

ORIGINAL ARTICLE

Association of APOE genotype and cerebrospinal fluid A β and tau biomarkers with cognitive and motor phenotype in amyotrophic lateral sclerosis

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Abstract

Objective: Little is known about amyotrophic lateral sclerosis (ALS)-nonspecific cognitive deficits – most notably memory disturbance – and their biological underpinnings. We investigated the associations of the Alzheimer's disease (AD) genetic risk factor APOE and cerebrospinal fluid (CSF) biomarkers A β and tau proteins with cognitive and motor phenotype in ALS.

Methods: APOE haplotype was determined in 281 ALS patients; for 105 of these, CSF levels of A β ₄₂, A β ₄₀, total tau (T-tau), and phosphorylated tau (P-tau₁₈₁) were quantified by chemiluminescence enzyme immunoassay (CLEIA). The Edinburgh Cognitive and Behavioural ALS Screen (ECAS) was employed to evaluate the neuropsychological phenotype.

Results: APOE-E4 allele was associated with worse ECAS memory score (median, 14.0 in carriers vs. 16.0 in non-carriers) and lower CSF A β ₄₂ (−0.8 vs. 0.1, log-transformed values) and A β ₄₂/40 ratio (−0.1 vs. 0.3). Some 37.1% of ALS patients showed low A β ₄₂ levels,

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possibly reflecting cerebral A β deposition. While lower A β 42/40 correlated with lower memory score ($\beta=0.20$), A β 42 positively correlated with both ALS-specific ($\beta=0.24$) and ALS-nonspecific ($\beta=0.24$) scores. Although A β 42/40 negatively correlated with T-tau ($\beta=-0.29$) and P-tau181 ($\beta=-0.33$), we found an unexpected positive association of A β 42 and A β 40 with both tau proteins. Regarding motor phenotype, lower levels of A β species were associated with lower motor neuron (LMN) signs (A β 40: $\beta=0.34$; A β 42: $\beta=0.22$).

Conclusions: APOE haplotype and CSF A β biomarkers are associated with cognitive deficits in ALS and particularly with memory impairment. This might partly reflect AD-like pathophysiological processes, but additional ALS-specific mechanisms could be involved.

KEYWORDS

Alzheimer's disease, amyloid beta, amyotrophic lateral sclerosis, APOE genotype, tau proteins

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder characterized by loss of upper (UMNs) and lower motor neurons (LMNs) and leading to progressive paralysis of voluntary muscles [1].

Increasing evidence indicates that, beyond motor symptoms, almost 50% of ALS patients show neuropsychological impairment, mainly concerning language, verbal fluency, and executive functions [2]. However, little is known about ALS-nonspecific cognitive deficits – particularly memory disturbance – and their biological underpinnings. Since memory impairment represents the distinctive feature of Alzheimer's disease (AD) [3], some studies have investigated biological AD hallmarks in relation to cognitive symptoms in ALS patients and the possible involvement of A β and tau proteins in ALS pathophysiology [4]. Moreover, the acknowledged co-occurrence of proteinopathy in neurodegenerative disorders demonstrated the importance of mixed pathology as an underrated but key element to unveil complexity behind neurodegeneration [5]. There is evidence for a potential role of amyloid precursor protein (APP) in the cellular response to axonal damage, with increased immunoreactivity of this protein in the perikarya of anterior horn cells suggesting an early protective effect [6, 7]. However, intracellular A β deposition might also be a late deleterious event leading to oxidative stress, activation of proapoptotic pathways [8], and TDP-43 accumulation [9]. Concerning the other AD protein hallmarks, evaluation of the diagnostic potential of cerebrospinal fluid (CSF) total (T-tau) and phosphorylated tau (P-tau₁₈₁) has provided conflicting results [10, 11]; nevertheless, T-tau might serve as prognostic biomarker reflecting the entity of motor neuron (MN) degeneration [12] similarly to serum levels of neurofilament light chain (NFL). Recent studies have reported increased plasma phosphorylated P-tau₁₈₁ levels in ALS patients which were proposed as a novel marker specific to LMN degeneration [13–15]. Finally, a major risk factor for AD is represented by the E4 allele of the APOE gene. Whereas a pathogenic role of E4 in frontotemporal dementia (FTD) is still a matter of debate [16], with a recent article pointing out an unexpectedly increased risk in carriers

of the APOE-E2 allele [17], inconsistent data are available for ALS [18]. Indeed, while some evidence suggests a deleterious role of the E4 allele in ALS pathogenesis [19], other works failed to confirm an influence of APOE on clinical phenotype [20, 21].

In this work, we investigated the potential association of APOE haplotype, as well as CSF A β and tau biomarkers, with motor and cognitive/behavioral features in ALS. Specifically, we explored the incidence of amyloid and tau pathology in a deep-phenotyped ALS cohort to estimate the occurrence of co-pathology and to verify whether AD-related mechanisms may be involved in ALS pathogenesis.

METHODS

Study cohort

A cohort of 281 Italian inpatients, diagnosed with ALS according to the El Escorial revised criteria [22], was recruited at IRCCS Istituto Auxologico Italiano between 2014 and 2022.

The following demographic and clinical data were collected: sex; age at onset; disease duration; survival; family history of ALS; motor phenotype (classic, bulbar, respiratory, flail arm, flail leg, UMN-predominant [UMN-p], primary lateral sclerosis [PLS], progressive muscular atrophy [PMA]) [23]; revised ALS Functional Rating Scale (ALSFRS-R) scores at evaluation and disease progression rate (Δ FS) [24, 25]; and presence of oculomotor abnormalities as previously described [26]. Motor impairment was assessed in all patients using the following scoring systems: the Penn Upper Motor Neuron Score (PUMNS) to account for UMN regional involvement [27] and a modified version of the Lower Motor Neuron Score to account for LMN signs as previously described [28, 29]. Spinal LMN involvement was also measured using the Medical Research Council (MRC) muscle scale assessing the strength of three muscle groups for each limb (shoulder abductors, elbow flexors, wrist dorsiflexors, hip flexors, knee extensors, and ankle dorsiflexors; total score 0–60). The Edinburgh Cognitive and Behavioural ALS Screen (ECAS)-Italian

version was used to perform an extensive evaluation of both cognitive and behavioral profile of the study population [30]. Behavioral symptoms were further investigated with the Frontal Behavior Inventory (FBI) [31]. Detailed descriptions of neuropsychological scores are provided in Supplementary Methods.

APOE haplotype analysis

APOE haplotype was determined by imputing rs7412 and rs429358 from previously generated genotyping data [32] or by direct sequencing of APOE exon 4. A full description of the methodology is reported in the Supplementary Methods.

