* (%)	68 (68.0)	20 (86.9)	21 (95.5)	**0.0095**[b]
A+T+N+, *n* (%)	8 (8.4)	2 (8.7)	3 (13.0)	0.786
A+T+, *n* (%)	10 (10.6)	3 (13.0)	3 (13.0)	0.907
A+T-, *n* (%)	18 (18.9)	6 (26.1)	8 (34.6)	0.224
$A\beta_{42}/A\beta_{40}$	0.8 (0.7–0.9)	0.8 (0.7–0.9)	0.7 (0.5–0.8)	0.055
p-tau, pg/mL	37 (28–47)	35 (26–50)	41 (34–51)	0.212
p-tau/$A\beta_{42}$	0.6 (0.4–0.9)	0.6 (0.6–0.9)	0.8 (0.6–1.5)	**0.0008**[e]
t-tau, pg/mL	292 (219–376)	236 (182–329)	285 (247–363)	0.112
NfL, pg/mL	542 (412–751)	540 (339–751)	606 (507–794)	0.489

Follow-up information was available in 147 patients, CSF AD core biomarkers in 95 non-converters, 23 PD converters, and 23 DLB converters; CSF NfL in 98 non-converters, 22 PD converters, and 22 DLB converters.

p values indicating statistically significant differences between groups are highlighted in bold.

LP lumbar puncture, *IRBD* isolated REM sleep behavior disorder, *PD* Parkinson disease, *DLB* dementia with Lewy bodies, *AS* misfolded α-synuclein, $A\beta_{42}$ 42 amino acid isoform of amyloid-beta, $A\beta_{40}$ amino acid isoform of amyloid-beta, *p-tau* phosphorylated tau protein, *A* amyloid-beta, *T* tau, *NfL* neurofilament light chain, *t-tau* total tau protein.

[a]DLB converters vs non-converters, $p < 0.05$.

[b]DLB converters vs non-converters, $p < 0.01$.

[c]PD converters vs non-converters, $p < 0.01$.

[d]DLB converters vs non-converters, $p < 0.0001$.

[e]DLB converters vs PD converters, $p < 0.001$.

Table 5 | Univariable and multivariable Cox regression analyses for prognostic factors of conversion to combined PD and DLB

Univariable analysis		**HR (95% CI)**	***P* value**
Age at LP		1.05 (1.00–1.10)	**0.030**
Female sex		1.60 (0.83–3.07)	0.158
Mild cognitive impairment at LP		2.81 (1.58–5.02)	**<0.001**
Prodromal PD rate at LP		1.03 (1.01–1.06)	**0.001**
Abnormal DAT-SPECT		2.66 (1.37–5.16)	**0.004**
UPSIT-40 score		0.94 (0.88–1.00)	0.053
AS positivity		4.51 (1.60–12.7)	**0.004**
t-tau value		0.99 (0.99–1.00)	0.283
p-tau/$A\beta_{42}$ ratio		1.17 (0.85–1.61)	0.339
A+		1.55 (0.83–2.92)	0.169
A + T+		0.99 (0.42–2.37)	0.999
NfL	Low tertile	Ref.	Ref.
	Intermediate tertile	1.85 (0.86–3.99)	0.118
	High tertile	1.92 (0.89–4.13)	0.098
Multivariable analysis[a]		**HR (95% CI)**	***P* value**
Age		1.03 (0.98–1.09)	0.251
Mild cognitive impairment at LP		3.86 (1.96–7.61)	**<0.001**
Abnormal DAT-SPECT		2.31 (1.09–4.91)	**0.030**
AS positivity		4.05 (1.26–12.99)	**0.019**

p values indicating statistically significant differences between groups are highlighted in bold.

Ref. reference group, *LP* lumbar puncture, *PD* Parkinson disease, *AS* α-synuclein, $A\beta_{42}$ 42 amino acid isoform of amyloid-beta, *p-tau* phosphorylated tau protein, *A* amyloid-beta, *T* tau, *NfL* neurofilament light chain, *t-tau* total tau protein.

[a]In the multivariable analysis, the prodromal PD rate was not included since it accounts for other variables (e.g., age, reduced DAT-SPECT uptake) already included in the model.

