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

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Abstract

Background and Objectives
Despite recent advances, it is not clear whether the various genes/genetic variants related to amyotrophic lateral sclerosis (ALS) interact in modifying patients’ phenotype. The aim of this study was to determine whether the copresence of genetic variants related to ALS has interactive effects on the course of the disease.

Methods
The study population includes 1,245 patients with ALS identified through the Piemonte Register for ALS between 2007 and 2016 and not carrying superoxide dismutase type 1, TAR DNA binding protein, and fused in sarcoma pathogenic variants. Controls were 766 Italian participants age-matched, sex-matched, and geographically matched to cases. We considered Unc-13 homolog A (UNC13A) (rs12608932), calmodulin binding transcription activator 1 (CAMTA1) (rs2412208), solute carrier family 11 member 2 (SLC11A2) (rs407135), and zinc finger protein 512B (ZNF512B) (rs2275294) variants, as well as ataxin-2 (ATXN2) polyQ intermediate repeats (≥31) and chromosome 9 open reading frame 72 (C9orf72) GGGGCC intronic expansions (≥30).

Results
The median survival time of the whole cohort was 2.67 years (interquartile range [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 UNC13A C/C (2.3 years, IQR 1.3–3.9; p < 0.001) significantly reduced survival. In Cox multivariable analysis, CAMTA1 also emerged to be independently related to survival (hazard ratio 1.13, 95% CI 1.001–1.30, p = 0.048). The copresence of 2 detrimental alleles/expansions was correlated with shorter survival. In particular, the median survival of patients with CAMTA1G/G+G/T and UNC13A C/C alleles was 1.67 years (1.16–3.08) compared with 2.75 years (1.67–5.26) of the patients not carrying these variants (p < 0.001); the survival of patients with CAMTA1G/G+G/T alleles and ATXN2 ≥31 intermediate polyQ repeats was 1.75 years (0.84–2.18) (p < 0.001); the survival of patients with ATXN2 ≥31 polyQ repeats and UNC13A C/C allele was 1.33 years (0.84–1.75) (p < 0.001); the survival of patients with C9ORF72 ≥30 and UNC13A C/C allele was 1.66 years (1.41–2.16). Each pair of detrimental alleles/expansions was associated to specific clinical phenotypes.

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Glossary

ALS = amyotrophic lateral sclerosis; ALSFRS-R = Revised ALS Functional Rating Scale; ATXN2 = ataxin-2; C9orf72 = chromosome 9 open reading frame 72; CAMTA1 = calmodulin binding transcription activator 1; FTD = frontotemporal dementia; FUS = fused in sarcoma; GP = general practitioner; HR = hazard ratio; IQR = interquartile range; SLC11A2 = solute carrier family 11 member 2; SOD1 = superoxide dismutase type 1; TARDBP = TAR DNA binding protein; TDP-43 = TAR DNA binding protein-43; UNCI3A = Unc-13 homolog A; WGS = whole genome sequencing; ZNF512B = zinc finger protein 512B.

Discussion

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 1 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.

Methods

The study population includes the patients with ALS diagnosed between 2007 and 2016 and identified through an Italian prospective population-based register (Piemonte and Valle d’Aosta Register for ALS).11,12 All patients were diagnosed as definite, probable, probable laboratory-supported, or possible ALS according to El Escorial revised criteria.13 More details concerning the epidemiologic register are reported in eMethods (links.lww.com/WNL/C799). Controls were randomly identified from the lists of patients’ general practitioners (GPs) and matched to the cases by sex and age (±5 years). Because the list of GP assisted persons is by definition in the same community of their assisted patients with ALS, a geographical matching was ensured.

Whole Genome Analysis

Whole genome sequencing (WGS) methods are reported in detail as eMethods (links.lww.com/WNL/C799). WGS of 1,029 patients with ALS and 766 controls have already been reported.14 An additional 290 ALS cases underwent WGS as described elsewhere.15

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). Both methodologies are described in detail as eMethods and eAppendix 1 (links.lww.com/WNL/C799).

