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Association of Copresence of Pathogenic Variants Related to Amyotrophic Lateral Sclerosis and Prognosis

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Abstract

Objective. Despite recent advances, it is not clear whether the various genes/genetic variants related to ALS interact in modifying patients phenotype. The aim of this study was to determine if the co-presence of genetic variants related to ALS has interactive effects on the course of the disease.

Methods. The study population includes 1,245 ALS patients identified through the Piemonte Register for ALS between 2007 and 2016 and not carrying SOD1, TARDBP and FUS pathogenic variants. Controls were 766 Italian subjects age-, sex-, and geographically-matched to cases. We considered UNCI3A (rs12608932), CAMTA1 (rs2412208), SLC11A2 (rs407135) and ZNF512B (rs2275294) variants, as well as ATXN2 polyQ intermediate repeats (≥31) and C9orf72 GGGGCC intronic expansions(≥30).
**Results.** The median survival time of the whole cohort was 2.67 years (IQR 1.67-5.25). In univariate analysis only C9orf72 (2.51 years, IQR 1.74-3.82; p=0.016), ATXN2 (1.82 years, IQR 1.08-2.33; p<0.001) and UNCI3A<sup>C/C</sup> (2.3 years, IQR 1.3-3.9; p<0.001) significantly reduced survival. In Cox multivariable analysis, also CAMTA1 emerged to be independently related to survival (HR 1.13, 95% c.i. 1.001-1.30, p=0.048). The co-presence of two detrimental alleles/expansions was correlated with shorter survival. In particular, the median survival of patients with CAMTA1<sup>G/G+G/T</sup> and UNCI3A<sup>C/C</sup> alleles was 1.67 years (1.16-3.08) years compared to 2.75 years (1.67-5.26) of the patients not carrying these variants (p<0.001); the survival of patients with CAMTA1<sup>G/G+G/T</sup> alleles and ATXN2<sup>≥31</sup> intermediate polyQ repeats was 1.75 years (0.84-2.18) (p<0.001); the survival of patients with ATXN2<sup>≥31</sup> polyQ repeats and UNCI3A<sup>C/C</sup> allele was 1.33 years (0.84-1.75) (p<0.001); the survival of patients with C9ORF72<sup>≥30</sup> and UNCI3A<sup>C/C</sup> allele was 1.66 years (1.41-2.16). Each pair of detrimental alleles/expansions was associated to specific clinical phenotypes.

**Conclusions.** We showed that gene variants acting as modifiers of ALS survival or phenotype can act on their own or in unison. Overall, 54% of patients carried at least one detrimental common variant or repeat expansion, emphasizing the clinical impact of our findings. In addition, the identification of the interactive effects of modifier genes represents a crucial clue for explaining ALS clinical heterogeneity and should be considered when designing and interpreting clinical trials results.
Introduction

Amyotrophic lateral sclerosis (ALS) is a progressive degenerative disorder of the CNS, characterized by the involvement of upper motor neurons and lower motor neurons, as well as the cortical neurons of the frontotemporal cortices. ALS is considered a multifactorial disorder caused by an interaction between genetics and the environment. While relatively little is known about the environmental contributions to ALS, pathogenic variants in more than thirty genes have been linked to the disease, the most common being C9orf72, SOD1, TARDBP and FUS. Overall, the genetic etiology is known for about 70% of ALS patients with a familial history of ALS (fALS) and 10% of apparently sporadic ALS patients (sALS).

In addition to disease-causing genes, several other genes have been reported to be modifiers of ALS phenotype, especially patients’ survival. Among these, the most relevant are UNC13A (rs12608932 variant), CAMTA1 (rs2412208 variant), ATXN2 (intermediate polyQ repeats), SLC11A2 (rs407135 variant), and ZNF512B (rs2275294 variant). Interestingly, UNC13A variant has been demonstrated to be also a modifier of the response to drugs. These observations are important from the clinical trial perspective. Not only does it provide additional new targets for drug development, but it also suggests that these data should be incorporated into the clinical trial design; their effect on survival often equals the anticipated therapeutic effect, meaning balancing of genotypes in the treatment and placebo arms is needed to avoid false positive findings.