The cohort was subdivided into two groups according to the presence of at least one E4 allele. Considering the putative protective role of the E2 allele against AD and the indetermined significance of the E2|E4 genotype, patients carrying this specific genotype were excluded from analyses [33].

CSF and serum biomarker measurement

A subcohort of patients underwent lumbar puncture as part of the diagnostic process. Measurement of CSF $A\beta_{42}$, $A\beta_{40}$, T-tau, and P-tau₁₈₁ was performed by chemiluminescence enzyme immunoassay (CLEIA) on the Lumipulse G600II platform (Fujirebio Europe, Ghent, Belgium). An $A\beta_{42}/A\beta_{40}$ ($A\beta_{42/40}$) ratio ≤ 0.069 enabled classification of patients as $A\beta$ -positive (A+) while an $A\beta_{42/40}$ ratio > 0.069 denoted $A\beta$ -negative (A-); positivity of tau pathology (T) and neurodegeneration (N) was defined by P-tau₁₈₁ and T-tau levels ≥ 56.5 pg/mL and ≥ 404 pg/mL, respectively [34]. NFL measurement was performed on the Simoa SR-X platform (Quanterix, Lexington, MA, USA) as previously described [35].

Statistical analysis

Analyses were performed with statistical software R version 4.1.1. Descriptive statistics were reported as means and standard deviations for quantitative variables or frequencies (%) for categorical ones. Paired sample t-test was used to compare demographic and clinical features of the whole cohort versus the CSF subcohort. Log transformation was applied to all CSF and serum biomarkers values to obtain a normal distribution, and derived measures were used in the regression analyses. Dependent and independent variables were standardized prior to regression analyses to achieve standardized beta values. Linear regression was employed for modeling the association of CSF biomarkers with motor and cognitive variables of interest, indexes of disease progression, as well as serum levels of NFL, and to investigate differences in the distribution of these variables in APOE-E4 carriers and non-carriers. Accordingly, separate comparisons between patients stratified according to amyloid and tau status were also performed. Binary logistic regression was used to assess if CSF biomarkers predicted presence or absence of

cognitive impairment in different ECAS subdomains. Chi squared test was employed to compare the distribution of APOE genotypes in cognitively impaired and unimpaired patients. Age at evaluation was introduced as a covariate when appropriate. A sensitivity analysis was performed to assess if the association of APOE status with ECAS scores retained significance after covariation for variables known to be associated with cognitive impairment (gender, Δ FS, region of onset, and C9orf72 expansion). Survival analysis was performed with Kaplan–Meier curves and log-rank test was used to compare survival curves across groups. Values of $p < 0.05$ were considered statistically significant.

RESULTS

Demographic and clinical features of ALS cohort

We studied a cohort of 281 ALS patients (179 males). Mean age at onset was 61.6 (± 11.7) years, while median survival was 49.3 (41.1–57.4) months. A positive family history was reported by 41 (14.5%) patients. Site of onset was bulbar in 63 (22.4%) and spinal in 218 (77.6%) patients. Mutations in ALS-associated genes were observed in 39 patients (21 C9orf72, 11 TARDBP, 6 SOD1, 1 FUS). According to the Strong revised criteria [36], 75 (26.7%) patients had cognitive impairment, 52 (18.5%) had behavioral impairment, and 35 (12.4%) had both cognitive and behavioral impairment, while 119 (42.3%) were cognitively and behaviorally unimpaired. Measurement of CSF biomarkers was available for 105 ALS patients (70 males) (Table 1).

Association of APOE alleles with ALS phenotype

In the whole cohort, genetic analysis revealed the presence of the E2|E3 genotype in 32 (11.4%), E2|E4 in 4 (1.4%), E3|E3 in 188 (66.9%), E3|E4 in 53 (18.9%), and E4|E4 in 4 (1.4%) patients. No individuals homozygous for the E2 allele were found. Allele frequencies were 6.1% for E2, 82.6% for E3, and 10.9% for E4. Regarding the CSF subcohort, the E4 allele was reported in 15/105 (14.3%) patients (14 E3|E4 and 1 E4|E4). After excluding patients with the E2|E4 genotype, individuals carrying at least one E4 allele had a lower median ECAS total score compared with non-carriers (104.0 vs. 106.0; $p = 0.037$) indicating worse cognitive performances. This is explained by E4 carriers being more severely impaired in ALS-nonspecific cognitive domains (25.0 vs. 27.0; $p = 0.009$), specifically memory (14.0 vs. 16.0; $p = 0.005$) (Figure 1). After stratifying for covariates that are known to influence cognitive performances (i.e., gender, site of onset, Δ FS, and C9orf72 expansion), we still observed an effect of allele E4 on ECAS total score (104.0 vs. 106.0; $p = 0.028$), ECAS ALS-nonspecific (25.0 vs. 27.0; $p = 0.012$), and memory subdomain (14.0 vs. 16.0; $p = 0.008$). Furthermore, when considering ECAS cut-offs, E4 carriers presented more frequently with memory impairment compared with non-carriers [6/15 (40.0%) vs. 12/88 (13.6%); $p = 0.036$]. Conversely, the E4 allele was not associated with ALS-specific

TABLE 1 Comparison of demographic and clinical features between the whole cohort and the cerebrospinal fluid subcohort.

Feature	Total ALS cohort	CSF subcohort	P value
Patients, <i>n</i>	281	105	
Age at onset, mean ± SD	61.6 ± 11.7	62.8 ± 10.6	0.428
Sex, <i>n</i> (%)			
M	179 (63.7)	70 (66.7)	0.672
F	102 (36.3)	35 (33.3)	0.672
Family history, <i>n</i> (%)			
SALS	240 (85.4)	91 (86.7)	0.90
FALS	41 (14.6)	14 (13.3)	0.90
Disease duration, mean ± SD	16.5 ± 13.9	14.1 ± 10.6	0.258
Site of onset, <i>n</i> (%)			
Bulbar	63 (22.4)	25 (23.8)	0.891
Spinal	218 (77.6)	80 (76.2)	0.892
ALSFRS-R, mean ± SD	39.8 ± 6.7	39.5 ± 6.5	0.351
PUMNS, mean ± SD	9.4 ± 7.2	9.7 ± 7.0	0.751
MRC, score mean ± SD	52.0 ± 7.9	52.4 ± 7.5	0.614
APOE status, <i>n</i> (%)			
E2 E3	32 (11.4)	14 (13.3)	0.643
E2 E4	4 (1.4)	1 (1.0)	0.644
E3 E3	188 (66.9)	75 (71.4)	0.645
E3 E4	53 (18.9)	14 (13.3)	0.646
E4 E4	4 (1.4)	1 (1.0)	0.647
C9orf72 expansion, <i>n</i> (%)	21 (7.4)	2 (1.9)	0.266
TARDBP mutation, <i>n</i> (%)	11 (3.9)	4 (3.8)	0.005
Cognitive phenotype, <i>n</i> (%)			
ALScn	119 (42.3)	46 (44.2)	0.550
ALSci	52 (18.5)	34 (32.4)	0.551
ALSbi	35 (12.4)	15 (14.3)	0.552
ALScbi	75 (26.7)	10 (9.6)	0.553
ECAS cognitive subdomains impairment, <i>n</i> (%)			
Language	59 (21.0)	21 (20)	0.962
Fluency	56 (23.5)	20 (19.0)	0.981
Executive	66 (23.5)	27 (25.7)	0.725
Memory	41 (14.6)	18 (17.1)	0.628
Visuospatial	19 (6.7)	10 (9.5)	0.474

Note: Demographic and clinical features of the entire cohort and the CSF subcohort. Paired sample *t*-test to compare differences between the two groups.