Table 6 | Univariable and multivariable Cox regression analysis for prognostic factors of short-term conversion to DLB

Univariate analysis variable (s)	HR (95% CI)	*P* value
Age at LP	1.08 (1.01–1.16)	**0.024**
Female sex	1.49 (0.57–3.87)	0.412
Mild cognitive impairment at LP	6.52 (2.75–15.44)	**<0.001**
Prodromal PD rate at LP	1.05 (1.01–1.09)	**0.017**
Abnormal DAT-SPECT	1.78 (0.74–4.25)	0.197
UPSIT-40 score	0.89 (0.81–0.98)	**0.031**
AS positivity	9.18 (1.22–68.7)	**0.031**
t-tau value	1.00 (0.99–1.00)	0.774
p-tau/$A\beta_{42}$ ratio	1.46 (1.01–2.11)	**0.046**
A+	2.31 (0.99–5.36)	0.050
A+T+	1.02 (0.30–3.46)	0.975
Neurofilament light chain	1.00 (0.99–1.00)	0.187
Multivariate analysis variable (s)[a]	**HR (95% CI)**	***P* value**
Age	0.97 (0.89–1.07)	0.606
Mild cognitive impairment at LP	11.23 (3.06–41.21)	**<0.0001**
UPSIT-40 score	1.01 (0.89–1.15)	0.835
p-tau/$A\beta_{42}$ ratio	1.25 (0.67–2.37)	0.482
AS positivity	9.40 (0.88–100.65)	0.064

p values indicating statistically significant differences between groups are highlighted in bold.
LP lumbar puncture, *PD* Parkinson disease, *AS* α-synuclein, $A\beta_{42}$ 42 amino acid isoform of amyloid-beta, *p-tau* phosphorylated tau protein, *A* amyloid-beta; *T* tau; *t-tau* total tau protein.
[a]In the multivariable analysis, the prodromal PD rate was not included since it accounts for other variables (i.e., age, etc.) that are already included in the model.

Table 7 | Univariable Cox regression analysis for prognostic factors of short-term conversion to PD

Univariate analysis variable(s)	HR (95% CI)	*P* value
Age at LP	1.03 (0.96–1.09)	0.414
Female sex	1.70 (0.70–4.18)	0.243
Mild cognitive impairment at LP	1.16 (0.46–2.94)	0.749
Prodromal PD rate at LP	1.03 (1.00–1.06)	**0.034**
Abnormal DAT-SPECT	4.35 (1.48–12.75)	**0.007**
UPSIT-40 score	0.98 (0.90–1.08)	0.642
AS positivity	2.95 (0.87–10.02)	0.083
t-tau value	0.99 (0.99–1.00)	0.071
p-tau/$A\beta_{42}$ ratio	0.73 (0.35–1.55)	0.422
A+	0.97 (0.36–2.63)	0.959
A+T+	0.98 (0.29–3.32)	0.973
Neurofilament light chain	0.99 (0.99–1.00)	0.673

p values indicating statistically significant differences between groups are highlighted in bold.
LP lumbar puncture, *PD* Parkinson disease, *AS* α-synuclein, $A\beta_{42}$ 42 amino acid isoform of amyloid-beta, *p-tau* phosphorylated tau protein, *A* amyloid-beta, *T* tau, *t-tau* total tau protein.

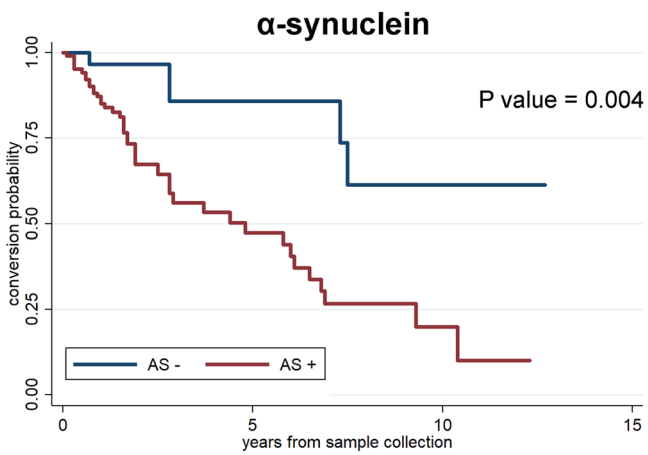

Fig. 2 | Predictive value of CSF AS from the time of lumbar puncture in the conversion from IRBD to PD or DLB. Survival curves in patients with IRBD according to the AS status (negative = blue; positive = red). CSF cerebrospinal fluid, IRBD isolated REM sleep behavior disorder, AS misfolded α-synuclein.