Despite these advances, it is not clear whether the various ALS genes/genetic variants interact in modifying the phenotype of patients. This study aimed to determine if the co-presence of variants related to disease has interactive effects on the course of ALS in a population-based cohort.

Methods

The study population includes the ALS patients diagnosed between 2007 and 2016 and identified through an Italian prospective population-based register (Piemonte and Valle d’Aosta Register for ALS, PARALS). All patients were diagnosed as definite, probable, probable laboratory supported or possible ALS according to El Escorial revised criteria (Brooks et al 2020). More details concerning the epidemiological register are reported in the Supplemental data. Controls were randomly identified from the lists of patients’ general practitioners (GPs) and matched to the cases by sex, age (±5 years). Since the list of GPs...
assisted persons is by definition in the same community of their assisted ALS patients, a geographical matching was ensured.

**Whole Genome Analysis.** WGS methods are reported in detail as eMethods in the Supplement. Whole genome sequencing (WGS) of 1029 ALS subjects and 766 controls have already been reported. An additional 290 ALS cases underwent whole genome sequencing as described elsewhere.

**ATXN2 CAG and C9orf72 repeat analysis.** C9ORF72 intronic expansions were determined using an established repeat-primed PCR method. ATXN2 polyQ repeat in exon 1 (NM_002973.3) was amplified using a fluorescent primer and sized by capillary electrophoresis on an ABI3130 genetic analyzer (Applied Biosystems, Foster City, CA, USA). Both methodologies are described in detail as eMethods and eAppendix 1 in the Supplement.

**Survival modifiers genes.** For the present study, we considered the following genes reported to be related to ALS outcome: UNC13A, CAMTA1, SLC11A2, and ZNF512B. We also considered the interaction between these genes and C9orf72 repeat expansion and ATXN2 polyQ intermediate repeats, which also affects the survival of patients with ALS. Gene variants were dichotomized as follows: UNC13A<sup>C/C</sup> vs UNC13A<sup>A/A+A/C</sup>; CAMTA1<sup>G/G+G/T</sup> vs CAMTA1<sup>T/T</sup>; SLC11A2<sup>A/C+C/C</sup> vs SLC11A2<sup>A/A</sup>; ZNF512B<sup>C/C+G/T</sup> vs. ZNF512B<sup>T/T</sup>. The first allele(s) reported here is the detrimental one. All dichotomies were based on the original papers reporting the gene in ALS or subsequent studies and were confirmed in our cohort (data not shown).

**Clinical variables.** The mean monthly decline of ALSFRS-R (ΔALSFRS-R) was calculated using the following formula: (48 – ALSFRS-R score at diagnosis)/(time from onset to diagnosis, in months). Similarly, the mean monthly decline of weight (ΔWeight) was calculated as (Weight at diagnosis – healthy body weight)/(time from onset to diagnosis, in months). Finally, to have a proxy of disease spread, the mean monthly decline of King’s staging (ΔKing’s) was calculated as (King’s staging at diagnosis)/(time from onset to diagnosis, in months).

A total of 909 patients underwent cognitive assessment at the time of diagnosis using an extensive test battery. Cases were classified into five categories according to the Consensus Criteria for diagnosing frontotemporal cognitive and behavioral syndromes in ALS. The battery assessed visuospatial function, language, executive function, memory, and social
cognition, as well as anxiety and depression, is reported in detail as eMethods in the Supplement.\textsuperscript{17}

**Statistical analysis.** Hardy-Weinberg equilibrium was calculated for all the considered variants. The effect of survival of each gene was firstly evaluated in isolation. Second, all genes were assessed together in Cox multivariable analysis. Third, the interaction of alleles on survival and other phenotypic characteristics was evaluated by pair of genes. Differences between continuous variables were assessed with the Mann-Whitney U test. Differences between discrete variables were assessed with the $\chi^2$ test. Kaplan-Meier curves were used to calculate survival and were compared with the log-rank test, setting the onset date as day 0 and the date of death or tracheostomy as the endpoint. The last day of follow-up for censored cases was December 31, 2021.