Survival Modifiers Genes

For this study, we considered the following genes reported to be related to ALS outcome: UNCI3A, 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: UNCI3A/C/C vs UNCI3A/A/A/A/C;
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+C/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 articles reporting the gene in ALS or subsequent studies<sup>3-5,8,9</sup> and were confirmed in our cohort (data not shown).

Clinical Variables

The mean monthly decline of Revised ALS Functional Rating Scale (ALSFRS-R) (ΔALSFRS-R) was calculated using the following formula: (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 5 categories according to the Consensus Criteria for diagnosing frontotemporal cognitive and behavioral syndromes in ALS<sup>16</sup>. The battery that assessed visuospatial function, language, executive function, memory, and social cognition, as well as anxiety and depression, is reported in detail as eMethods (links.lww.com/WNL/C799)<sup>17</sup>.

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 χ<sup>2</sup> 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 end point. 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 <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 vs female), site of onset (bulbar vs spinal), King’s staging, ΔALSFRS-R (continuous), forced vital capacity % at diagnosis (continuous), ΔWeight (continuous), ΔKing’s (continuous), and chronic obstructive pulmonary disease (yes vs no). The SPSS 28.0 statistical package was used for the analyses (SPSS, Chicago, IL).
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

The individual-level sequence data are available on the database of Genotypes and Phenotypes (accession number: phs001963.v1.p1). Phenotypic data will be available on 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 because of 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 male patients [55.3%], median age at onset = 68.0 years [interquartile range (IQR) 60.3–74.3]). A flow diagram summarizing the patient selection is reported as eFigure 1 (links.lww.com/WNL/C799). 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 eTable 1 (links.lww.com/WNL/C799). 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. In addition, 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

Table 2 Cox Multivariable Analysis

<table>
<thead>
<tr>
<th>Factors</th>
<th>Values</th>
<th>HR (95% CI)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at onset, y</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 1 (reference)</td>
<td>1.484 (1.288–1.709)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>∆ALSFRS-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>∆King'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>∆Weight</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&lt;sup&gt;29&lt;/sup&gt;</td>
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<tr>
<td>C9orf72&lt;sup&gt;30&lt;/sup&gt;</td>
<td>1.645 (1.302–2.079)</td>
<td>&lt;0.001</td>
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<td>ATXN2 polyQ repeats</td>
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<td>ATXN&lt;sup&gt;231&lt;/sup&gt;</td>
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<tr>
<td>UNC13A</td>
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<td>COPD</td>
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<tr>
<td>Yes</td>
<td>1.330 (1.068–1.656)</td>
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<tr>
<td>CAMTA1</td>
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<tr>
<td>CAMTA1&lt;sup&gt;G/G+G/T&lt;/sup&gt;</td>
<td>1.135 (1.001–1.287)</td>
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</tr>
</tbody>
</table>

Abbreviations: ALSFRS-R = Revised Amyotrophic Lateral Sclerosis Functional Rating Scale; ATXN2 = ataxin-2; C9orf72 = chromosome 9 open reading frame 72; CAMTA1 = calmodulin binding transcription activator 1; COPD = chronic obstructive pulmonary disease; HR = hazard ratio; UNC13A = Unc-13 homolog A.
cases and in 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, IQR 1.59–5.58, p = 0.665), and ZNF512B C/C+C/T (median survival 2.66 years, IQR 1.66–5.16; p = 0.325) did not influence ALS outcome (eFigures 2–7, links.lww.com/WNL/C799). In the Cox multivariable analysis, C9orf72 (hazard ratio [HR] 1.65, 95% CI 1.30–2.08, p < 0.001), ATXN2 (HR 1.65, 95% CI 1.18–2.29, p = 0.003), UNC13A (HR 1.31, 95% CI 1.09–1.58, p = 0.005), and CAMTA1 (HR 1.13, 95% CI 1.001–1.30, p = 0.048) were independently related to survival (Table 2). Therefore, we assessed the combined effects of C9orf72, ATXN2, UNC13A, and CAMTA1 on ALS outcome in patients with deleterious alleles or expansions compared with 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 with 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 CAMTA1 G/G+G/T and UNC13A C/C alleles. Their median survival was 1.67 (1.16–3.08) years compared with 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 CAMTA1 G/G+G/T and UNC13A C/C alleles were characterized by a 4-year older age at onset, a higher ΔWeight, and a more frequent bulbar onset (eTable 2, links.lww.com/WNL/C799).

