CSF α-synuclein contributes to the differential diagnosis of Alzheimer disease - Supplementary Materials

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I. Supplementary Methods

Tissue collection and neuropathological assessment

Tissue collection procedures have been previously described [1]. Briefly, with the routine diagnostic procedure the sections were stained with hematoxylin-eosin, in order to quantify neuron loss and gliosis the following primary antibodies were used for the detection of abnormal proteins: nab228 [monoclonal antibody (mAb), 1:8,000, generated in the Center for Neurodegenerative Disease Research (CNDR) at the University of Pennsylvania] [2] to detect amyloid deposits and Thal staging [3], phosphorylated tau PHF-1 (mAb, 1:1,000, a gift of Dr. Peter Davies) to detect phosphorylated tau deposits, pS409/410 (mAb, 1:500, a gift of Dr. Manuela, Neumann) to detect phosphorylated TDP43 deposits [4], and Syn303 (mAb, 1:16,000, generated in the CNDR) to detect the presence of oxidized/nitrated α-syn [5]. Primary antibody binding was visualized with the avidin–biotin complex detection method (VECTASTAIN ABC kit; Vector Laboratories, Burlingame, CA, USA) with ImmPACT diaminobenzidine peroxidase substrate (Vector Laboratories, Burlingame, CA, USA) as the chromogen. In addition, Thioflavin S was used to grade the presence of neuritic plaques and Consortium to Establish a Registry for Alzheimer's disease (CERAD) score [6, 7]. All changes were rated using a semi-quantitative scale (0 = no changes, 0.5 = rare, 1 = mild, 2 = moderate and 3 = severe), and these gradings were entered in the Integrated Neurodegenerative Disease Database (INDD) established in the CNDR. All cases were reviewed by a board-certified neuropathologist for quality assurance and accurate grading. Briefly, neurofibrillary tangles and senile plaques were graded according to NIA-Reagan criteria using Braak staging [8, 9] and the CERAD protocol, without considering subject age [6]. A neuropathological diagnosis of Alzheimer disease (AD) was assigned if the probability was intermediate or high [10]. The diagnoses of frontotemporal lobar degeneration (FTLD)-TAU, FTLD-TDP and dementia with Lewy bodies (DLB) were based on established criteria [11, 12]. FTLD-TAU cases included cases with a diagnosis of argyrophilic grain disease (AGD), progressive supranuclear palsy (PSP), tangle predominant senile dementia (TPSD), and corticobasal degeneration (CBD).

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II. Supplementary Tables

Supplementary Table 1. Characteristics of the whole clinical cohort.

	HC	AD	MCI	FTD	CBS	DLB	PD	ALS	PSP
N	69	165	105	60	10	16	63	41	11
Age at CSF sampling	68 [(61)-(74)]	72 [(63)-(78)]	72 [(65)-(76)]	64 [(56)-(67)]	67 [(62)-(73)]	67.5 [(64.5)-(74.5)]	66 [(61.5)-(72)]	56 [(50)-(66)]	71 [(63)-(74)]
Sex (% male)	36.2	40	49.5	61.7	50	50	79.4	78	54.5
Disease duration at CSF (yr)	-	2 [(1)-(4)]	2 [(1)-(3)]	2 [(1)-(4)]	2 [(1)-(2)]	2 [(1)-(3)]	7 [(4)-(11)]	1 [(0)-(1)]	2 [(2)-(4.5)]
APOE ε4 presence (%)	31.2	63.2	44.8	32.2	20	30	28.1	21.1	9.1
CSF $A\beta_{42}$ (pg/mL)	244.0 [(202)-(286.21)]	140.6 [(112)-(163)]	183.0 [(122.81)-(228.1)]	196.8 [(147.12)-(289.12)]	232.0 [(214.75)-(292)]	156.5 [(138)-(182.49)]	231.8 [(180)-(269.36)]	276.0 [(219)-(318)]	181 [(140.5)-(223)]
CSF t-tau (pg/mL)	49.6 [(36.5)-(62.59)]	95 [(70.28)-(145)]	71.8 [(43.5)-(104.95)]	62.0 [(43.64)-(87.25)]	69 [(62)-(93.25)]	70.9 [(45.75)-(97.96)]	39.0 [(29)-(54.92)]	55 [(39)-(67)]	43.0 [(34)-(53)]
CSF p-tau (pg/mL)	18 [(14.0)-(24.7)]	35.4 [(23.1-(51.2)]	24 [(13.9-(48.8)]	17 [(12)-(25.5)]	19.5 [(16.5)-(23.8)]	24.29 [(16.0)-(39.0)]	20 [(15)-(27.0)]	11 [(8.0)-(14.0)]	14 [(12.5)-(19.5)]