Multivariable analysis for survival was performed with the Cox proportional hazards model (stepwise backward) with a retention criterion of a p-value less than 0.1. In the final model we considered significant a p-value $<$0.05. Besides the examined genes, the following variables were included in the model: age at onset (continuous), time from onset to diagnosis (continuous), genetic sex (male versus female), site of onset (bulbar versus spinal), King’s staging, ΔALSFRS-R (continuous), FVC% at diagnosis (continuous), ΔWeight (continuous), ΔKing’s (continuous), and chronic obstructive pulmonary disease (COPD) (yes versus no).

The SPSS 28.0 statistical package was used for the analyses (SPSS, Chicago, IL, USA).

**Standard Protocol Approvals, Registrations, and Patient Consents.** The study was approved by the Ethics Committees of the ALS Expert Centers of Torino and Novara (Comitato Etico Azienda Ospedaliero-Universitaria Città della Salute e della Scienza, Torino, and Comitato Etico Azienda Ospedaliero-Universitaria Maggiore della Carità, Novara #0038876). Patients and controls provided written informed consent before enrollment. The databases were anonymized according to Italian law for the protection of privacy.

**Data Availability Statement.** The individual-level sequence data are available on dbGaP (accession number: phs001963.v1.p1). Phenotypic data will be available upon motivated request by interested researchers.
Results

During the 2007-2016 period a total of 1,445 patients were diagnosed with ALS in the study area. Of these, 1,319 (91.2%) had available DNA and were therefore whole genome sequenced. A total of 74 patients carrying SOD1 (n=45), TARDBP (n=22) and FUS (n=7) pathogenic variants were excluded from the analysis due to the heterogeneous clinical course of the different missense and nonsense pathogenic variants of these genes. Therefore, the final study population included 1,245 patients (n=689 males [55.3%], median age at onset = 68.0 years [IQR 60.3-74.3]). A flow diagram summarizing the patients selection is reported as eFigure 1 in the Supplement. A total of 766 matched controls were included in the study. The clinical and demographic characteristics of cases and controls are detailed in Table 1.

The frequency of the alleles of the examined variants is reported in the eTable 1 in the Supplement. For UNC13A, the allele frequency was significantly different among cases and controls (p=0.037); the allele frequency did not deviate from the Hardy-Weinberg Equilibrium among controls (p=0.29), while a deviation observed among patients (p=0.015), reflecting an increase of risk associated with the C allele. Also, for ZNF512B, allele frequency was significantly different among cases and controls (p=0.027); however, for patients and controls, the allele frequency of ZNF512B did not deviate from the Hardy-Weinberg Equilibrium. CAMTA1 and SLC11A2 allele frequencies were not different among cases and controls (p=0.60 and p=0.33, respectively), and allele frequency did not deviate from the Hardy-Weinberg Equilibrium both in cases and controls. A total of 40 patients (3.2%) had ATXN2 polyQ repeats ≥31, and 91 patients (7.3%) carried the C9orf72 repeat expansion. The frequency of the combination of genetic variants and expansions did not deviate from the expected figures.