Abbreviations: ALS, amyotrophic lateral sclerosis; ALSbi, ALS behaviorally impaired; ALScbi, ALS cognitively and behaviorally impaired; ALSci, ALS cognitively impaired; ALScn, ALS cognitively normal; ALSFRS-R, ALS Functional Rating Scale Revised; APOE, apolipoprotein E; CSF, cerebrospinal fluid; ECAS, Edinburgh Cognitive and Behavioural ALS Screen; F, female; FALS, familial amyotrophic lateral sclerosis; M, male; MRC, Medical Research Council; PUMNS, Penn Upper Motor Neuron Score; SALS, sporadic amyotrophic lateral sclerosis; SD, standard deviation.

cognitive domains, nor with behavioral symptoms assessed using both ECAS and FBI. Finally, no differences were observed regarding age at onset, site of onset, survival, and motor features.

CSF and serum biomarkers analysis

Based on CSF biomarker values, 17 (16.2%) patients were classified as A+, 15 (14.2%) as T+, and 24 (22.8%) as N+, with 7 patients (6.6%) displaying an A+T+N+ profile (Table S1). Remarkably, a significant number of ALS patients (*N*=39, 37.1%) displayed CSF A β_{42} levels below 599 pg/mL, that is, the cut-off used to define A positivity when A β_{40} is not measured and the A $\beta_{42/40}$ ratio cannot be calculated. The presence of at least one E4 allele was associated with significantly lower A $\beta_{42/40}$ (-0.1 vs. 0.3 ; $p < 0.001$) and A β_{42} (-0.8 vs. 0.1 ; $p = 0.038$), while no association was found with other CSF biomarkers (Figure 2). Moreover, the E4 allele was more frequently observed in patients with a full AD neurochemical pattern (A+T+N+) compared with the remaining CSF cohort (4/7 [57.1%] vs. 11/97 [11.3%]; $p = 0.005$).

As for the relationship among CSF biomarkers, lower values of A $\beta_{42/40}$ correlated with higher levels of both P-tau $_{181}$ ($\beta = -0.33$; 95% CI = -0.49 , -0.17 ; $p < 0.001$) and T-tau ($\beta = -0.29$; 95% CI = -0.47 , -0.12 ; $p = 0.001$) (Figure 3a,b). Moreover, A+ individuals displayed significantly higher levels of both T-tau and P-tau $_{181}$ compared with A- ones (for T-tau: 0.54 vs. -0.25 ; $p = 0.002$; for P-tau $_{181}$: 0.79 vs. -0.25 ; $p < 0.001$), thus reproducing the pattern observed in AD. Conversely, we found that both A β_{42} and A β_{40} , when considered individually, positively correlated with P-tau $_{181}$ (A β_{42} : $\beta = 0.38$; 95% CI = 0.22 , 0.52 ; $p < 0.001$; A β_{40} : $\beta = 0.71$; 95% CI = 0.59 , 0.83 ; $p < 0.001$) (Figure 3c-e) and T-tau (A β_{42} : $\beta = 0.32$; 95% CI = 0.16 , 0.49 ; $p < 0.001$; and A β_{40} : $\beta = 0.63$; 95% CI = 0.48 , 0.77 ; $p < 0.001$) (Figure 3d-f). We did not observe any association between the presence of ALS-associated mutations and CSF biomarkers.

NFL serum levels were available for 86 of 105 patients with CSF biomarkers. The A $\beta_{42/40}$ was inversely associated with NFL ($\beta = -0.19$; 95% CI = -0.37 , -0.02 ; $p = 0.027$) while T-tau displayed a positive association ($\beta = 0.23$; 95% CI = 0.04 , 0.43 ; $p = 0.015$). No association was observed between the others CSF biomarkers and NFL.

Association of CSF biomarkers with neuropsychological domains explored by ECAS

Lower A $\beta_{42/40}$ correlated with lower ECAS memory scores ($\beta = 0.20$; 95% CI = 0.003 , 0.39 ; $p = 0.044$), while no correlation was observed with ALS-specific cognitive functions. When applying ECAS cut-off values for individual cognitive domains, we again observed lower A $\beta_{42/40}$ in patients with memory impairment compared with unimpaired ones (-0.82 vs. 0.34 ; $p = 0.006$).

Conversely, A β_{42} showed a positive correlation with both specific and nonspecific ECAS cognitive domains: total score ($\beta = 0.27$; 95% CI = 0.10 , 0.43 ; $p = 0.001$), ALS-nonspecific ($\beta = 0.24$; 95%

