

Elevated plasma p-tau₁₈₁ levels unrelated to Alzheimer's disease pathology in amyotrophic lateral sclerosis

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SUPPLEMENTARY METHODS

Genetic Analyses

Genomic DNA (gDNA) was extracted from peripheral blood by standard procedures. gDNA was quantified using the Quantus Fluorometer (Promega) with QuantiFluor doublestranded DNA system (Promega). Patients were screened for mutations in ALS major genes: *SOD1* (all exons), *FUS* (exons 6 and 15), *TARDBP* (exons 2, 3, and 5) genes and for pathogenic repeat expansion (RE) in the *C9orf72* gene as previously reported.[1]

Assessment of cognitive functions

Presence of FTD was assessed through the clinical history, neurological examination, and a neuropsychological assessment including the Frontal Assessment Battery,[2] the Brief Mental Deterioration Battery (BMDB) [3] and the Edinburgh Cognitive and Behavioural ALS Screen (ECAS).[4]

Supplementary Table 1 – Core AD biomarkers values distribution across the diagnostic groups

	ALS (n=148)	ALS A+ (n=20)	AD (n=88)	P value^c
CSF t-tau^a	255.5 (199.8-357.5) ^b	382.0 (281.5-579.0)	635.5 (494.3-971.5)	<0.0001 ^d
CSF p-tau^a	33.0 (26.2-42.6) ^b	58.1 (43.7-80.1)	109.0 (82.0-159.5)	<0.0001 ^e
CSF Aβ42/Aβ40 ratio^a	0.97 (0.92-1.00) ^b	0.52 (0.40-0.61)	0.43 (0.37-0.49)	<0.0001 ^f

a: Data are expressed as median (interquartile range); b: data are available in 130 patients; c: refer to the ANOVA-test (biomarker values were transformed into a logarithmic scale to obtain a normal data distribution); p values of the statistically significant comparisons of the post-hoc test (Tukey test) are further detailed in the table legend; d: ALS vs. ALS A+ p=0.001, ALS vs. AD and ALS A+ vs. AD p<0.0001; e: ALS vs. ALS A+, ALS vs. AD and ALS A+ vs. AD p<0.0001; f: ALS vs. ALS A+ and ALS vs. AD p<0.0001. Key: ALS, amyotrophic lateral sclerosis; AD, Alzheimer's disease; A+, amyloid positive; CSF, cerebrospinal fluid, p-tau, phosphorylated tau protein; t-tau, total tau protein.

Supplementary Table 2 - Multivariate model for the assessment of the association of plasma p-tau and the extent of LMN involvement.

Variable		β coefficients (95% CI)	p-value
LMN degeneration	Three regions	Ref	Ref
	Two regions	-0.26 (-0.53– -0.01)	0.063
	One region	-0.46 (-0.89– -0.03)	0.036
	Zero region	-0.001 (-0.89– -0.87)	0.98
Age at sample collection		0.129 (0.004-0.02)	0.007
Sex		0.4 (0.16-0.64)	0.002
Genetic status	Wild type	Ref	Ref
	<i>SOD1</i>	0.399 (-0.32-1.12)	0.28
	<i>TARDBP</i>	-1.34 (-2.29– -0.39)	0.006
	<i>C9Orf72</i>	-0.29 (-0.68-0.10)	0.15
FTD		-0.47 (-0.86– -0.084)	0.018
ALSFRS-R scale		-0.006 (-0.03-0.02)	0.61
ALS phenotype	Classic	Ref	Ref
	Bulbar	-0.26 (-0.75-0.24)	0.31
	PLMN	-0.12 (-0.44-0.21)	0.47
	PUMN	0.22 (-0.37-0.82)	0.46
MRC score		-0.21 (-0.46-0.04)	0.1
King's score		0.14 (-0.09-0.37)	0.24

Type of onset	spinal	Ref	Ref
	bulbar	-0.30 (-0.73-0.13)	0.17
	pyramidal	-0.91 (-1.66– -0.16)	0.017
	pseudopolyneuritic	0.21 (-0.23-0.65)	0.34

The results are presented as β coefficients and 95% confidence intervals. Key: ALS, amyotrophic lateral sclerosis; CI, confidence interval; FTD, frontotemporal dementia; LMN, lower motor neuron; MRC, Medical Research Council; PLMN, predominant lower motor neuron; PUMN, predominant upper motor neuron; Ref, reference.