The median survival time of the entire cohort was 2.67 years (IQR 1.67-5.25). The examined variants in C9orf72 (median survival 2.51 years, IQR 1.74-3.82; p=0.016), ATXN2 (median survival 1.82 years, IQR 1.08-2.33; p<0.001) and UNC13A C/C (median survival 2.3 years, IQR 1.3-3.9; p<0.001) were significantly related to shorter survival in univariate analysis, while the variants in CAMTA1 G/G+G/T (median survival 2.58 years, IQR 1.59-5.08; p=0.231), SLC11A2 A/C+C/C (median survival 2.66 years , IQR1.59-5.58, p=0.665) and ZNF512B C/C+G/T (median survival 2.66 years, IQR 1.66-5.16; p=0.325) did not influence ALS outcome (eFigures 2 to 7 in the Supplement). In the Cox multivariable analysis, C9orf72 (Hazard Ratio [HR] 1.65, 95% c.i. 1.30-2.08, p<0.001), ATXN2 (HR 1.65, 95% c.i. 1.18-2.29,
p=0.003), UNCI3A (HR 1.31, 95% c.i. 1.09-1.58, p=0.005), and CAMTA1 (HR 1.13, 95% c.i. 1.001-1.30, p=0.048) were independently related to survival (Table 2). Therefore, we assessed the combined effects of C9orf72, ATXN2, UNCI3A, and CAMTA1 on ALS outcome in patients with deleterious alleles or expansions compared to those without, on a pairwise basis.

When assessing the interaction by pairs of genes, we found that, in most gene pairs, the presence of both detrimental alleles/repeat expansion was correlated with significantly shorter survival compared to other cases. The partial exception was the interaction between C9orf72 and CAMTA1, which was only marginally significant (p=0.052).

Specifically, a total of 68 cases (5.5%) carried the \( \text{CAMTA1}^{G/G+G/T} \) and \( \text{UNCI3A}^{C/C} \) alleles. Their median survival was 1.67 (1.16-3.08) years compared to 2.75 (1.67-5.26) for patients who did not carry detrimental alleles at both genes (p<0.001) (Figure 1). From the phenotypic perspective, patients with both \( \text{CAMTA1}^{G/G+G/T} \) and \( \text{UNCI3A}^{C/C} \) alleles were characterized by a 4-year older age at onset, a higher ∆Weight and a more frequent bulbar onset (eTable 2 in the Supplement).

A total of 20 cases (1.6%) carried the \( \text{CAMTA1}^{G/G+G/T} \) allelles and the \( \text{ATXN2}^{\geq 31} \) intermediate polyQ repeats. Their median survival was 1.75 (0.84-2.18) years compared to 2.67 (1.67-5.25) for patients who did not carry detrimental alleles at both genes (p<0.001) (Figure 2). The phenotype of patients with both \( \text{CAMTA1}^{G/G+G/T} \) alleles and \( \text{ATXN2}^{\geq 31} \) CAG repeats was characterized by a more frequent bulbar onset and a higher ∆ALSFRS-R and ∆King’s (eTable 3 in the Supplement).

A total of 38 cases (3.1%) carried both the \( \text{CAMTA1}^{G/G+G/T} \) alleles and the \( \text{C9ORF72}^{\geq 30} \) repeat expansion. Their median survival was 2.33 (1.49-3.84) years compared to 2.67 (1.67-5.25) for patients who did not carry any of these detrimental alleles (p=0.052) (Figure 3). Patients with \( \text{CAMTA1}^{G/G+G/T} \) alleles and \( \text{C9ORF72}^{\geq 30} \) had an 8-year younger age at onset and were more frequently affected by co-morbid FTD (34.4% vs. 15.3%) (eTable 4 in the Supplement).

Six patients (0.5%) carried both \( \text{ATXN2}^{\geq 31} \) polyQ repeats and \( \text{UNCI3A}^{C/C} \) variant; their median survival time was 1.33 (0.84-1.75) vs. 2.67 (1.67-5.25) for those who carried \( \text{ATXN2}^{\leq 30} \) CAG repeats and \( \text{UNCI3A}^{A/A+A/G} \) (p<0.001) (Figure 4).
Five patients (0.4%) carried both \textit{C9ORF72}≥30 and \textit{UNC13A}\textsubscript{CC} alleles; their median survival time was 1.66 (1.41-2.16) vs. 2.67 (1.67-5.25) for those who carried \textit{C9ORF72}≥30 and \textit{UNC13A}\textsubscript{A/A+AG} (p<0.019) (Figure 5).