pure autonomic failure who eventually develop MSA[18,29]. We found that in IRBD the CSF NfL levels are associated with age, abnormal AD biomarkers and AS positivity. However, given the higher mean age of the patients with positive AD markers and AS positivity, the association between the neurodegenerative markers did not hold after correction for age due to the small number of IRBD patients that we found having AD pathology in the CSF.

At the end of our study, 47 (31.8%) patients developed PD or DLB after a mean follow-up of 2.5 years. At the time of lumbar puncture, the short-term risk of developing PD and DLB was associated with demographic and clinical variables and biomarker changes such as older age, a high prodromal PD score, mild cognitive impairment, abnormal DAT-SPECT, and CSF AS positivity. Abnormal CSF levels of $A\beta_{42}$, p-tau, t-tau, and NfL were not predictors of short-term conversion to PD or DLB. Nevertheless, an elevated p-tau/$A\beta_{42}$ ratio increased the risk of conversion to DLB. These findings suggest that AS is the pathologic substrate that mainly drives phenoconversion, but AD pathology may also contribute to precipitating the short-term onset of cognitive decline in a subset of IRBD patients. Our findings in patients converting to DLB are in line with previous literature showing that mild cognitive impairment, reduced cortical cholinergic activity demonstrated by PET imaging and slowness in the background EEG activity predict the development of dementia rather than parkinsonism, whereas abnormal DAT-SPECT, hyposmia and dysautonomia do not distinguish between dementia-first and parkinsonism first phenoconverters[6]. The mean patient follow-up in the present study was relatively short, of only 2.5 years. However, even when we only

considered the patients with a follow-up longer than 5 years, we did not see a significant change in the percentage of AS-positive patients (8/10, 80% vs 41/45, 91.1%), likely because the few ($n = 4$) AS-negative converters developed PD or DLB at variable times after the LP (0.7, 2.8, 7.3, and 7.5 years, respectively). Patients with IRBD may develop either PD, DLB, or MSA with time. The conversion to MSA is much less common than to PD and DLB, as it only accounts for about 5% of all IRBD cases that develop a synucleinopathy[6]. In our current study, none of our IRBD patients developed MSA. It can be speculated that our IRBD patients with very high NfL levels in the CSF represent prodromal MSA patients, as MSA advances faster and is a more aggressive condition than PD, PDD, and DLB[18,29]. Follow-up of our two IRBD patients with 3900 pg/mL and 1484 pg/mL NfL concentrations will elucidate whether they develop MSA.

Our study has some limitations. First, there was no postmortem confirmation of our findings. It should be noted that a major drawback of autopsy studies is that they involve patients with end-stage disease, whereas, in our study, we examined living subjects with IRBD who are in an earlier stage of the neurodegenerative process. Thus, postmortem and CSF antemortem assessments of the presence and severity of AS and AD pathologies may not be comparable. Second, we could not evaluate all the biomarkers in eight (5.7%) patients because the available CSF did not contain enough volume for all the measurements. Third, the study was retrospective, and the biomarkers were not assessed longitudinally to determine, for example, whether AS and AD negativities changed to positivity over time. Fourth, our mean follow-up was 2.5 years. It can be speculated that longer follow-up will show that baseline $A\beta_{42}$, p-tau, and t-tau concentrations may predict the late development of dementia. Finally, patients had different follow-up durations ranging from six months to 12 years between CSF collection and the time of the study.

Our study also has strengths. First, we evaluated a well-characterized IRBD cohort. Second, in all the instances, the diagnosis of IRBD was confirmed by video-polysomnography, which revealed increased electromyography activity in REM sleep linked to dream-enacting behaviors[30]. Third, patients underwent a comprehensive assessment that included DAT-SPECT and the UPSIT-40 smell test at the time of lumbar puncture. This allowed us to calculate the likelihood ratio of prodromal PD in each patient. Fourth, all the CSF measurements were performed blindly to the subject's condition.