Data shown is median [(25^{th} percentile)-(75^{th} percentile)], except for Sex of subject and APOE ε4 presence. AD, Alzheimer disease; ALS, amyotrophic lateral sclerosis; bvFTD, behavioral variant (social/executive) FTD; CBS, corticobasal syndrome; DLB, dementia with Lewy bodies; FTD, frontotemporal degeneration; HC, healthy control; LBD, Lewy body disorders; MCI, mild cognitive impairment; PD, Parkinson disease; PSP, progressive supranuclear palsy.

Supplementary Table 2. Characteristics of the whole autopsy cohort.

	AD	FTLD	FTLD-AD	PD	PD-AD	LRP-AD	LRP-TDP	ALS	HC
N	40	17	6	3	4	21	2	6	3
Age at CSF sampling	75 [(64)-(79.5)]	71.0 [(64.0)-(72.0)]	66 [(58.75)-(68.75)]	77 [(77)-(82.5)]	73 [(66.25)-(80.25)]	72 [(62)-(76)]	63.5 [(61.25)-(65.75)]	57 [(54.25)-(61.25)]	80 [(78)-(86.5)]
Sex (% male)	60	52.9	83.3	66.7	100	52.4	50	16.7	66.7
Disease duration at CSF	3 [(1)-(4.25)]	2.5 [(1.0)-(5.3)]	3 [(2.25)-(3.75)]	17 [(14)-(18.5)]	6.5 [(5)-(7)]	4 [(3)-(4)]	1.5 [(1.25)-(1.75)]	1 [(1)-(1)]	-
Age at Death	79 [(70)-(85.25)]	73.0 [(68.0)-(76.0)]	70.5 [(60.5)-(73.75)]	80 [(79.5)-(84.5)]	76.5 [(70.75)-(83)]	75 [(68)-(85)]	69 [(67)-(71)]	59 [(56.25)-(61.75)]	86 [(83)-(93.5)]
Survival after CSF	5.2 [(3.5)-(6.6)]	3.7 [(2.1)-(5.6)]	4.4 [(2.9)-(5.07)]	1.4 [(1.25)-(1.8)]	3.9 [(2.88)-(4.85)]	5.8 [(4.5)-(8.3)]	5.0 [(4.52)-(5.38)]	2.2 [(0.75)-(2.38)]	8.0 [(4.1)-(8.95)]
APOE ε4 presence (%)	55	11.8	16.7	33.3	75	61.9	50	16.7	0
CSF $A\beta_{42}$ (pg/mL)	130.2 [(104)-(151.6)]	199.9 [(171.5)-(227.0)]	164.0 [(132.5)-(206.75)]	269.7 [(236.76)-(271.87)]	186.1 [(157.7)-(214.25)]	139.9 [(123.17)-(151)]	213.8 [(209.01)-(218.53)]	280.0 [(259.25)-(314.25)]	239.0 [(178.5)-(252)]
CSF t-tau (pg/mL)	99.5 [(71.43)-(152.5)]	49.0 [(43.0)-(63.6)]	40.9 [(24)-(84.18)]	45.9 [(44.47)-(52.69)]	66.2 [(57)-(71.08)]	110.0 [(65.42)-(133.71)]	61.5 [(44.97)-(77.96)]	36.0 [(30.75)-(69.75)]	39.0 [(29.5)-(77.5)]
CSF p-tau (pg/mL)	27.98[(19.3)-(41.76)]	11.4 [(7.8)-(13.5)]	17.81 [(13.2)-(19.8)]	9.18 [(9.1)-(16.8)]	25.91 [(19.4)-(32.0)]	28.63[(13)-(42.05)]	5.22 [(4.4)-(6.0)]	8[(6.5)-(9.5)]	9.6 [(6.2)-(10.3)]

Data shown is median [(25^{th} percentile)-(75^{th} percentile)], except for Sex of subject and APOE ε4 presence. AD, Alzheimer disease; ALS, amyotrophic lateral sclerosis; FTLD, frontotemporal lobar degeneration; HC, healthy control with an unremarkable burden of any significant brain pathology; LRP, Lewy-related pathology; PD, Parkinson disease; TDP, TAR DNA-binding protein 43 (TDP-43) pathology .