Finally, in the present cohort, no cases with \textit{C9orf72} expansion carried also an \textit{ATXN2} intermediate repeat expansion.

We also evaluated the effect on ALS outcome in patients with one, two or three deleterious alleles. The 573 patients (46.0%) with no deleterious allele had a median survival time of 3.0 years (1.67-5.92) compared to 2.67 years (1.75-5.0) for those carrying one deleterious variants/expansion (543 cases, 43.6%), 1.84 years (1.25-3.25) for those carrying two deleterious variants/expansions (125 cases, 10.0%), and 0.84 years (0.33-1.33) for the 4 (0.3%) patients carrying three variants (p<0.001). The corresponding survival curves are reported in eFigure 8 in the Supplement. Clinical details of the 4 patients carrying three deleterious alleles are reported in eTable 5 in the Supplement. These patients are characterized by an age at onset over 70 years, a short time from onset to diagnosis (3 to 8 months), a wide range of \textit{ALSFRS-R} scores at diagnosis (10 to 42) and a rapid disease, as indicated by the ∆\textit{ALSFRS-R} and the ∆King’s. Three out of the four patients had comorbidity for dyslipidemia. Only one was a former cigarette smoker. Due to these characteristics, it is likely that patients carrying multiple deleterious variant can be missed due to their extremely rapid clinical course.

**Discussion**

In our cohort, we have found that the co-presence of selected detrimental alleles at common variants or repeat expansions that individually are detrimental to survival in patients with ALS has an additive effect. In particular, the co-presence of \textit{CAMTA1}\textsubscript{G/G+G/T} variants with either \textit{UNC13A}\textsubscript{CC} variant or \textit{ATXN2} polyQ intermediate expansion or \textit{C9ORF72} expansion was related to a significantly worse patients’ outcome. This effect was also found when assessing the co-presence of \textit{UNC13A}\textsubscript{CC} variant with either \textit{C9orf72} GGGGCCC expansion or \textit{ATXN2} polyQ intermediate expansion. In our cohort, 672 patients (54%) carried at least one deleterious variant/expansion.

Genetic modifiers of ALS phenotype have been generally studied in isolation.\textsuperscript{3,4,5,8,9} Notable exceptions are two studies reporting that the co-occurrence of the \textit{C9orf72} repeat expansion...
and UNC13A<sup>C/C</sup> variant significantly worsened the prognosis of patients with ALS<sup>19,20</sup>. However, identifying the mechanisms underlying the wide phenotypic heterogeneity of ALS, which hinders the discovery of effective therapies<sup>2</sup>, remains one of the significant unmet goals of ALS research. ALS heterogeneity is likely due to an interplay between genetics, age, sex<sup>1,21</sup>, and environmental factors, both related to lifestyle (i.e., physical activity, smoking)<sup>22,23</sup> and metabolic factors (i.e., lipid metabolism, gut microbiome).<sup>24-26</sup> In this study, we have shown that another element determining ALS phenotypic heterogeneity is the co-presence of two or more different genetic modifiers of survival.

We did not confirm the prognostic role of two of the examined variants, SLC11A2 (rs407135) and ZNF512B (rs2275294). The prognostic effect of SLC11A2 has been reported in only one study on a small cohort and has never been replicated afterwards.<sup>8</sup> Similarly, ZNF512B has been evaluated in only two small cohorts of patients of Asian ancestry, accounting for a total of 388 subjects.<sup>9,27</sup> Therefore, for both of these genes further study is needed to explore their possible prognostic role on ALS.

