ADDITIONAL FILE 1

SUPPLEMENTARY MATERIALS

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Table S1. Summary of evaluations in the included cohorts.

	NPH Pro-Hydro	NPH Kuopio	CJD	DLB
Demographic	√	√	√	√
CSF α-syn RT-QuIC	√	√	√	√
CSF Aβ	√	√		
CSF NfL	√			
Association CSF biomarkers and clinical variables	√			
Outcome evaluation	√			
Neuropathology			√	

Legend=: NPH: normal pressure hydrocephalus; CJD, Creutzfeldt-Jakob disease; DLB, dementia with Lewy bodies; RT-QuIC, real-time quaking-induced conversion; α-syn, α-synuclein; Aβ, amyloid-beta; NfL, neurofilament light chain.

Table S2. Demographic data and summary of α-syn RT-QuIC results in the CJD cohort.

	CJD group
N	89
Female, n (%)	46 (51.7)
Age at lumbar puncture (years)	75.2±4.9
Definite/probable*, n (%)	60/29 (67.4/32.6)
Positive α-syn RT-QuIC, n (%)	
- Definite	4 (6.7)
- Probable	2 (6.9)

*Definite and probable CJD diagnoses were formulated according with the updated European diagnostic criteria (https://www.eurocjd.ed.ac.uk/node/833).

Legend= CJD: Creutzfeldt-Jakob disease; RT-QuIC: real-time quaking-induced conversion; α-syn: α-synuclein.

Table S3. Summary of demographic and clinical features and results of indicative biomarkers of DLB individuals.

	DLB group
N	45
Female, n (%)	17 (37.8)
Age at lumbar puncture (years)	73.7±5.9
Time between disease onset and lumbar puncture (years)	5.4±4.6
Clinical features, n (%)	
- Dementia (*essential feature*)	45 (100)
- Visual hallucinations	31 (68.9)
- Fluctuations	17 (37.8)
- Parkinsonism	40 (88.9)
- REM sleep behavior disorder	20 (44.4)
Indicative biomarkers, positive/tested (%)	
- SPECT DaTscan	20/22 (90.9)
- ^{123}I-MIBG myocardial uptake	13/13 (100)
- REM sleep without atonia at polysomnography	7/14 (50.0)

Legend= DLB: dementia with Lewy bodies; MIBG: metaiodobenzylguanidine.

Table S4. Baseline clinical features of the Bologna PRO-HYDRO cohort and comparisons between α-syn[LB] + and α-syn[LB] -.