In conclusion, our IRBD study showed that CSF contains AS in an important number of patients (75%), while AD biomarkers are found in a subset (15–22%). Although the main pathological driver in IRBD is AS pathology, AD pathology may likely contribute to the late development of cognitive decline.

As the IRBD represents the prodromal stage of PD and DLB, in vivo identification of CSF biomarkers could be useful for understanding the underlying neuropathological process during its earliest phase and for selecting patients for future neuroprotective clinical trials, not only to target AS but also to implement anti-amyloid and anti-tau therapies.

Methods

Participants

We assessed CSF biomarkers of neurodegeneration (AS, $A\beta_{42}$, $A\beta_{40}$, p-tau, t-tau, and NfL) in 148 IRBD patients diagnosed at the Hospital Clinic of Barcelona, Spain. The diagnosis of IRBD was made by the current diagnostic criteria, including video-polysomnography, in subjects without dementia or parkinsonism[30]. Some IRBD patients had coexistent mild cognitive impairment as they had: (1) complaints of subjective cognitive decline in at least one relevant cognitive domain (attention, memory, visual perception,

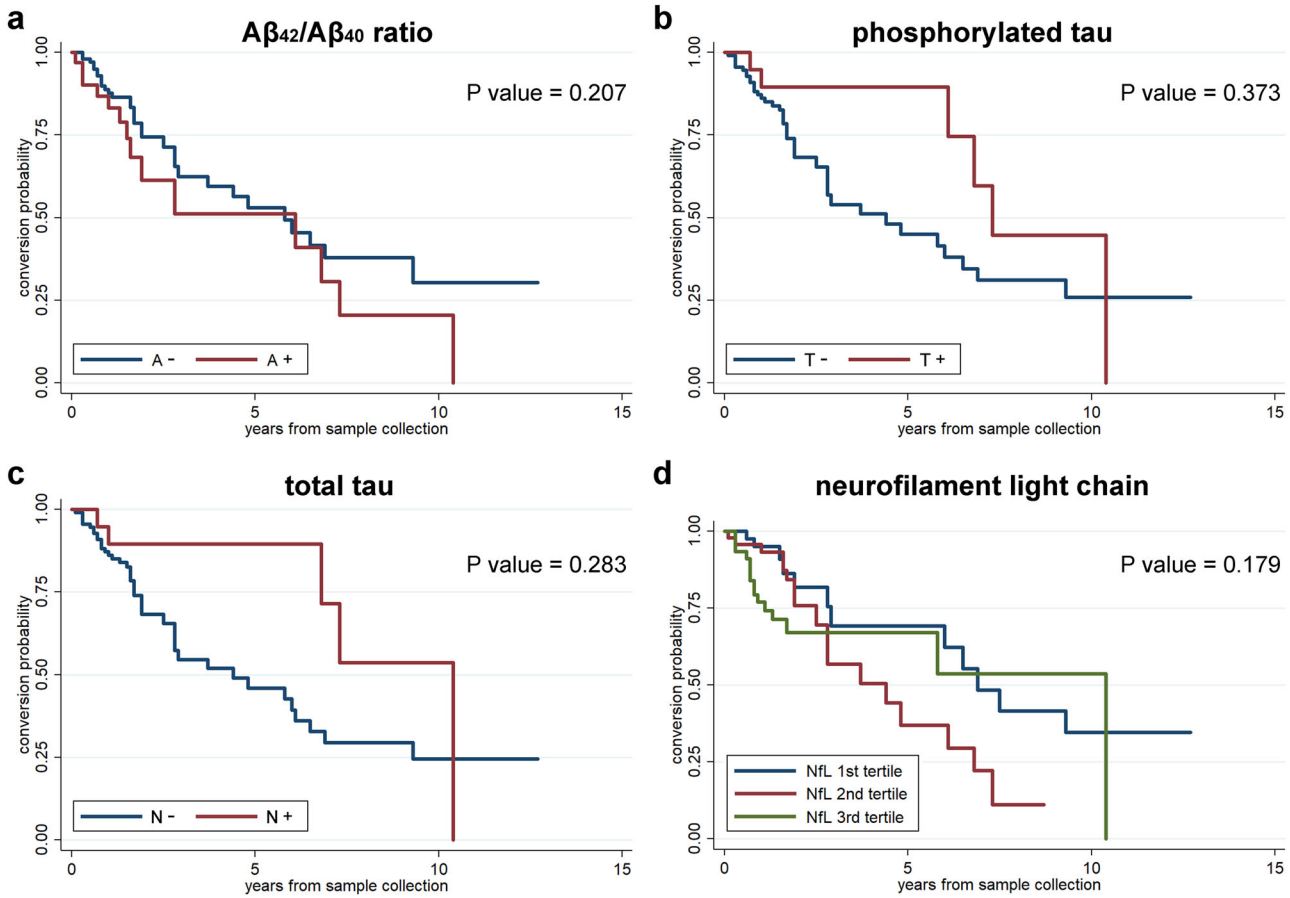

Fig. 3 | Predictive value of CSF AD core markers and NfL in the conversion from IRBD to PD or DLB. Survival curves in patients with IRBD according to the amyloid-beta (**a**), tau (**b**), and neurodegeneration (**c**) status (negative = blue; positive = red), and NfL value tertiles (**d**).

language, executive function, and praxis), (2) objective impairment in at least one test in one or more cognitive domains as evident by scores 1.5 standards below the appropriate norms adjusted for age and education, and 3) preserved activities of daily living.

Lumbar punctures were performed between March 2008 and March 2023. At the time of lumbar puncture, patients underwent demographic and clinical history, neurological examination, the UPSIT-40 smell test, and DAT-SPECT, which were used to calculate the likelihood ratio of prodromal PD in each patient[31]. At the time of this study, when the biomarkers were measured in the CSF (May 2023), we reviewed whether patients who underwent lumbar puncture were disease-free or if they were diagnosed with PD, DLB or MSA according to current clinical criteria[32–34]. The CSF of 40 individuals without RBD at video-polysomnography served as a blind element for the personnel at the Istituto delle Scienze Neurologiche di Bologna, Italy, who examined the presence of biomarkers in the samples from the IRBD patients.

CSF biomarker analyses