CAMTA1<sup>G/G+G/T</sup> alleles in our cohort appear to interact with all other examined genes in shortening ALS survival. The interaction between CAMTA1<sup>G/G+G/T</sup> and UNC13A<sup>C/C</sup> variants, accounting for 68 patients (5.5%), besides the strong negative effect on survival, is also phenotypically characterized by an older age at onset, a more frequent bulbar onset, and a higher reduction of weight (∆Weight). The interaction between CAMTA1<sup>G/G+G/T</sup> and ATXN2<sup>≥31</sup> (20 cases, 1.6%) is characterized by an increased ΔALSFRS-R and ΔKing’s, indicating a faster spreading and worsening of motor symptoms. Finally, patients with both CAMTA1<sup>G/G+G/T</sup> and C9ORF72<sup>≥30</sup> were younger, had an increased ΔKing’s and a higher frequency of FTD. The number of cases with the interaction between UNC13A<sup>C/C</sup> and C9ORF72<sup>≥30</sup> (5 cases, 0.4%) and UNC13A<sup>C/C</sup> and ATXN2<sup>≥31</sup> (6 cases, 0.5%) was too low to detect any significant phenotypic difference.

The biological reasons for these interactions remain to be elucidated. The proteins encoded by these genes may interact at a molecular level. It has been reported that TDP-43 protein, cytoplasmic inclusions of which are a pathological hallmark of the disease, enhances translation of CAMTA1 and Mig12 via a gain-of-function mechanism operating through their 5'UTRs;<sup>28</sup> however, this paper did not assess if the occurrence of CAMTA1<sup>G/G+G/T</sup> variant differentially influences the observed effect. More recently, it has been shown that TDP-43 represses a cryptic exon-splicing event in UNC13A, causing a reduction in UNC13A protein.
expression. In addition, the C/C genetic variation in **UNC13A** promotes cryptic exon inclusion upon nuclear depletion of TDP-43. Independently from the previously reported mechanism, CAMTA1 was found to be a relevant ‘Master Regulator’ of neurodegenerative disease transcriptional programs in a cultured motor neuron-based ALS model. Biological studies on preclinical models are therefore necessary to understand how these genes do interact.

This study is not without limitations. First, not all patients were tested for cognitive function, reducing the possibility of assessing the genetic interactions on cognition. However, the clinical and demographic characteristics of tested and non-tested patients were similar, limiting the possible selection bias. Second, very few patients carried both **ATXN2** CAG repeats and **UNC13A** C/C detrimental alleles or **C9ORF72** and **UNC13A** C/C detrimental alleles, reducing the possibility of assessing their phenotype and limiting the power of these analyses. Similarly, due to the reduced number of patients carrying more than two variants, we could not evaluate the effect of more than two variants. Larger patient cohorts are necessary to analyze these combinations and to calculate a polygenic risk score for survival. In addition, external replication of our finding would be necessary to confirm them in other populations. A remarkable aspect of our study is its population-based nature since it included some 90% of the incident cohort patients in the Piemonte and Valle d’Aosta regions. It has been demonstrated that prevalent and incident populations strongly differ from the clinical point of view, including survival, supporting the notion that studies derived from incident, population-based cohorts better represent the ALS population.

We demonstrated that gene variants and expansions acting as genetic modifiers of ALS survival can act on their own or in unison. Overall, 54% of patients carried at least one detrimental allele at common variant or repeat expansion, highlighting the clinical impact of our findings. This observation has several implications. First, identifying the interactive effects of modifier genes represents a crucial clue for explaining ALS clinical heterogeneity. Second, the interactive effect of these variants is likely to have profound effects on clinical trial design and interpretation, in particular for relatively common combinations, such as the association of **CAMTA1** G/G+G/T and **UNC13A** C/C variants which accounted in our series for 5.5% of patients and reduced patients’ survival by more than one year. Third, our study indicates that variants acting as phenotypic modifiers should be included in ALS genetic panels to provide patients and their families with a better prediction of the course of the disease and improve the planning of therapeutic interventions.
References