	Bologna iNPH cohort	α-syn[LB] +	α-syn[LB] -	*p-value*
N	127	28	99	
Males, *n (%)*	83 (65.4)	16 (57.1)	67 (67.7)	0.208
Age at evaluation, *years*	76.0 (72.0-79.0)	76.0 (72.0-80.5)	76.0 (72.0-79.0)	0.760
Age at disease onset, *years*	73.0 (69.0-76.0)	74.5 (69.0-78.0)	72.0 (69.0-76.0)	0.313
Disease duration <1 year, *n (%)*	21 (16.5)	6 (21.4)	15 (15.2)	0.447
Symptoms at first evaluation				
Gait disorders, *n (%)*	119 (98.4)	25 (89.3)	94 (94.9)	0.385
Urinary dysfunctions, *n (%)*	100 (82.6)	23 (82.1)	77 (77.8)	0.560
Cognitive impairment, *n (%)*	83 (68.6)	20 (71.4)	63 (63.6)	0.349
Symptom of disease onset				
Gait disorders, *n (%)*	77 (64.2)	17 (60.7)	60 (60.6)	
Balance disorders, *n (%)*	14 (11.7)	1 (3.6)	13 (13.1)	0.581
Urinary dysfunctions, *n (%)*	14 (11.7)	4 (14.3)	10 (10.1)	
Cognitive impairment, *n (%)*	15 (12.5)	3 (10.7)	12 (12.1)	
Fall in the last year, *n (%)*	88 (72.7)	20 (71.4)	68 (68.7)	0.804
Urinary dysfunctions*				
Neurological examination				
Partial MDS-UPDRS, motor section score	6.0 (3.0-14.0)	10.0 (4.0-18.0)	6.0 (3.0-12.5)	0.164
Rest tremor, *n (%)*	23 (18.1)	7 (25.0)	16 (16.2)	0.262
Gait impairment, *n (%)*	2 (1-2)	2 (1-2)	2 (1-2)	0.302
Festination, *n (%)*	15 (12.4)	3 (10.7)	12 (12.1)	0.592
Heel height reduction, *n (%)*	89 (70.1)	20 (71.4)	69 (69.7)	0.443
Petit-pas gait, *n (%)*	62 (52.1)	20 (71.4)	42 (42.4)	**0.007**
Varus, *n (%)*	59 (46.5)	9 (32.1)	50 (50.5)	0.066
Wide-based gait, *n (%)*	78 (61.4)	18 (64.3)	60 (60.6)	0.326
Arm swing reduction, *n (%)*	70 (59.3)	18 (64.3)	52 (52.5)	0.174
Freezing, *n (%)*	15 (11.8)	6 (21.4)	9 (9.1)	0.074
Start hesitation, *n (%)*	13 (10.2)	6 (21.4)	7 (7.1)	**0.036**
TUGT *(seconds)*	4.7 (3.7-13.3)	4.8 (4.0-20.0)	4.7 (3.7-12-7)	0.367
18-meters walking test				
Time 18-m *(seconds)*	25.5 (19.8-33.0)	29.4 (20.9-33.4)	23.9 (19.5-32.5)	0.212
Gait Speed *(meters for second)*	0.7 (0.5-0.9)	0.6 (0.5-0.8)	0.8 (0.5-0.9)	0.123
Step length *(meters)*	1.4 (0.5-2.1)	1.5 (0.4-2.0)	1.3 (0.5-2.2)	0.636
Number of steps (180° turn)	4 (3-6)	5 (4-6)	4 (3-6)	0.414
Postural Stability	2 (1-3)	2 (1-3)	2 (2-3)	0.289
Scores				
iNPH grading scale (max 12)	6 (4-7)	6 (5-7)	5 (4-7)	0.330
Gait status scale (max 16)	5 (3-8)	5 (3-9)	5 (2-8)	0.172
Tinetti balance	13 (10-15)	12 (10-14)	13 (10-15)	0.222
Tinetti gait	8 (5-9)	7 (4-8)	8 (6-10)	**0.017**
Tinetti total score (max 28)	21 (15-24)	19 (14-21)	22 (16-25)	0.052
FIM scale (max 126)	112 (100-121)	107 (98-117)	114 (100.5-121.5)	0.225
mRankin Scale (max 6)	2 (1-3)	3 (2-3)	2 (1-3)	0.075
MMSEc (max 30)	26.3 (23.7-27.7)	23.4 (19.4-26.3)	26.7 (24.7-27.7)	**0.003**
Brief Mental Deterioration Battery	0.71 (-0.34-1.35)	0.67 (-1.04-1.24)	0.76 (-0.55-1.40)	0.365
Dopaminergic treatment				
Levodopa treatment, *n (%)*	36 (28.4)	12 (42.9)	24 (24.2)	0.061
Levodopa max daily posology (mg)	300 (300-450)	400 (300-600)	300 (200-450)	0.226
Response to Levodopa, *n (%)*	15 (11.8)	7 (25.0)	8 (8.1)	0.151
Magnetic resonance imaging:				
ARWMC score	8 (4-12)	8.5 (4.5-12)	7.5 (4-12)	0.915
Fazekas score	3 (2-4)	2 (1.5-3.5)	3 (2-4)	0.561

Continuous variables are expressed as median (interquartile range).

*Urinary dysfunctions include the following: nycturia, urinary incontinence, pollakiuria, and urinary urgency.

Legend= α-syn-LB: Lewy body-associated α-synuclein seeding activity; ARWMC: Age-Related White Matter Changes; FIM: Functional Independence Measure; iNPH: idiopathic Normal Pressure Hydrocephalus; MDS-UPDRS: Movement Disorder Society-Unified Parkinson's Disease Rating Scale; MMSEc: corrected Mini-Mental State Examination; mRankin: Modified Rankin Scale; MRI: magnetic resonance imaging; TUGT: timed up and go test.

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Table S5. Comparisons between Aß+ vs. Aß– subgroups of patients from the Bologna PRO-HYDRO cohort.