CSF was collected during a standardized lumbar puncture procedure. Storage and shipment from Barcelona, Spain, to Bologna, Italy, were performed as described previously[22]. All CSF samples were analyzed by researchers blinded to the individual clinical condition. As previously reported, the presence of AS seeding activity was analyzed by an in-house RT-QuIC assay and dichotomised as AS-positive (present) or AS-negative (absent)[13,22]. All other biomarkers were measured by commercially available kits. The levels of $A\beta_{42}$, $A\beta_{40}$, p-tau181, and t-tau were measured by an automated chemiluminescent enzyme immunoassay (CLEIA) on the Lumipulse G600II platform (Fujirebio, Gent, Belgium). The mean intra-assay and interassay CVs for these markers were <8%. The $A\beta_{42}/A\beta_{40}$ and p-tau/$A\beta_{42}$ ratios were also calculated., NfL was measured by a validated ELISA assay (NfL ELISA kit, IBL, Hamburg, Germany), as described previously[18]. The biomarker levels were analyzed either as continuous values or dichotomised as positive (abnormally increased or decreased) or negative (within the normal range) according to our in-house following cutoffs: abnormally positive when $A\beta_{42}/A\beta_{40}$ ratio was <0.65, p-tau was >60 pg/mL, t-tau was >450 pg/mL, and NfL was >550 pg/ml[19]. Patients were classified according to the A/T/N framework based on the combined $A\beta_{42}/A\beta_{40}$ ratio (A), p-tau (T), and t-tau (N) dichotomized results as abnormal (+) and normal (−).

Statistical analysis

Data are presented as mean (standard deviation), median (interquartile interval), or number (percentage), as appropriate. The normality of the distribution of continuous variables was assessed using the Kolmogorov–Smirnov test and Shapiro–Wilk test. The data were compared using the Student's *t*-test, the Mann–Whitney *U* test, the Kruskal-Wallis test (followed by the Dunn post hoc test), the X^2 test, and Fisher's exact test. We used NfL levels as the dependent variable in a multivariable general linear model to test the association with other biomarkers correcting for age. We used the Kaplan-Meier method to estimate the cumulative time-dependent probability of conversion to PD and DLB for the prognostic analysis. The time of entry into the analysis was the date of lumbar puncture, and the time of the endpoint was the date of phenoconversion to a neurodegenerative disease or the date of the last follow-up visit in the disease-free IRBD patients. We performed univariable and multivariable Cox regression models to study prognostic factors in the IRBD group of patients who developed either PD or DLB. The results are presented as hazard ratios (HRs) and 95% CI. The assumption of proportional hazard was assessed by Schoenfeld residuals. Differences were considered significant at a two-sided $p < 0.05$.