Table 1. Demographic and clinical characteristics of patients and controls

<table>
<thead>
<tr>
<th></th>
<th>Cases (n=1245)</th>
<th>Controls (n=766)</th>
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<td>Age at onset (years, median, IQR)</td>
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<td>Sex (female)</td>
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<td>368 (48.0%)</td>
<td>0.14</td>
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<td>Site of onset (bulbar)</td>
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<td>n.a.</td>
<td>-</td>
</tr>
<tr>
<td>Time from onset to diagnosis (months, median, IQR)</td>
<td>9.0 (5.1-14.0)</td>
<td>n.a.</td>
<td>-</td>
</tr>
<tr>
<td>Education (median, IQR) °</td>
<td>8 (5-11)</td>
<td>8 (5-11)</td>
<td>0.87</td>
</tr>
<tr>
<td>ALSFRS-R at diagnosis (median, IQR)</td>
<td>42 (37-45)</td>
<td>n.a.</td>
<td>-</td>
</tr>
<tr>
<td>FVC% at diagnosis * (median, IQR)</td>
<td>90 (71-104)</td>
<td>n.a.</td>
<td>-</td>
</tr>
<tr>
<td>BMI at diagnosis § (median, IQR)</td>
<td>24.1 (21.9-26.8)</td>
<td>n.a.</td>
<td>-</td>
</tr>
<tr>
<td>ΔALSFRS-R (points/month, median, IQR)</td>
<td>0.68 (0.33-1.35)</td>
<td>n.a.</td>
<td>-</td>
</tr>
<tr>
<td>ΔWeight § (kg/month, median, IQR)</td>
<td>0.27 (0-0.97)</td>
<td>n.a.</td>
<td>-</td>
</tr>
<tr>
<td>ALS-FTD ^</td>
<td>146 (16.1%)</td>
<td>n.a.</td>
<td>-</td>
</tr>
<tr>
<td>King’s stage (1/2/3/4A+4B) at diagnosis</td>
<td>524/392/283/46</td>
<td>n.a.</td>
<td>-</td>
</tr>
<tr>
<td>MiToS stage (0/1/2/3/4) at diagnosis</td>
<td>822/368/41/12/2</td>
<td>n.a.</td>
<td>-</td>
</tr>
<tr>
<td>ΔKing’s</td>
<td>0.2 (0.11-0.34)</td>
<td>n.a.</td>
<td>-</td>
</tr>
</tbody>
</table>

* Available for 1162 patients; § available for 1223 patients; ° available for 1238 patients; ^ available for 909 patients
<table>
<thead>
<tr>
<th>Factors</th>
<th>Values</th>
<th>Hazard Ratio (95% c.i.)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at onset (years)</td>
<td>Per each year of age at onset</td>
<td>1.029 (1.023-1.036)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Time from onset to diagnosis</td>
<td>Per each month</td>
<td>0.955 (0.946-0.964)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Site of onset</td>
<td>Spinal</td>
<td>1 [reference]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Bulbar</td>
<td>1.484 (1.288-1.709)</td>
<td></td>
</tr>
<tr>
<td>ΔALSFRS-R</td>
<td>Per each point loss/month</td>
<td>1.329 (1.267-1.394)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ΔKing’s</td>
<td>Per each point loss/month</td>
<td>1.663 (1.259-2.197)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ΔWeight</td>
<td>Per each kg loss/month</td>
<td>1.071 (1.028-1.116)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>C9orf72 repeats</td>
<td>C9ORF72\textsuperscript{≤29}</td>
<td>1 [reference]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>C9ORF72\textsuperscript{≥30}</td>
<td>1.645 (1.302-2.079)</td>
<td></td>
</tr>
<tr>
<td>ATXN2 polyQ repeats</td>
<td>ATXN2\textsuperscript{≤30}</td>
<td>1 [reference]</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td>ATXN2\textsuperscript{≥31}</td>
<td>1.645 (1.181-2.292)</td>
<td></td>
</tr>
<tr>
<td>UNC13A</td>
<td>UNC13A\textsuperscript{A/A+A/C}</td>
<td>1 [reference]</td>
<td>0.005</td>
</tr>
<tr>
<td></td>
<td>UNC13A\textsuperscript{C/C}</td>
<td>1.309 (1.087-1.578)</td>
<td></td>
</tr>
<tr>
<td>COPD</td>
<td>No</td>
<td>1 [reference]</td>
<td>0.011</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>1.330 (1.068-1.656)</td>
<td></td>
</tr>
<tr>
<td>CAMTA1</td>
<td>CAMTA1\textsuperscript{T/T}</td>
<td>1 [reference]</td>
<td>0.048</td>
</tr>
<tr>
<td></td>
<td>CAMTA1\textsuperscript{C/C,H/H}</td>
<td>1.135 (1.001-1.287)</td>
<td></td>
</tr>
</tbody>
</table>
Figure legends