	Aß+	Aß-	*p-value*
N	32	94	
Males, *n (%)*	22 (68.8)	61 (64.9)	0.830
Age at disease onset, *years*	74.0 (70.0-77.0)	72.0 (69.0-76.0)	0.258
Age at evaluation, *years*	77.5 (72.5-79.0)	76.0 (72.0-79.0)	0.213
Disease duration <1 year, *n (%)*	6 (18.8)	14 (14.9)	0.125
Symptom of disease onset:			
Gait disorders, *n (%)*	17 (53.1)	59 (62.8)	
Balance disorders, *n (%)*	3 (9.4)	11 (11.7)	0.107
Urinary dysfunctions, *n (%)*	1 (3.1)	13 (13.8)	
Cognitive impairment, *n (%)*	7 (21.9)	8 (8.5)	
Symptoms at first evaluation:			
Gait disorders, *n (%)*	28 (87.5)	90 (95.7)	0.426
Urinary dysfunctions, *n (%)*	21 (65.6)	78 (83.0)	0.560
Cognitive impairment, *n (%)*	23 (71.9)	59 (62.8)	0.108
Fall in the last year, n (%)	19 (59.4)	68 (72.3)	0.231
Number of urinary dysfunctions*	3 (1-4)	3 (2-4)	0.233
Neurological examination:			
Partial MDS-UPDRS, motor section score	6.5 (2.0-14.5)	6.0 (4.0-13.0)	0.984
Rest tremor, *n (%)*	6 (19.4)	16 (17.8)	0.196
Gait impairment, *n (%)*	2 (1-2)	2 (1-2)	0.302
Festination, *n (%)*	2 (6.3)	13 (13.8)	0.218
Heel height reduction, *n (%)*	23 (71.9)	66 (70.2)	0.587
Petit-pas gait, *n (%)*	18 (56.3)	43 (45.7)	0.200
Varus, *n (%)*	13 (40.6)	46 (48.9)	0.263
Wide-based gait, *n (%)*	19 (59.4)	58 (61.7)	0.452
Arm swing reduction, *n (%)*	19 (59.4)	51 (54.3)	0.409
Freezing, *n (%)*	2 (6.3)	12 (12.8)	0.253
Start hesitation, *n (%)*	5 (15.6)	7 (7.5)	0.155
TUGT *(seconds)*	5.3 (4.3-15.6)	4.7 (3.5-12.9)	0.301
18-meters walking test:			
Time 18-m *(seconds)*	28.1 (19.7-38.8)	25.4 (20.4-32.8)	0.818
Gait Speed *(meters for second)*	0.6 (0.5-0.9)	0.7 (0.53-0.9)	0.924
Step length *(meters)*	0.9 (0.5-2.0)	1.4 (0.5-2.2)	0.616
Number of steps (180° turn)	4 (3-7)	4 (3-6)	0.663
Postural Stability	2 (1-3)	2 (2-3)	0.472
Scores:			
iNPH grading scale (max 12)	6 (5-7)	5.5 (4-7)	0.293
Gait status scale (max 16)	5 (3-7)	5 (2-8)	0.692
Tinetti balance	13.5 (10-15)	13 (10-14)	0.257
Tinetti gait	7.5 (5-9)	8 (5-9)	0.813
Tinetti total score (max 28)	21.5 (16-24)	21 (15-24)	0.600
FIM scale (max 126)	105 (96-123)	114.5 (102-121)	0.259
mRankin Scale (max 6)	2 (1-3)	2 (1-3)	0.566
MMSEc (max 30)	24.5(19.3-27.4)	26.4(24.7-27.7)	**0.037**
Brief Mental Deterioration Battery	-0.6 (-2.2-0.8)	0.9 (0.3-1.5)	**0.0003**
Levodopa treatment	9 (28.1)	26 (27.7)	0.564
Magnetic resonance imaging:			
ARWMC score	6.0 (4.0-11.0)	9.0 (4.0-13.0)	0.161
Fazekas score	2.0 (2.0-3.0)	3.0 (1.0-4.0)	0.326

Data were available for 126 out 127 patients of the Bologna PRO-hydro cohort.

Continuous variables are expressed as median (interquartile range).

*Urinary dysfunctions include the following: nycturia, urinary incontinence, pollakiuria, and urinary urgency.

Legend= ARWMC: Age-Related White Matter Changes; FIM: Functional Independence Measure; iNPH: idiopathic Normal Pressure Hydrocephalus; MDS-UPDRS: Movement Disorder Society-Unified Parkinson's Disease Rating Scale; MMSEc: corrected Mini-Mental State Examination; mRankin: Modified Rankin Scale; TUGT: timed up and go test.

Table S6. Comparisons between responders and non-responders 6 months after surgery.