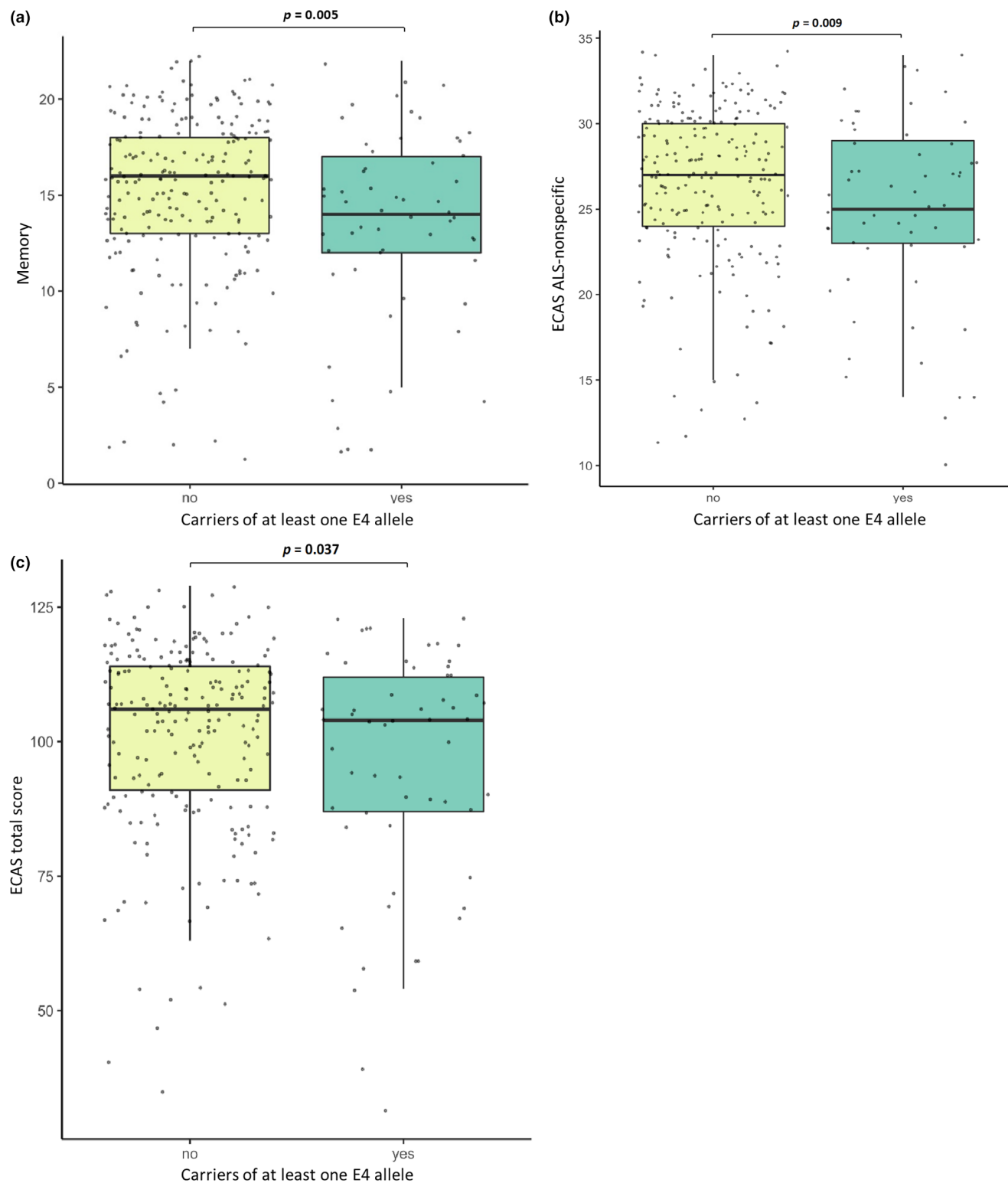


FIGURE 1 Distribution of Edinburgh Cognitive and Behavioural ALS Screen (ECAS) total (a), amyotrophic lateral sclerosis (ALS)-nonspecific (b), and memory scores (c) in carriers and non-carriers of at least one APOE-E4 allele. For each group, the bold line shows the median, the coloured box includes the middle 50% of the values, and the extreme points of the vertical line indicate the minimum and maximum values. Black dots represent single individual scores.

CI=0.06, 0.42; $p=0.008$), ALS-specific ($\beta=0.24$; 95% CI=0.08, 0.41; $p=0.004$), memory ($\beta=0.22$; 95% CI=0.03, 0.40; $p=0.018$), and fluency ($\beta=0.22$; 95% CI=0.04, 0.41; $p=0.016$). These relationships

held true also when using ECAS cut-offs for individual domains, with ALS patients with verbal fluency or memory impairment displaying lower $A\beta_{42}$ levels compared with unimpaired ones (verbal

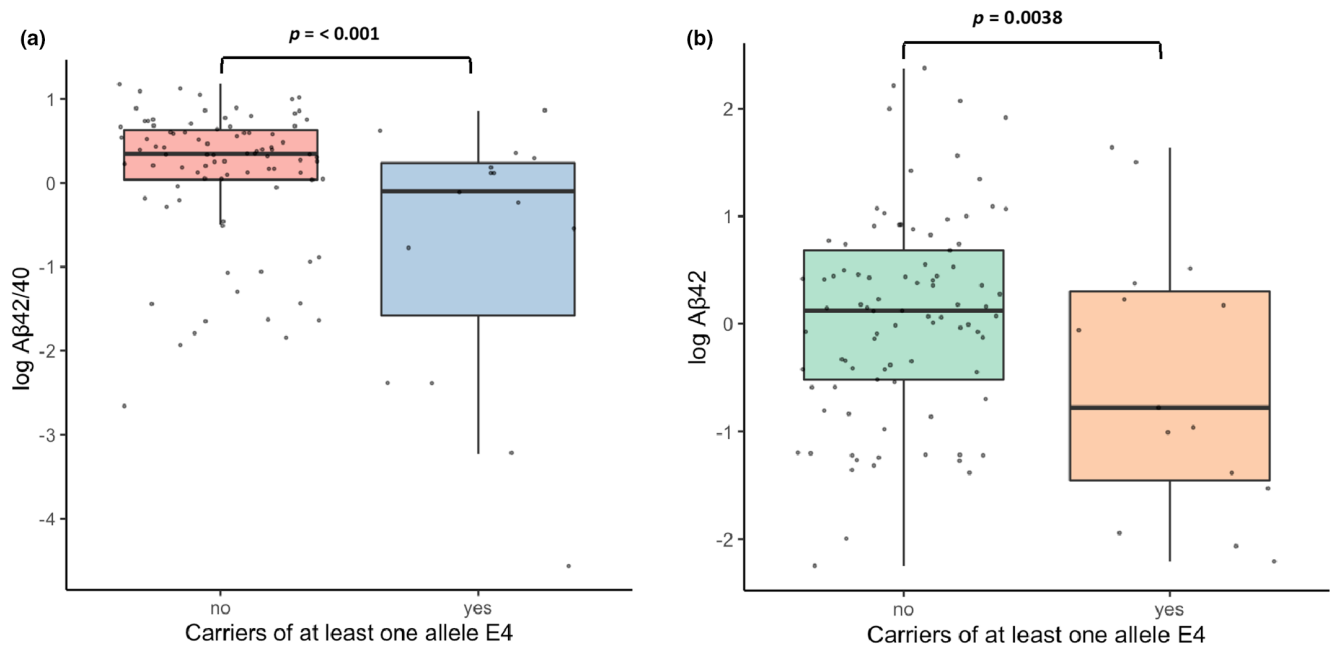


FIGURE 2 Distribution of cerebrospinal fluid (CSF) $A\beta_{42/40}$ and $A\beta_{42}$ levels between carriers and non-carriers of at least one APOE-E4 allele. For each group, the wide horizontal line shows the median, the coloured box includes the middle 50% of the values, and the extreme points of the vertical line show the minimum and maximum values. Black dots represent single individual scores.

fluency: -0.36 vs. 0.17 ; $p=0.016$; memory: -0.05 vs. 0.12 ; $p=0.022$). Interestingly, after splitting the cohort according to the $A\beta_{42}$ cut-off, we again observed that patients with lower $A\beta_{42}$ levels obtained significantly lower ECAS total (102.0 vs. 104.0 ; $p=0.025$), ALS-specific (75.0 vs. 79.0 ; $p=0.032$), and fluency scores (16.0 vs. 18.0 ; $p=0.015$) compared with those with normal $A\beta_{42}$ (Figure S1).