A total of 20 cases (1.6%) carried the CAMTA1 G/G+G/T alleles and the ATXN2≥31 intermediate polyQ repeats. Their median survival was 1.75 (0.84–2.18) years compared with 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 CAMTA1 G/G+G/T alleles and ATXN2≥31 CAG repeats was characterized by a more frequent bulbar onset and a higher ΔALSFRS-R and ΔKing’s (eTable 3, links.lww.com/WNL/C799).

A total of 38 cases (3.1%) carried both the CAMTA1 G/G+G/T alleles and the C9orf72 repeat expansion. Their median survival was 2.33 (1.49–3.84) years compared with 2.67 (1.67–5.25) for patients who did not carry any of these detrimental alleles (p = 0.052) (Figure 3). Patients with CAMTA1 G/G+G/T alleles and C9orf72≥30 had an 8-year younger survival compared with other cases. The partial exception was the interaction between C9orf72 and CAMTA1, which was only marginally significant (p = 0.052).
age at onset and were more frequently affected by comorbid frontotemporal dementia (FTD) (34.4% vs 15.3%) (eTable 4, links.lww.com/WNL/C799).

Six patients (0.5%) carried both ATXN2 ≥ 31 polyQ repeats and UNC13A 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

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<30 and CAMTA1 T/T (1,225 cases, green line) 2.67 years (1.67–5.25), p < 0.001. Ticks represent censored patients. ATXN2 = ataxin-2; CAMTA1 = calmodulin binding transcription activator 1.

Figure 3 Survival Curves (Kaplan-Meier) for the Interaction Between CAMTA1 rs2412208 Variant and C9orf72 GGGGCC Expansion

Median survival time: CAMTA1 G/G+G/T and C9orf72 ≥30 (38 cases, blue line) 2.33 years (1.49–3.84), CAMTA1 T/T and C9orf72 ≤29 (1,207 cases, green line) 2.67 years (1.67–5.25), p = 0.052. Ticks represent censored patients. C9orf72 = chromosome 9 open reading frame 72; CAMTA1 = calmodulin binding transcription activator 1.
ATXN2 ≤30 CAG repeats and UNC13A A/A+A/G (p < 0.001) (Figure 4).

Five patients (0.4%) carried both C9ORF72 ≥30 and UNC13A C/C alleles; their median survival time was 1.66 (1.41–2.16) vs 2.67 (1.67–5.25) for those who carried C9ORF72 ≥30 and UNC13A A/A+A/G (p < 0.019) (Figure 5).

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

We also evaluated the effect on ALS outcome in patients with 1, 2, or 3 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 with 2.67 years (1.75–5.0) for those carrying 1 deleterious variants/expansion (543 cases, 43.6%), 1.84 years (1.25–3.25) for those carrying 2 deleterious variants/expansions (125 cases, 10.0%), and 0.84 years (0.33–1.33) for the 4 (0.3%) patients carrying 3 variants (p < 0.001). The corresponding survival curves are reported in eFigure 8 (links.lww.com/WNL/C799). Clinical details of the 4 patients carrying 3 deleterious alleles are reported in eTable 5. These patients are characterized by an age at onset over 70 years, a short time from onset to diagnosis (3–8 months), a wide range of ALSFRS-R scores at diagnosis (10–42) and a rapid disease, as indicated by the ΔALSFRS-R and the ΔKing’s. Three of the 4 patients had comorbidity for dyslipidemia. Only 1 was a former cigarette smoker. Owing 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 copresence 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 copresence of CAMTA1 G/G+G/T variants with either UNC13A C/C variant or ATXN2 polyQ intermediate expansion or C9ORF72 expansion was related to a significantly worse patients’ outcome. This effect was also found when assessing the copresence of UNC13A C/C variant with either C9orf72 GGGGCC expansion or ATXN2 polyQ intermediate expansion. In our cohort, 672 patients (54%) carried at least 1 deleterious variant/expansion.

