Amyloid-beta co-pathology is a major determinant of the elevated plasma GFAP values in amyotrophic lateral sclerosis

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SUPPLEMENTARY RESULTS

Table S1. Neuropsychological results in the whole ALS cohorts and in the AD/ALS and not-AD/ALS subcohorts

	Total ALS (n=156)	**AD/ALS (n=9)**	**not-AD/ALS (n=147)**
ECAS total scores (age-and education-adjusted), **n**	108.2 (96.1-116.8),88	89.2 (79.9-112.0),4	108.4 (96.8-116.8),84
ECAS total scores (equivalent scores), **n**	4.0 (3.0-4.0),88	2.0 (1.2-3.5),4	4.0 (3.0-4.0),84
ECAS ALS-specific scores (age-and education-adjusted), **n**	79.7 (71.9-86.1),88	64.4 (61.2-84.4),4	79.9 (72.6-86.1),84
ECAS ALS-specific scores (equivalent scores), **n**	4.0 (3.0-4.0),88	2.0 (2.0-3.5),4	4.0 (3.0-4.0),84
ECAS language sub-scores (age-and education-adjusted), **n**	25.5 (23.2-27.0),87	22.8 (20.4-28.0),3	25.6 (23.3-27.0),84
ECAS verbal fluency sub-scores (age-and education-adjusted), **n**	18.7 (15.2-21.1),86	18.3 (15.2-21.2),3	18.7 (15.0-21.1),83
ECAS executive functions sub-scores (age-and education-adjusted), **n**	36.3 (32.1-40,1),87	29.3 (22.9-40.3)	36.7 (32.4-40.1),84
ECAS ALS-nonspecific scores (age-and education-adjusted), **n**	27.3 (23.9-30.6),88	24.8 (18.4-27.8),4	27.3 (24.1-30.6),84
ECAS ALS-nonspecific scores (equivalent scores), **n**	4.0 (2.0-4.0),88	3.0 (1.2-4.0),4	4.0 (2.0-4.0),84
ECAS memory sub-scores (age-and education-adjusted), **n**	16.0 (13.0-18.44),87	15.7 (9.1-17.1),3	16.0 (13.0-18.6),84
ECAS visuo-spatial sub-scores (age-and education-adjusted), **n**	11.9 (11.4-12.2),87	11.8 (10.9-12.5),3	11.9 (11.4-12.2),84
MMSE, n	29.0 (28.0-30.0),114	28.0 (22.5-29.0),5	29.0 (28.0-30.0),109
MMSE (age-and education-adjusted), **n**	28.1 (26.7-29.0),113	25.7 (22.9-29.4),5	28.8 (27.5-30),108
Rey Auditory Verbal Learning test (immediate recall)**, n***	40.2 (32.9-46.3),114	34.6 (21.1-40.4),5	40.3 (33.0-46.3),109
Rey Auditory Verbal Learning test (delayed recall)**, n***	8.0 (5.9-9.7),113	9.4 (2.7-10.5),5	7.9 (6.0-9.7),108
Short-term visual memory, n*	19.6 (17.2-20.9),114	15.8 (14.2-18.0),5	19.7 (17.4-20.9),109
Simple verbal analogies test, n*	16.3 (13.7-17.6),114	15.7 (13.4-17.3),5	16.4 (13.7-17.7),109
BDMB, n*	1.8 (1.1-2.3),107	1.1 (-0.5-2.1),5	1.8 (1.2-2.3),102
FAB, n*	16.4 (14.6-17.4),27	16.9 (16.4-17.4),2	16.0 (14.5-17.6),25
Freehand copy of drawings test, n*	10.4 (9.5-11.4),96	9.5 (7.2-10.0),4	10.6 (9.5-11.5),92
Category fluency test, n*	38.0 (31.0-46.0),111	29.3 (18.2-39.9),4	38.0 (31.2-46.0),107
Letter fluency test, n*	30.3 (22.4-37.4),110	23.0 (14.3-33.9),4	30.4 (23.9-37.6),106

*Test scores are age- and education-adjusted;
Test scores are expressed as median (interquartile range).

Abbreviations: AD, Alzheimer's Disease; ALS, amyotrophic lateral sclerosis; BMDB, Brief Mental Deterioration Battery; ECAS, Edinburgh Cognitive and Behavioural ALS Screen; FAB, Frontal assessment Battery; MMSE, Mini Mental State Examination.