Analyses were performed with SPSS version 27.0 and Stata SE 14.2.

The ethical committees of HCB (HCB/2022/1089) and Area Vasta Emilia Centro (EM1173–2021–20226) approved this study. All participants provided written informed consent.

Data availability

The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

Received: 4 March 2024; Accepted: 26 July 2024;
Published online: 15 August 2024

References

1. Goedert, M., Jakes, R. & Spillantini, M. G. The synucleinopathies: twenty years on. *J. Parkinsons Dis.* **7**, S51–S69 (2017).
2. Aarsland, D. et al. Parkinson disease-associated cognitive impairment. *Nat. Rev. Dis. Prim.* **7**, 1–21 (2021).
3. Colom-Cadena, M. et al. Regional overlap of pathologies in Lewy body disorders. *Neuropathol. Exp. Neurol.* **76**, 216–224 (2017).
4. Jellinger, K. A. Multiple system atrophy. A clinicopathological update. *Free Neuropathol*. **1**, 1-17 (2020).
5. Iranzo, A., Santamaria, J. & Tolosa, E. Idiopathic rapid eye movement sleep behavior disorder: diagnosis, management, and the need for neuroprotective interventions. *Lancet Neurol.* **15**, 405–419 (2016).
6. Postuma, R. B. et al. Risk and predictors of dementia and parkinsonism in idiopathic REM sleep behavior disorder: a multicenter study. *Brain* **142**, 744–759 (2019).
7. Videnovic, A. et al. Clinical trials in REM sleep behavior disorder. Challenges and opportunities. *J. Neurol. Neurosurg. Psychiatry* **91**, 740–749 (2020).
8. Uchiyama, M. et al. Incidental Lewy body disease in a patient with REM sleep behavior disorder. *Neurology* **45**, 709–712 (1995).
9. Boeve, B. F. et al. Insights into REM sleep behavior disorder pathophysiology in brainstem-predominant Lewy body disease. *Sleep Med.* **8**, 60–64 (2007).
10. Gaig, C. et al. Pathologically description of a nonmotor variant of multiple system atrophy. *J. Neurol. Neurosurg. Psychiatry* **79**, 1399–1400 (2008).
11. Iranzo, A. et al. Neuropathology of prodromal Lewy body disease. *Mov. Disord.* **29**, 410–415 (2014).
12. Maya, G. et al. Neuropathological alterations in subjects initially diagnosed by polysomnography with isolated REM sleep behavior disorder. *J. Sleep. Res* **31**, 78 (2022).
13. Rossi, M. et al. Ultrasensitive RT-QuIC assay with high sensitivity and specificity for Lewy body-associated synucleinopathies. *Acta Neuropathol.* **140**, 49–62 (2020).
14. Blennow, K., Biscetti, L., Eusebi, P. & Parnetti, L. Cerebrospinal fluid markers in Alzheimer's disease and Parkinson's disease. From pathophysiology to clinical practice. *Mov. Disord.* **31**, 836–847 (2016).
15. Farotti, L., Paoletti, F. P., Simoni, S. & Parnetti, L. Unraveling pathophysiological mechanisms of Parkinson's disease: contribution of CSF biomarkers. *Biomark. Insights* **15**, 1–9 (2020).
16. van Steenoven, I. et al. Cerebrospinal fluid Alzheimer's disease biomarkers across the spectrum of Lewy bodies diseases: results from large multicenter cohort. *J. Alzheimers Dis.* **54**, 287–295 (2016).
17. Lemstra, A. W. et al. Concomitant AD pathology affects clinical manifestation and survival in dementia with Lewy bodies. *J. Neurol. Neurosurg. Psychiatry* **88**, 113–118 (2017).
18. Quadalti, C. et al. Neurofilament light chain and α-synuclein RT-QuIC as differential diagnostic biomarkers in parkinsonisms and related syndromes. *NPJ Parkinsons Dis.* **7**, 93 (2021).
19. Irwin, D. J. et al. Evolution of Alzheimer's disease cerebrospinal fluid biomarkers in early Parkinson's disease. *Ann. Neurol.* **88**, 574–587 (2020).
20. Iranzo, A. et al. Detection of α-synuclein in CSF by RT-QuIC in patients with isolated rapid-eye-movement sleep behavior disorder: a longitudinal observational study. *Lancet Neurol.* **20**, 203–212 (2021).
21. Poggiolini, I. et al. Diagnostic value of cerebrospinal fluid alpha-synuclein seed quantification in synucleinopathies. *Brain* **145**, 584–595 (2022).