Bologna iNPH cohort - 6 months follow-up	Non-Responders (n=47)	Responders (n=42)	p-value
Males, *n (%)*	37 (78.7)	22 (52.4)	**0.009**
Age at evaluation, *years*	76 (72-79)	75 (72-78)	0.599
Age at disease onset, *years*	72 (67-75)	73 (69-76)	0.645
Symptoms at first evaluation			
Gait disorders, *n (%)*	45 (95.7)	42 (100.0)	0.176
Urinary dysfunctions, *n (%)*	36 (76.6)	35 (30.1)	0.430
Cognitive impairment, *n (%)*	36 (76.6)	25 (59.5)	0.083
Symptom at disease onset			
Gait disorders, *n (%)*	29 (61.7)	27 (64.3)	
Balance disorders, *n (%)*	4 (8.5)	7 (16.7)	0.127
Urinary dysfunctions, *n (%)*	3 (6.4)	5 (11.9)	
Cognitive impairment, *n (%)*	11 (23.4)	3 (7.1)	
Fall in the last year, *n (%)*	30 (63.8)	35 (83.3)	**0.038**
Number of urinary dysfunctions	3 (1-4)	2 (1-4)	0.316
Neurological examination			
Rigidity, *n (%)*	30 (63.8)	18 (42.9)	**0.035**
Partial MDS-UPDRS score, motor section	10 (4-15)	5.5 (2-11)	0.071
Gait impairment, *n (%)*	2 (1-2)	2 (1-3)	**0.035**
Festination, *n (%)*	6 (12.8)	5 (11.9)	0.936
Petit-pas gait, *n (%)*	22 (46.8)	23 (54.8)	0.516
Varus, *n (%)*	26 (55.3)	16 (38.1)	0.084
Wide-based gait, *n (%)*	30 (63.8)	24 (57.1)	0.521
Freezing, *n (%)*	7 (14.9)	5 (11.9)	0.651
Start hesitation, *n (%)*	5 (10.6)	6 (14.3)	0.628
Timed up to go test *(seconds)*	5.7 (4.0-18.7)	5.0 (4.0-15.0)	0.773
18-meter walking test			
Time *(seconds)*	23.3 (18.9-31.0)	28.7 (20.9-34.3)	0.189
Gait Speed *(meters for second)*	0.8 (0.6-0.9)	0.6 (0.5-0.8)	0.123
Postural Stability	2 (1-3)	3 (2-3.5)	0.124
Scores			
iNPH grading scale (max 12)	6 (4-6)	6 (4-7)	0.724
Gait status scale (max 16)	3.5 (3-6)	6 (4-8)	**0.025**
Tinetti Balance	14 (11-15)	12 (10-14)	0.139
Tinetti Gait	8 (6-9)	7 (4-9)	0.203
Tinetti total score (max 28)	22 (17-24)	20 (14-23)	0.150
mRankin Scale (max 6)	2 (1-3)	3 (2-3)	**0.008**
MMSEc (max 30)	26.3 (24-27.7)	25.8 (23.2-27.3)	0.370
Brief Mental Deterioration Battery	0.53 (-0.59-1.05)	0.88 (0.08-1.47)	0.160
Levodopa treatment	19 (40.4)	9 (21.4)	0.069
ARWMC score - MRI	8 (6-13)	6.5 (4-10)	0.184
Fazekas score - MRI	3 (2-4)	2 (1-3)	0.200
CSF biomarkers			
NfL *(pg/ml)*	1063.1 (810.5-1671)	1054.9 (730.5-1274.0)	0.360
total-tau *(pg/ml)*	191.0 (142.0-278.0)	196.0 (135.0-254.0)	0.754
phospho-tau *(pg/ml)*	27.7 (20.1-35.6)	23.0 (19.9-33.0)	0.345
Aβ42/40 ratio	0.9 (0.7-1.0)	0.9 (0.6-1.0)	0.475
Positive α-syn RT-QuIC, *n (%)*	9 (19.2)	9 (21.4)	0.789

Data are expressed as median (interquartile range).

Legend= Aβ: amyloid-beta; α-syn: α-synuclein; ARWMC: Age-Related White Matter Changes; MDS-UPDRS: Movement Disorder Society-sponsored revision of the Unified Parkinson's Disease Rating Scale; MMSEc: corrected Mini-Mental State Examination; mRankin: Modified Rankin Scale; MRI: magnetic resonance imaging; NfL: neurofilament-light chain protein; RT-QuIC: real-time quaking-induced conversion.