Genetic modifiers of ALS phenotype have been generally studied in isolation. Notable exceptions are 2 studies reporting that the co-occurrence of the C9orf72 repeat expansion and UNC13A C/C variant significantly worsened the prognosis of patients with ALS. However, identifying the mechanisms underlying the wide phenotypic heterogeneity of ALS, which hinders the discovery of effective therapies, remains one of the significant unmet goals of ALS research. ALS heterogeneity is likely due to an interplay between genetics, age, sex, and environmental factors, both related to lifestyle.
and metabolic factors (i.e., lipid metabolism, gut microbiome). In this study, we have shown that another element determining ALS phenotypic heterogeneity is the copresence of 2 or more different genetic modifiers of survival.

We did not confirm the prognostic role of 2 of the examined variants, SLC11A2 (rs407135) and ZNF512B (rs2275294). The prognostic effect of SLC11A2 has been reported in only 1 study on a small cohort and has never been replicated afterward. Similarly, ZNF512B has been evaluated in only 2 small cohorts of patients of Asian ancestry, accounting for a total of 388 participants. Therefore, for both of these genes, further study is needed to explore their possible prognostic role on ALS.

CAMTA1G/G+G/T alleles in our cohort seem to interact with all other examined genes in shortening ALS survival. The interaction between CAMTA1G/G+G/T and UNC13AC/C 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 (ΔWeight). The interaction between CAMTA1G/G+G/T and ATXN2≥31 (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 CAMTA1G/G+G/T and C9ORF72≥30 were younger, had an increased ΔKing’s, and had a higher frequency of FTD. The number of cases with the interaction between UNC13A C/C and C9ORF72≥30 (5 cases, 0.4%) and UNC13A C/C and ATXN2≥31 (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 TAR DNA binding protein-43 (TDP-43), cytoplasmic inclusions of which are a pathologic hallmark of the disease, enhances translation of CAMTA1 and midline-1-interacting G12-like protein through a gain-of-function mechanism operating through their 5’ untranslated regions; however, this article did not assess if the occurrence of CAMTA1G/G+G/T 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. 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≥31 CAG

Figure 5 Survival Curves (Kaplan-Meier) for the Interaction Between C9orf72 GGGCC Expansion and UNC13A rs12608932 Variant

Median survival time: C9orf72≥30 and UNC13AC/C (5 cases, blue line) 1.66 years (1.41–2.16), C9orf72<30 and UNC13A≥31 (1,240 cases, green line) 2.67 years (1.67–5.25), p < 0.019. Ticks represent censored patients. C9orf72 = chromosome 9 open reading frame 72; UNC13A = Unc-13 homolog A.
repeats and UNC13A\textsuperscript{C/C} detrimental alleles or C9orf72\textsuperscript{30} and UNC13A\textsuperscript{C/C} detrimental alleles, reducing the possibility of assessing their phenotype and limiting the power of these analyses. Similarly, owing to the reduced number of patients carrying more than 2 variants, we could not evaluate the effect of more than 2 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 because it included some 90% of the incident cohort patients in the Piemonte and Valle d’Aosta regions.\textsuperscript{14} 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.\textsuperscript{32,33}

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 1 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\textsuperscript{G/G+T} and UNC13A\textsuperscript{C/C} variants which accounted in our series for 5.5% of patients and reduced patients’ survival by more than 1 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.

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**Disclosure**

A. Chiò serves on scientific advisory boards for Mitsubishi Tanabe, Biogen, Roche, Denali Pharma, Cytokinetics, Lilly, and Amylyx Pharmaceuticals and has received a research grant from Biogen. C. Moglia, A. Canosa, U. Manera, M. Grassano, R. Vasta, F. Palumbo, S. Gallone, M. Brunetti, F. De Marchi, C.L. Dalgaard, R. Chia, G. Mora, L. Corrado, S. D’Alfonso, and L. Mazzini report no disclosures. B.J. Traynor holds the US, Canadian, and European patents on the clinical testing and therapeutic intervention for the hexanucleotide repeat expansion in C9orf72. A. Calvo has received a research grant from Cytokinetics. Go to Neurology.org/N for full disclosures.

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### References