22. Iranzo, A. et al. Misfolded α-Synuclein assessment in the skin and CSF by RT-QuIC in isolated REM sleep behavior disorder. *Neurology* **100**, e1944–e1954 (2023).
23. Concha-Marambio, L. et al. Accurate detection of α-Synuclein seeds in cerebrospinal fluid from isolated rapid eye movement sleep behavior disorder and patients with Parkinson's disease in the DeNovo Parkinson (DeNoPa) cohort. *Mov. Disord.* **38**, 567–578 (2023).
24. Fernandes, M. et al. Cerebrospinal-fluid biomarkers for predicting phenoconversion in patients with isolated rapid-eye movement sleep behavior disorder. *Sleep* **47**, zsad198 (2024).
25. Aamodt, W. W. et al. Neurofilament light chain as a biomarker for cognitive decline in Parkinson disease. *Mov. Disord.* **36**, 2945–2950 (2021).
26. Bentivenga, G. M. et al. Performance of a seed amplification assay for misfolded alpha-synuclein in cerebrospinal fluid and brain tissue in relation to Lewy body disease stage and pathology burden. *Acta Neuropathol.* **147**, 18 (2024).
27. Irwin, D. J. et al. Neuropathological and genetic correlates of survival and dementia onset in synucleinopathies: a retrospective analysis. *Lancet Neurol.* **16**, 55–65 (2017).
28. Bridel, C. et al. Diagnostic value of cerebrospinal fluid neurofilament light protein in neurology: a systematic review and meta-analysis. *JAMA Neurol.* **76**, 1035–1048 (2019).
29. Singer, W. et al. Alpha-synuclein oligomers and neurofilament light chain predict phenoconversion of pure autonomic failure. *Ann. Neurol.* **89**, 1212–1220 (2021).
30. American Academy of Sleep Medicine. *International Classification Of Sleep Disorders.* 3rd edn. test revision. (American Academy of Sleep Medicine, 2023).
31. Heinzel, S. et al. Update of the MDS research criteria for prodromal Parkinson's disease. *Mov. Disord.* **34**, 1464–1470 (2019).
32. Postuma, R. B. et al. MDS clinical diagnostic criteria for Parkinson's disease. *Mov. Disord.* **30**, 1591–1601 (2015).
33. McKeith, I. G. et al. Diagnosis and management of dementia with Lewy bodies: fourth consensus report of the DLB Consortium. *Neurology* **89**, 88–100 (2017).
34. Wenning, G. K. et al. The Movement Disorder Society Criteria for the diagnosis of multiple system atrophy. *Mov. Disord.* **37**, 1131–1148 (2022).

Acknowledgements

Gerard Mayà was funded by BBVA-Baselga grant. The study was supported by the grant #NextGenerationEU (NGEU), funded by the Italian Ministry of University and Research (MUR), National Recovery and Resilience Plan (NRRP), project MNESYS (PE0000006). The publication of this article was supported by the "Ricerca Corrente" funding from the Italian Ministry off Health.

Author contributions

A.I., A.M.L., M.B., P.P., and S.B. contributed to the conception and design of the study. A.I., A.M., A.M.L., A.T., C.G., C.Z., G.M., J.S., M.B., M.R., M.S., P.P., S.B., and S.D. contributed to the acquisition and analysis of the data. A.I., A.M.L., C.Z., M.B., P.P., and S.B. contributed to drafting the text and preparing the figures. All authors read and approved the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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