The association between $A\beta$ species and cognitive performance was also confirmed by the observation that lower CSF $A\beta_{40}$ levels were positively correlated with ECAS total ($\beta=0.26$; 95% CI= $0.07, 0.44$; $p=0.005$), ALS-specific ($\beta=0.25$; 95% CI= $0.07, 0.43$; $p=0.007$), and fluency scores ($\beta=0.36$; 95% CI= $0.17, 0.56$; $p<0.001$), and were more frequently observed in ALS individuals with fluency impairment compared with the remaining cohort (-0.43 vs. 0.20 ; $p=0.002$).

Surprisingly, we found that both CSF T-tau and P-tau₁₈₁ positively correlated with ECAS fluency score (T-tau: $\beta=0.30$; 95% CI= $0.10, 0.51$; $p=0.003$; P-tau₁₈₁: $\beta=0.32$; 95% CI= $0.11, 0.54$; $p=0.003$), and that lower levels of both tau proteins were observed in individuals with pathological fluency scores compared with those with normal scores (P-tau₁₈₁: -0.36 vs. 0.02 ; $p=0.005$; T-tau: -0.47 vs. 0.05 ; $p=0.005$). Nevertheless, after adding $A\beta_{40}$ as covariate in the regression analysis, the actual determinant of fluency score was $A\beta_{40}$ (model with T-tau: $\beta=0.30$; 95% CI= $0.04, 0.55$; $p=0.022$; model with P-tau₁₈₁: $\beta=0.30$; 95% CI= $0.02, 0.63$; $p=0.036$), while T-tau and P-tau₁₈₁ lost statistical significance. Conversely, no differences in cognitive scores were observed between T+ and T- and between N+ and N- groups. (Table S2) reports correlations between CSF biomarkers and ECAS cognitive domains. Finally, we did not observe any correlations between CSF biomarkers and behavioral domains.

Association of CSF biomarkers with motor features

While $A\beta_{42/40}$ was not associated with motor features, we found positive correlations between both $A\beta_{42}$ and $A\beta_{40}$ and MRC score ($A\beta_{42}$: $\beta=0.22$; $p=0.046$; $A\beta_{40}$: $\beta=0.34$; $p=0.002$) and a negative correlation between $A\beta_{40}$ and LMN score ($\beta=-0.23$; $p=0.041$), indicating that CSF amyloid β species are related to the severity of LMN degeneration (Figure 4). Moreover, higher $A\beta_{42}$ levels characterized patients with bulbar phenotype compared with those with classic ALS (0.5 vs. -0.01 ; $p=0.007$) (Figure S2), whereas T-tau positively correlated with Δ FS ($\beta=0.28$; $p=0.016$) (Figure 5).

Conversely, no association of T-tau and P-tau₁₈₁ with motor phenotype and burden of UMN and LMN signs was observed, nor were CSF biomarkers associated with survival.

DISCUSSION

Our results indicate that APOE genotype and CSF $A\beta$ and tau biomarkers are associated with cognitive and motor features in ALS. Specifically, presence of at least one E4 allele and lower $A\beta_{42/40}$ were associated with memory impairment while lower $A\beta_{42}$ and $A\beta_{40}$ levels were associated with diffuse cognitive deficits involving ALS-specific functions. Lower $A\beta_{42}$ and $A\beta_{40}$ levels were observed in cases with more severe LMN involvement, while CSF T-tau positively correlated with Δ FS and NFL serum levels. Classic ALS was characterized by lower $A\beta_{42}$ levels compared with bulbar phenotype. When analyzing relationships between APOE and CSF biomarkers, we found associations partly explainable according to an AD-like