Table S2. Univariable Cox Regression analysis for prognostic value of plasma biomarkers and clinical-laboratory variables in ALS patients

Variable		**Univariable COX regression**	
		HR (95% CI)	**p-value**
Sex		1.34 (0.84-2.15)	0.224
Age at sampling		1.03 (1.009-1.05)	**0.005**
Time from clinical onset to sample		0.87 (0.72-1.05)	0.143
King's stage	**Stage 1**	Ref	**<0.001**
	Stage 2	1.88 (0.25-14.3)	0.543
	Stage 3	4.78 (0.66-34.59)	0.122
	Stage 4	32.99 (4.07-267.56)	**0.001**
BMI		0.99 (0.94-1.04)	0.617
DPR	**Slow progressors**	Ref	Ref
	Intermediate progressors	2.27 (1.31-3.94)	**0.004**
	Fast progressor	4.15 (2.37-7.27)	**<0.001**
FTD status		1.97 (1.01-3.85)	**0.048**
Onset type	**Spinal**	Ref	Ref
	Bulbar	2.34 (1.39-3.94)	**0.001**
	Pyramidal	1.13 (0.35-3.64)	0.837
	Pseudopolyneuritic	0.84 (0.33-2.12)	0.716
Clinical Phenotype	**Classic**	Ref	
	Bulbar	1.76 (0.96-3.21)	0.067
	PUMN	1.03 (0.53-1.99)	0.938
	PLMN	1.09 (0.46-2.56)	0.843
ALSFRS-R scale		0.89 (0.86-0.92)	**<0.001**
Genetic status	**Wild type**	Ref	Ref
	SOD1	1.35 (0.33-5.52)	0.678
	C9Orf72	1.59 (0.81-3.1)	0.179
Blood-brain barrier index		1.02 (0.96-1.08)	0.58
CPK levels		0.99 (0.99-1)	0.183
Plasma GFAP		2.46 (1,91-3,01)	**<0.001**
Plasma GFAP	**Lowest tertile**	Ref	Ref
	Intermediate tertile	1.21 (0.68-2.13)	0.519
	Highest tertile	2.80 (1.59-4.92)	**<0.001**
Plasma p-Tau181		1.11 (1.02-1.2)	**0.016**
Plasma NfL		1.01 (1.006-1.01)	**<0.001**

Significant p-values are reported in bold.

Abbreviations: ALSFRS-R, amyotrophic lateral sclerosis functional rating scale revised; BMI, body mass index; CI, confidence interval; CPK, creatine phosphokinase; DPR, disease progression rate; FTD, frontotemporal dementia; GFAP, glial fibrillary acidic protein; HR, hazard ratio; NfL, neurofilament light chain; PLMN, prevalent lower motor neuron; p-tau181, phosphorylated tau 181; PUMN, prevalent upper motor neuron; Ref, reference.

Table S3. Multivariable Cox Regression analysis for plasma GFAP and clinical prognostic factors in ALS

Variable		HR (95% CI)	P-value
Plasma GFAP	**Lowest tertile**	Ref	Ref
	Intermediate tertile	0.83 (0.43-1.60)	0.58
	Highest tertile	0.96 (0.44-2.08)	0.92
Age at sampling		1.02 (0.99-1.05)	0.06
Onset type	**Spinal**	Ref	Ref
	Bulbar	2.23 (1.24-4.02)	**0.007**
	Pyramidal	0.48 (0.13-1.80)	0.28
	Pseudopolyneuritic	0.45 (0.17-1.22)	0.12
ALSFRS-R scale		0.91 (0.87-0.95)	**<0.001**
FTD status		1.22 (0.58-2.58)	0.60
DPR	**Slow progressors**	Ref	Ref
	Intermediate progressors	2.33 (1.27-4.24)	**0.006**
	Fast progressors	2.58 (1.36-4.89)	**0.004**

Significant p-values are reported in bold.

Abbreviations: ALSFRS-R, amyotrophic lateral sclerosis functional rating scale revised; CI, confidence interval; DPR, disease progression rate; FTD, frontotemporal dementia; GFAP, glial fibrillary acidic protein; HR, hazard ratio; Ref, reference.