Supplementary Table 3 - Multivariate model for the assessment of the association of plasma p-tau and the extent of LMN involvement including plasma NfL levels

Variable		β coefficients (95% CI)	p-value
LMN degeneration	Three regions	Ref	Ref
	Two regions	-0.25 (-0.52-0.02)	0.068
	One region	-0.48 (-0.91– -0.05)	0.029
	Zero region	0.02 (-0.86– -0.91)	0.96
Age at sample		0.012 (0.003-0.02)	0.012
Sex		0.42 (0.18-0.66)	0.001
Genetic status	Wild type	Ref	Ref
	<i>SOD1</i>	-0.05 (-0.93-0.82)	0.9
	<i>TARDBP</i>	-1.32 (-2.27– -0.37)	0.007
	<i>C9Orf72</i>	-0.31 (-0.69-0.08)	0.12
FTD		-0.48 (-0.86– -0.095)	0.015
ALSFRS-R scale		-0.006 (-0.03-0.02)	0.58
ALS phenotype	Classic	Ref	Ref
	Bulbar	-0.23 (-0.72-0.27)	0.37
	PLMN	-0.11 (-0.44-0.21)	0.49
	PUMN	0.20 (-0.39-0.80)	0.5
MRC score		-0.2 (-0.44-0.05)	0.12
King's score		0.16 (-0.07-0.39)	0.17

Plasma NfL levels		0.05 (-0.84-0.19)	0.44
Type of onset	spinal	Ref	Ref
	bulbar	-0.35 (-0.78-0.07)	0.11
	pyramidal	-0.93 (-1.68– -0.19)	0.014
	pseudopolineuritic	0.23 (-0.21-0.67)	0.29

The results are presented as β coefficients and 95% confidence intervals. Key: ALS, amyotrophic lateral sclerosis; CI, confidence interval; FTD, frontotemporal dementia; LMN, lower motor neuron; MRC, Medical Research Council; NfL, neurofilament light chain; PLMN, predominant lower motor neuron; PUMN, predominant upper motor neuron; Ref, reference.

Supplementary Table 4 - Univariate Cox Regression analysis for prognostic value of plasma p-tau and other clinical variables in ALS patients

Variable	Univariate COX regression	
	HR (95% CI)	p-value
Sex	1.17 (0.70-1.93)	0.54
Age at disease onset	1.02 (0.99-1.04)	0.06
Time from clinical onset to sample	0.99 (0.97-1.002)	0.09
King's stage	2.66 (1.71-4.14)	<0.001
MRC score	0.76 (0.55-1.03)	0.08
BMI	0.99 (0.93-1.04)	0.60
CVF	0.97 (0.96-0.98)	<0.001
DPR	1.89 (1.52-2.34)	<0.001
FTD status	1.92 (1.03-3.59)	0.042
Plasma p-Tau181	1.43 (1.04-1.97)	0.027
ALSFRS-R scale	0.94 (0.91-0.97)	<0.001
Creatinine	0.41 (0.09-1.93)	0.26
Onset type	Spinal	Ref
	Bulbar	2.24 (1.29-3.87) 0.004
	Pyramidal	0.85 (0.31-2.39) 0.76
	Pseudopolyneuritic	0.47 (0.11-1.95) 0.29
Genetic status	Wild type	Ref
	SOD1	0.58 (0.08-4.21) 0.59
	TARDBP	1.65 (-) 1
	C9Orf72	1.64 (0.81-3.33) 0.17

Data are presented as Hazard ratios and 95% CI. Key: ALS, amyotrophic lateral sclerosis; ALSFRS-R, Revised Amyotrophic Lateral Sclerosis Functional Rating; BMI, body mass index; CI, confidence interval; FVC, forced vital capacity; FTD, frontotemporal dementia; HR, hazard ratio; IQR, interquartile range; m, months; MRC, Medical Research Council; p-tau181, phosphorylated tau 181;

PLMN, predominant lower motor neuron; PUMN, predominant upper motor neuron; RE, repeats expansion; Ref, reference; y, years.