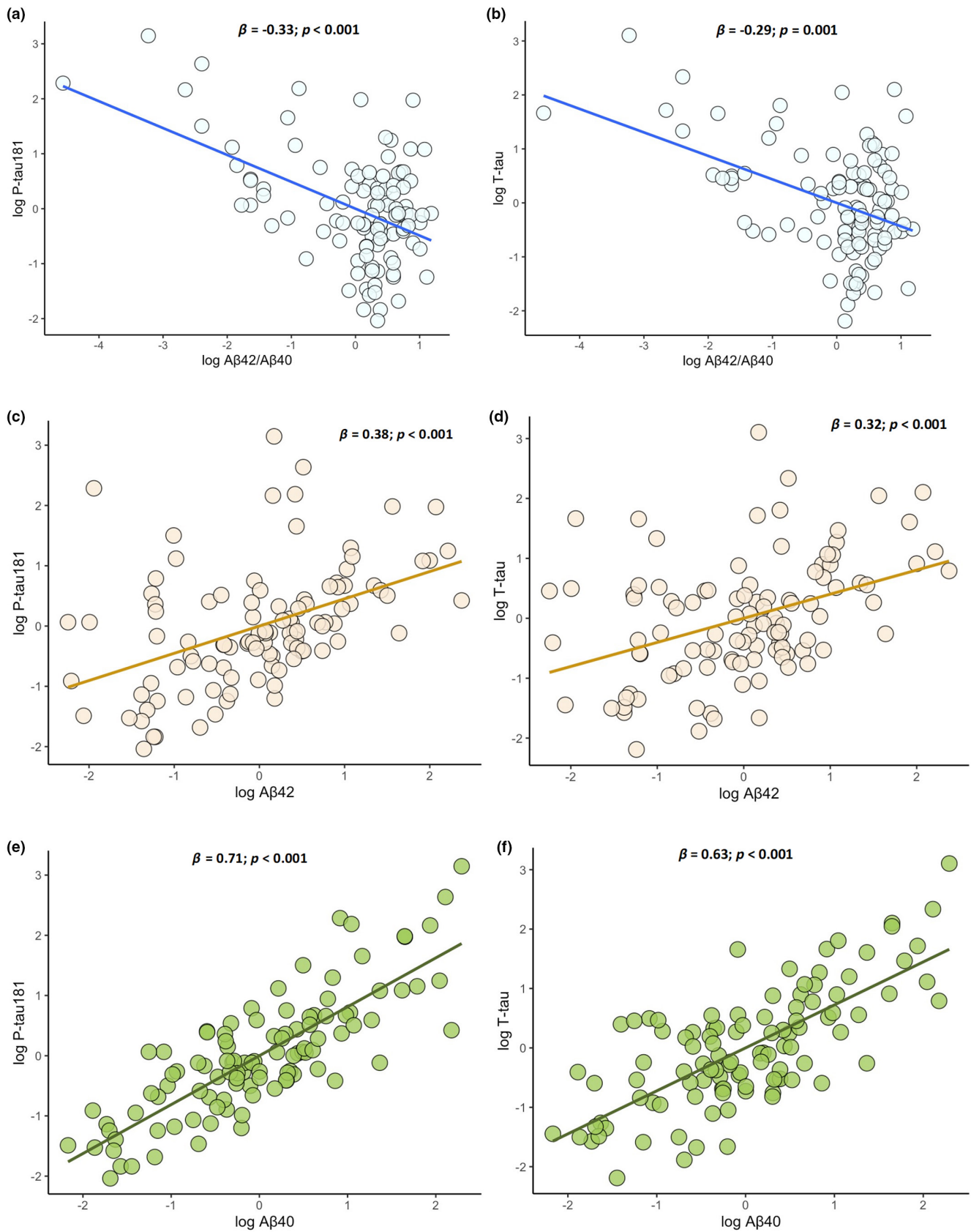


FIGURE 3 Simple dispersion with adjustment curve displaying significant negative correlations of $A\beta_{42/40}$ ratio with T-tau and P-tau₁₈₁ (a,b) and significant positive correlations of cerebrospinal fluid (CSF) $A\beta_{42}$ (c,d) and $A\beta_{40}$ (e,f) with T-tau and P-tau₁₈₁.

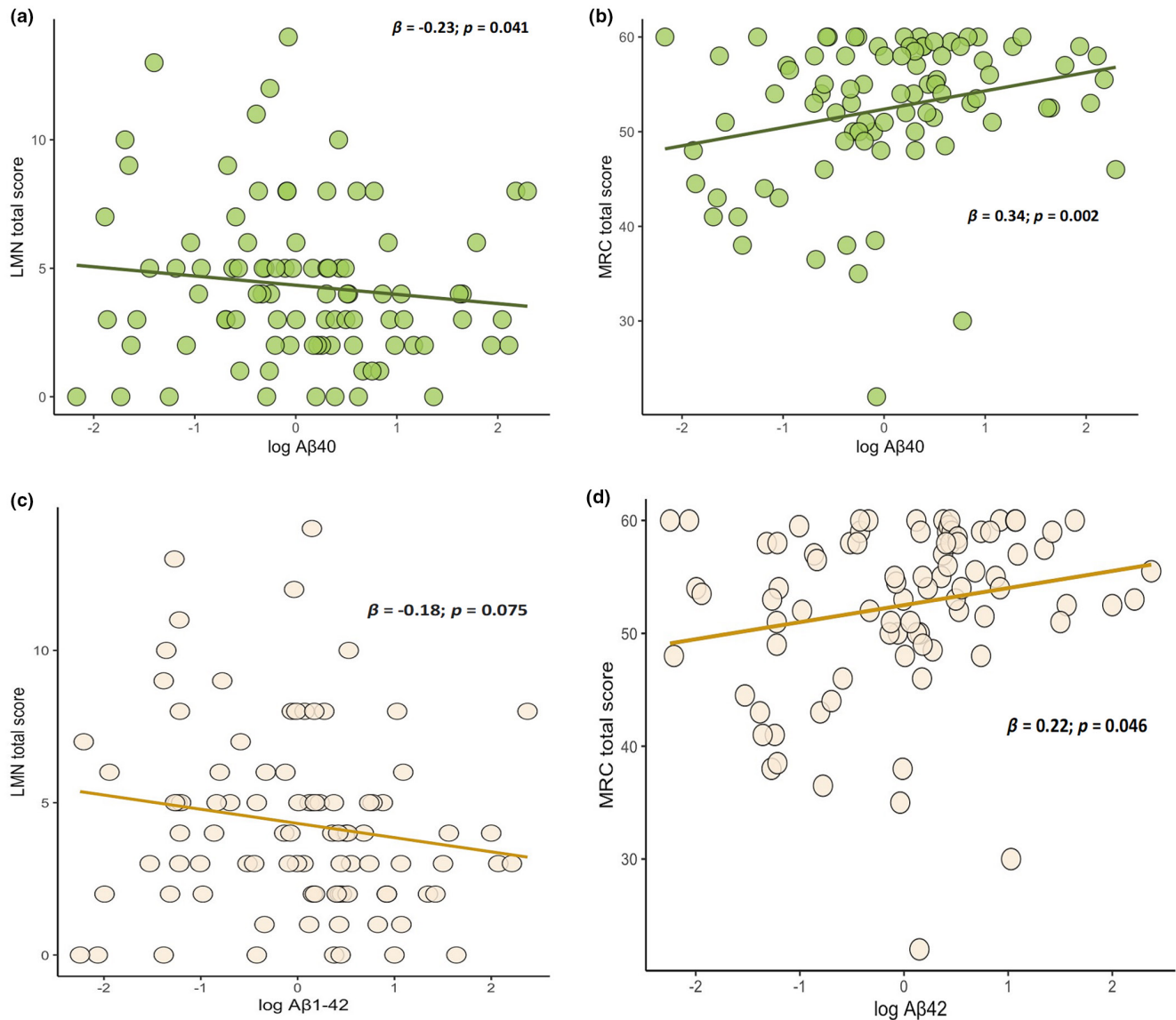


FIGURE 4 Simple dispersion with adjustment curve illustrating correlations of Aβ₄₀ and Aβ₄₂ with clinical indexes of lower motor neuron (LMN) impairment, namely LMN (a–c) and Medical Research Council (MRC) scores (b–d), respectively.

pathophysiological model. Indeed, presence of at least one E4 allele was associated with lower Aβ₄₂ and Aβ_{42/40} values. Furthermore, Aβ_{42/40} negatively correlated with both P-tau₁₈₁ and T-tau. Contrary to what is observed in AD, higher Aβ₄₂ and Aβ₄₀ levels were associated with higher values of both tau proteins.