**Figure 1.** Survival curves (Kaplan Meier) for the interaction between *CAMTA1* rs2412208 variant and *UNC13A* rs12608932 variant.

Median survival time: *CAMTA1*\(^{G/G+G/T}\) and *UNC13A*\(^{C/C}\) (68 cases, blue line) 1.67 years (1.16-3.08), *CAMTA1*\(^{T/T}\) and *UNC13A*\(^{A/A+A/C}\) (1177 cases, green line) 2.75 years (1.67-5.26), p<0.001. Ticks represent censored patients.
Figure 2. Survival curves (Kaplan Meier) for the interaction between ATXN2 polyQ repeats and CAMTA1 rs2412208 variant.

Median survival time: ATXN2$^{>31}$ and CAMTA1$^{G/G+G/T}$ (20 cases, blue line) 1.75 years (0.84-2.18), ATXN2$^{\leq30}$ and CAMTA1$^{T/T}$ (1225 cases, green line) 2.67 years (1.67-5.25), p<0.001. Ticks represent censored patients.
Figure 3. Survival curves (Kaplan Meier) for the interaction between *CAMTA1* rs2412208 variant and *C9orf72* GGGGCC expansion.

Median survival time: *CAMTA1*<sup>G/G+G/T</sup> and *C9orf72*<sup>≥30</sup> (38 cases, blue line) 2.33 years (1.49-3.84), *CAMTA1*<sup>T/T</sup> and *C9orf72*<sup>≤29</sup> (1207 cases, green line) 2.67 years (1.67-5.25), p=0.052. Ticks represent censored patients.
Figure 4. Survival curves (Kaplan Meier) for the interaction between ATXN2 polyQ repeats and UNC13A rs12608932 variant.

Median survival time: ATXN2$^{\geq 31}$ and UNC13A$^{C/C}$ (6 cases, blue line) 1.33 years (0.84-1.75) ATXN2$^{\leq 30}$ and UNC13A$^{A/A+A/C}$ (1239 cases, green line) 2.67 years (1.67-5.25), p <0.001.

Ticks represent censored patients.
Figure 5. Survival curves (Kaplan Meier) for the interaction between \textit{C9orf72} GGGCC expansion and \textit{UNC13A} rs12608932 variant.

Median survival time: \textit{C9orf72}^{\geq30} and \textit{UNC13A}^{C/C} (5 cases, blue line) 1.66 years (1.41-2.16), \textit{C9orf72}^{<29} and \textit{UNC13A}^{A/A+A/C} (1240 cases, green line) 2.67 years (1.67-5.25), p<0.019.

Ticks represent censored patients.
Association of Copresence of Pathogenic Variants Related to Amyotrophic Lateral Sclerosis and Prognosis
Adriano Chio, Cristina Moglia, Antonio Canosa, et al.
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