Supplementary Table 5 - Multivariate Cox Regression analysis for plasma p-Tau and clinical prognostic factors in ALS (plasma p-Tau expressed in tertiles).

Variable		HR (95% CI)	P-value
Plasma p-Tau181	Lowest tertile	Ref	Ref
	Intermediate tertile	2.66 (1.13-6.25)	0.025
	Highest tertile	3.57 (1.51-8.41)	0.004
Age at onset disease		1.02 (0.99-1.04)	0.09
Onset type	Spinal	Ref	Ref
	Bulbar	2.17 (1.09-4.35)	0.028
	Pyramidal	0.34 (0.98-1.22)	0.10
	Pseudopolineuritic	0.32 (0.07-1.36)	0.12
ALSFRS-R scale		0.96 (0.92-1.01)	0.10
FTD status		2.36 (0.96-5.79)	0.06
King's score		1.73 (0.99-2.99)	0.051
DPR	Slow progressors	Ref	Ref
	Intermediate progressors	2.29 (1.23-4.28)	0.009
	Fast progressors	4.44 (2.10-9.36)	<0.001

Data are expressed as Hazard Ratios and 95% CI. Key: ALSFRS-R, Revised Amyotrophic Lateral Sclerosis Functional Rating; DPR, disease progression rate; CI, confidence interval; FTD, frontotemporal dementia; HR, hazard ratio; p-tau181, phosphorylated tau 181; Ref, reference.

Supplementary Table 6 - Multivariate Cox Regression analysis for plasma p-Tau and clinical prognostic factors in ALS including plasma NfL levels.

Variable		HR (95% CI)	P-value
Plasma p-Tau181	Lowest tertile	Ref	Ref
	Intermediate tertile	2.11 (0.89-5)	0.089
	Highest tertile	3.18 (1.36-7.43)	0.007
Age at onset disease		1.02 (0.99-1.04)	0.1
Onset type	Spinal	Ref	Ref
	Bulbar	1.75 (0.85-3.64)	0.13
	Pyramidal	0.32 (0.89-1.14)	0.08
	Pseudopolineuritic	0.35 (0.08-1.51)	0.16
ALSFRS-R scale		0.96 (0.92-1.01)	0.14
FTD status		1.97 (0.80-4.84)	0.14
King's score		1.99 (1.10-3.58)	0.022
Plasma NfL levels		1.88 (1.24-2.85)	0.003
DPR	Slow progressors	Ref	Ref
	Intermediate progressors	1.98 (1.04-3.78)	0.038
	Fast progressors	2.45 (1.01-5.95)	0.047

Data are expressed as Hazard Ratios and 95% CI. Key: ALSFRS-R, Revised Amyotrophic Lateral Sclerosis Functional Rating; DPR, disease progression rate; CI, confidence interval; FTD, frontotemporal dementia; HR, hazard ratio; NfL, neurofilament light chain; p-tau181, phosphorylated tau 181; Ref, reference.

Supplementary Table 7 - Distribution of basal plasma p-tau181 values in ALS patients stratified in three groups (slow, intermediate and fast progressors), according to the b-DPR and the l-DPR

	SLOW progressors (N)	INTERMEDIATE progressors (N)	FAST progressors (N)	p-value
b-DPR^a	2.5 (1.3-3.5) (25)	1.6 (1.1-2.8) (8)	1.4 (0.6-2.9) (6)	0.14
l-DPR^a	1.8 (1.0-4.4) (13)	2.4 (1.7-3.9) (14)	1.3 (0.9-2.7) 12	0.24

a: Data are expressed as median (interquartile range). Key: b-DPR, basal disease progression rate; l-DPR, longitudinal disease progression rate.

References

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