Although a major ALS pathomechanism is represented by accumulation of TDP-43 protein within MNs [37], other, less investigated biological processes might be at play. Indeed, neuropathological studies identified Aβ pathology in up to one-half of autopsied ALS cases [38, 39]. Although this could be partly explained by the frequent occurrence of Aβ pathology in the elderly, in our cohort we found an unexpected prevalence of patients with low Aβ₄₂ levels, namely twice as high as that reported for cognitively unimpaired individuals of similar age by a meta-analysis [40], thus possibly reflecting increased cortical Aβ burden of potential pathogenic relevance. Interestingly, lower Aβ_{42/40} was associated with worse memory

scores, while Aβ₄₂ inversely correlated with scores in both ALS-specific and -nonspecific domains. Furthermore, the presence of at least one APOE-E4 allele was associated with lower values of both Aβ_{42/40} and Aβ₄₂ and with more severe cognitive impairment in ALS-nonspecific domains, particularly memory, supporting the role of APOE as major genetic determinant of cognitive impairment through Aβ-dependent mechanisms. Interestingly, association of APOE haplotypes with ALS-nonspecific domain of ECAS remained significant after covariation for variables influencing the cognitive profile. These findings are consistent with dynamics partly recapitulating AD pathophysiological processes, with Aβ deposition triggering tau accumulation as indicated by the inverse association between lower Aβ_{42/40} and tau protein levels. Therefore, Aβ pathology might contribute to cognitive impairment in ALS through AD-like mechanisms. Moreover, it cannot be completely ruled out that the observed effect of amyloid species on cognitive impairment in ALS could be driven

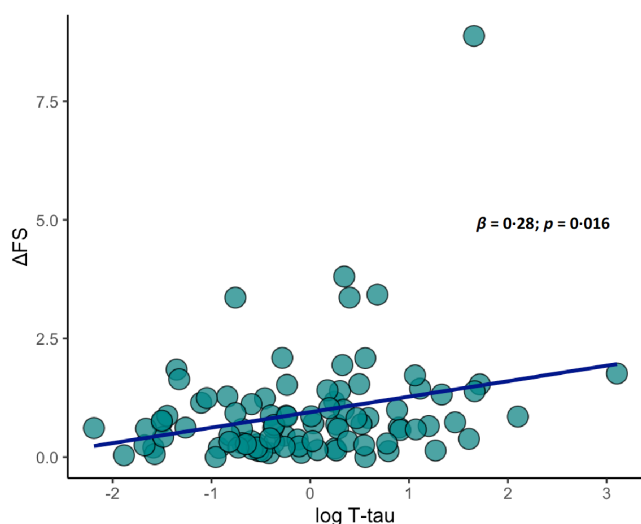


FIGURE 5 Simple dispersion with adjustment curve illustrating significant positive correlation between cerebrospinal fluid (CSF) T-tau levels and disease progression rate (Δ FS).

by an AD co-pathology that could be more frequent than generally considered [39, 41].

However, APOE could also contribute to cognitive abnormalities in ALS via different pathways, as suggested by the association between the E4 allele, TDP-43 pathology, and hippocampal sclerosis reported in a large neuropathological study [42]. Furthermore, a comprehensive analysis of our findings suggests that strictly applying the same biological paradigms used in AD to ALS might not be totally appropriate, with the risk of overlooking relevant biological clues. Indeed, contrary to AD, in our ALS cohort we found a positive correlation between CSF levels of A β and tau proteins. This only applied to single A β species and not to the A $\beta_{42/40}$ ratio. Therefore, it could be hypothesized that during the disease process, neuronal damage – reflected by increased T-tau, which in turn is associated with the index of disease progression Δ FS as well as serum levels of NFL – leads to an indirect increase of APP, and subsequently of A β_{42} and A β_{40} species, as a result of impaired axoplasmic transport or reactively enhanced APP synthesis [6, 43]. In fact, APP has been reported as a marker

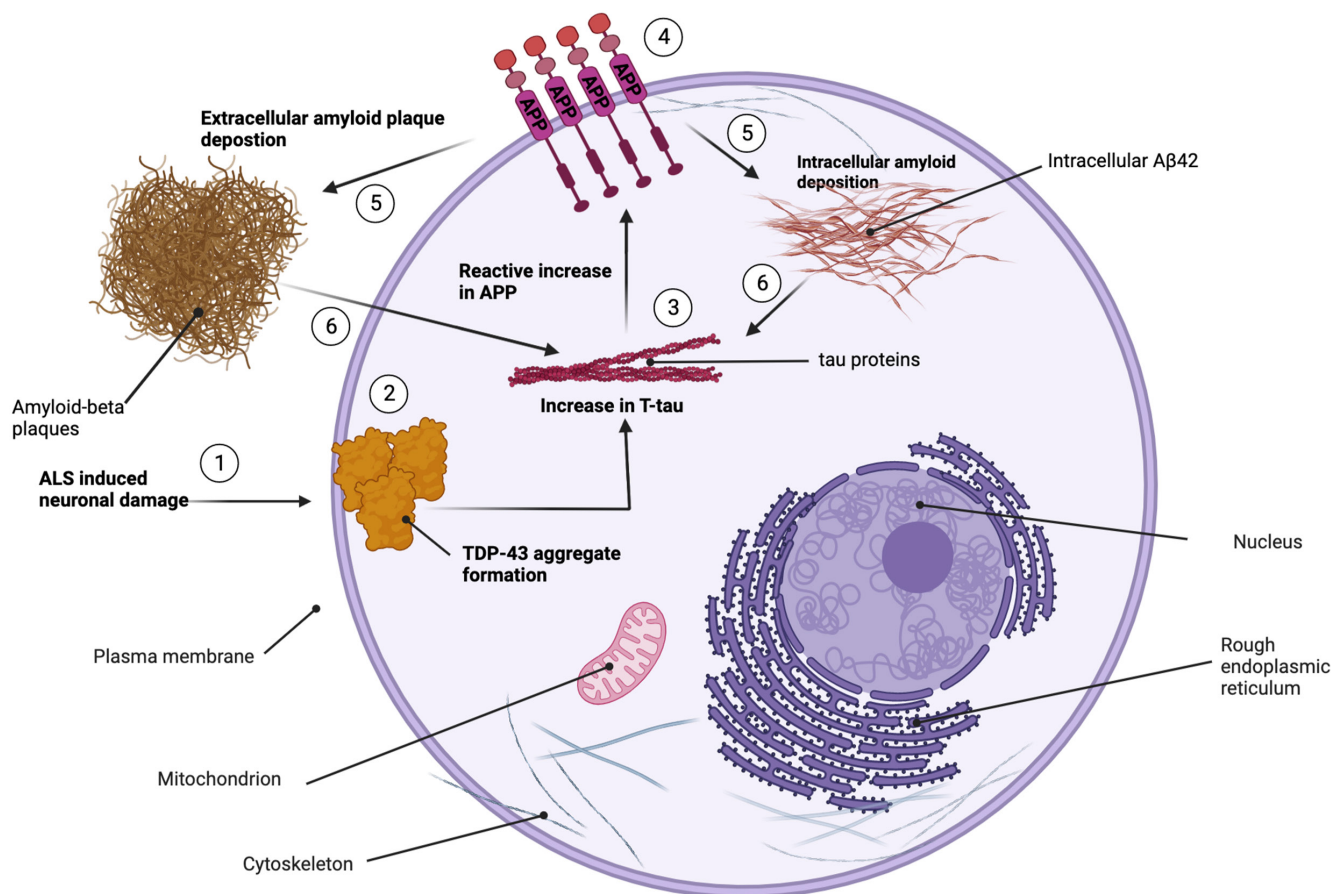


FIGURE 6 Illustration of proposed biological interplay between classic amyotrophic lateral sclerosis (ALS)-related mechanisms of damage and A β pathways. Neuronal damage caused by ALS (1) resulting in TDP-43 accumulation (2) is associated with progressive increase of T-tau protein (3). These cellular changes lead to an indirect increase of amyloid precursor protein (APP) (protein dysmetabolism or reactive increase) (4). The APP increase may subsequently cause intracellular amyloid accumulation or extracellular amyloid plaque deposition (5), circularly triggering further neuronal damage (6). Image created with BioRender. Adopted from "Structural overview of an animal cell template" by BioRender.com (2022). Retrieved from <https://app.biorender.com/biorender-templates>.

of axonal damage across different neurological conditions, including traumatic brain injury [44]. However, it is unclear whether the putative increase in APP following neuronal damage can be explained by impairment of cell structures involved in APP metabolism such as the Golgi apparatus, which is disrupted in ALS MNs [45], or rather represents a protective mechanism against glutamate excitotoxicity or proteasomal stress [46]. Regardless, the finding that lower $A\beta_{42}$ and $A\beta_{40}$ levels correlated with LMN involvement is consistent with the hypothesis that APP and its fragments might be part of a protective system whose deficiency accelerates disease progression [7]. Conversely, considering the unexpectedly high prevalence of low CSF $A\beta_{42}$ in our cohort, it could be speculated that a reactive increase in $A\beta$ species following MN damage might favor pathological $A\beta$ accumulation, thus leading to additional neurotoxicity. Finally, our study shows that $A\beta_{42}$ levels are higher in patients with bulbar compared with classic phenotype. This finding suggests that a more diffuse disease process enhances $A\beta$ accumulation, thus determining lower CSF $A\beta_{42}$ levels. Interestingly, a previous study found lower levels of soluble APP fragments sAPP α and sAPP β in limb-onset compared with bulbar-onset ALS patients [6]. In summary, our results suggest that: (1) AD pathology might contribute to cognitive dysfunction in ALS and more specifically to memory impairment; (2) it is likely that AD-related mechanisms do not fully explain the role of $A\beta$ species in ALS; (3) increased $A\beta$ production might represent either a protective mechanism against neuronal damage or a direct consequence of impaired $A\beta$ metabolism due to ALS pathological processes [47]; (4) $A\beta$ increase may trigger its intracellular accumulation or extracellular plaque formation, thus promoting neuronal death (Figure 6); and (5) CSF T-tau levels may represent a biomarker of disease progression in ALS.

The collateral finding that higher levels of tau proteins are associated with higher verbal fluency scores remains unclear. However, considering that the strongest association with fluency score was observed for $A\beta_{40}$ levels and not for tau proteins, we cannot exclude that $A\beta_{40}$ increase following early neuronal damage may elicit a protective response which initially succeeds in preserving cognitive functions before being overwhelmed by the disease.

Our study has some limitations. First, data were derived from a referral centre which is more susceptible to biases, such as longer median survival, compared with a registry population. Secondly, ECAS is tailored to assess primarily ALS-specific cognitive deficits, and may not represent the ideal tool to assess the presence of subclinical memory deficit or other cognitive features typical of AD. Moreover, although the measurement of CSF AD biomarkers complements the characterization of cognitive and motor symptoms in our ALS cohort, it does not allow a thorough exploration of the biological pathways through which $A\beta$ metabolism might contribute to ALS pathophysiology. In particular, it is not possible to establish whether low CSF levels of $A\beta_{42}$ are associated with extracellular amyloid plaque deposition as described in AD or with intracellular $A\beta$ accumulation as suggested by a previous neuropathological

study [8]. Moreover, sAPP α and sAPP β were not investigated. The lack of neuroimaging data prevented us from investigating whether amyloid and tau pathology were associated with atrophy of brain regions usually involved in AD, namely hippocampus and precuneus. Finally, despite the high concordance between CSF $A\beta_{42/40}$ levels and amyloid-positron emission tomography (PET) markers [48, 49], amyloid-PET derived data would be useful to have an additional confirmation of our findings and to further investigate this topic also in relation to spatial and regional distribution.

Our findings suggest that CSF levels of $A\beta$ and tau proteins might be associated with cognitive and motor features of ALS patients. Moreover, albeit preliminary, our results indicate that $A\beta$ species might play a more important role in ALS pathogenesis than previously thought. Further studies, using PET-derived data or directly neuropathological findings, are thus warranted to investigate the biological significance of these proteins in the pathological processes leading to ALS-related neurodegeneration.

AUTHOR CONTRIBUTIONS

Alessio Maranzano: Conceptualization; investigation; methodology; writing – review and editing; software; formal analysis; data curation; writing – original draft. **Federico Verde:** Conceptualization; investigation; methodology; data curation; supervision; writing – review and editing; funding acquisition. **Antonella Dubini:** Data curation; resources. **Silvia Torre:** Data curation; resources. **Eleonora Colombo:** Writing – review and editing. **Alberto Doretti:** Writing – review and editing. **Francesco Gentile:** Writing – review and editing; formal analysis. **Arianna Manini:** Writing – review and editing; formal analysis. **Ilaria Milone:** Data curation; resources. **Alberto Brusati:** Data curation; formal analysis. **Silvia Peverelli:** Data curation. **Serena Santangelo:** Writing – review and editing. **Edoardo Gioele Spinelli:** Writing – review and editing. **Erminio Torresani:** Writing – review and editing. **Davide Gentilini:** Writing – review and editing. **Stefano Messina:** Writing – review and editing. **Claudia Morelli:** Writing – review and editing. **Barbara Poletti:** Writing – review and editing; data curation; supervision; resources. **Federica Agosta:** Writing – review and editing. **Antonia Ratti:** Data curation; resources; writing – review and editing. **Massimo Filippi:** Writing – review and editing. **Vincenzo Silani:** Writing – review and editing. **Nicola Ticozzi:** Conceptualization; investigation; funding acquisition; writing – review and editing; methodology; formal analysis; supervision.

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CONFLICT OF INTEREST STATEMENT

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DATA AVAILABILITY STATEMENT

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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