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ORIGINAL COMMUNICATION

Disease survival and progression in TARDBP ALS patients from Sardinia, Italy

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Abstract

Background Common genes implicated in amyotrophic lateral sclerosis (ALS) development may also influence its progression rate. The C9orf72 mutations featured a faster progression rate while the European SOD1 mutations were associated with a slower progression. In this study, we assessed the relationship between TARDBP and ALS progression/survival.

Methods ALS incident patients (2010–2019) were diagnosed by El Escorial revised criteria and staged over the disease course by the King's staging system. Disease progression was analysed by Kaplan–Meier survival curves and Cox regression models, with survival measured from symptom onset to death/tracheostomy or censor date.

Results The study population included 76 patients carrying TARDBP mutations (A382T/G295S), 28 patients carrying the C9orf72 GGGGCC expansion, and 158 patients who had no evidence of causative genetic mutations (nmALS group). TARDBP patients reached death/tracheostomy later than C9orf72 and nmALS patients, independently of possible prognostic indicators (sex, age at ALS onset, diagnostic delay, phenotype at onset, and family history of ALS). On King's staging, the time elapsed between disease onset (King's stage 1) and involvement of the second body region (King's stage 2B) was similar in TARDBP and nmALS patients but longer in TARDBP than in C9orf72 patients. TARDBP patients reached King's stages 3 and 4 later than C9orf72 and nmALS patients.

Conclusions TARDBP patients have a better survival/prognosis than C9orf72-positive and nmALS patients. King's staging also suggested that the higher survival rate and the slower progression associated with the TARDBP mutation could mainly be attributed to the longer time elapsed between King's stages 2B to 3.

Keywords C9orf72 mutation · Amyotrophic lateral sclerosis · TARDBP · Survival

Introduction

Amyotrophic lateral sclerosis (ALS) is a chronic neurodegenerative disease which predominantly affects the upper and lower motor neurons and leads to progressive paralysis and death generally due to respiratory failure [1, 2]. The rate of ALS progression may be highly variable, with median survival time being 3–5 years since disease onset. Among prognostic factors that may influence survival, there are age at ALS onset, time from symptom onset to diagnosis (diagnostic delay), clinical phenotype at onset, progression rate, and cognitive impairment [3].

Approximately, 10–15% of ALS cases are associated with monogenic mutations in a number of genes. The commonest genes implied in ALS so far are C9ORF72, SOD1, TARDBP and FUS [4, 5], with marked differences between ethnic groups and geographical regions [6]. However, the genetic architecture of ALS is complex [7] and, in addition to disease-causing genes, several other genes have been reported to be modifiers of ALS phenotype and survival like the common variants12608932 located within an intron of UNC13A gene [8–10].

It has been demonstrated that some of the major genes implicated in the development of ALS can also influence ALS progression. Patients carrying the C9orf72 mutations,

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the most common cause of genetic ALS, have a faster progression rate as compared to patients without mutation [11, 12], while some European and Chinese SOD1 mutations are associated with a slower progression rate [13]. The relationship between TARDBP and the progression and survival of ALS patients remains to be better defined.

In this paper, we analysed the influence of the TARDBP gene on ALS progression over time and survival in an incident cohort of patients from Sardinia, a genetic isolate where the TARDBP p.A382T missense mutation has a higher frequency than expected [14, 15] and is the most common genetic cause of ALS [16].

Methods

The study population included ALS incident patients of Sardinian ancestry (i.e. defined as subjects with both parents of Sardinian origin) who were identified between 2010 and 2019 in a study area that covered about one-half of the whole island (9768 km^2), hosted nearly two-thirds of the whole Sardinian population and included three administrative subdivisions (Cagliari, South Sardinia and Oristano provinces). The ascertainment methodology, the demographic and clinical features of the study population and incidence data have been described elsewhere [17].

To identify individuals with ALS, we referred to the medical facilities across the study area that included one tertiary referral clinic located at the Institute of Neurology of the University of Cagliari, a genetic point reference, seven general neurology clinics and several field neurologists. All the aforementioned facilities and physicians were part of the National Health System Network with free access for the Sardinian population. Neurologists from the University center reviewed medical charts and checked for duplicate cases. The diagnosis was made according to El Escorial revised criteria [18].

Medical records were examined to collect clinical information on age, sex, age at symptom onset, diagnostic delay (time elapsing between onset of symptoms and diagnosis), clinical phenotype [19] and family history of ALS. According to the criteria by Byrne et al. [20], we could identify only patients with a probable and possible family history of ALS that were analysed together. On study time, patients were classified by King's staging system [21]: stage 1 was defined as symptom onset (functional involvement by weakness, wasting, spasticity, dysarthria or dysphagia of one CNS region defined as bulbar, upper limb, lower limb or diaphragmatic); stage 2A was defined by diagnosis; stage 2B was identified by functional involvement of a second region; stage 3 was defined by functional involvement of a third region; and stage 4 was characterized by needing gastrostomy and/or non-invasive ventilation. Timing of involvement was based on the date of onset of symptoms in the prior stage and the date of development of functionally significant symptoms in a second and third region. The need for gastrostomy/non-invasive ventilation was defined as the time gastrostomy or nasogastric feeding or non-invasive ventilation (NIV) was provided or refused.

Data on DNA analysis were available in 267/344 patients (78%) and included SOD1, TARDBP, ATXN2 and C9ORF72 gene. FUS was never identified in Sardinian patients [16]. To detect TARDBP mutations, Sanger sequencing was performed. The coding exon 6 of TARDBP has been PCR amplified, sequenced and run on an ABI 3500xL Genetic analyzer.

Statistical analysis was performed by the STATA 11 package. Data were expressed as mean and standard deviations (SD) unless otherwise indicated. Differences between groups were tested by the Mann–Whitney *U* test, Chi-square test or one-way ANOVA with subsequent post hoc Bonferroni test, as appropriate. Disease progression was analysed by time elapsing from King's stage 1 (involvement of the first region) to next King's stages 2B (involvement of a second region), 3 (involvement of a third region), and 4 (need for gastrostomy/non-invasive ventilation). Survival was analysed by Kaplan–Meier survival curves and Cox regression models, with survival measured from symptom onset to death/tracheostomy or censor date. The study was approved by the local Institutional Review Board and conducted in line with the ethical rules for data collection.

Results

During the study period, 344 incident individuals of Sardinian ancestry were diagnosed with ALS. Genetic analysis was performed in 267/344 patients (78%), of whom 76 carried the A382T (*n*. 69) or G295S mutation (*n*. 7) of the TARDBP gene. Although patients with the homozygous A382T mutation have been previously described [16, 22], all patients in the present study were heterozygous for the mutation. Among the other patients, 28 carried the C9orf72 GGG GCC expansion, 5 the ATXN2 mutation, and 158 patients had no evidence of causative genetic mutations (nmALS group). Family history of ALS was identified in 59 patients and classified as probable in 44 patients and possible in 15 patients [20]. Owing to the small size, the subgroup of ALS patients carrying the ATXN2 mutation was not analysed. The final study group therefore included 262 patients. Their demographic and clinical features were similar to those of the 77 patients (23%) who did not perform genetic analysis and did not participate in the study (Supplemental Table).

Demographic and clinical features at ALS onset of patients with and without TARDBP and C9orf72 mutations

Stratifying the study group by patients with ALS-related TARDBP mutation, patients with ALS-related C9orf72 mutation, and patients without evidence of a causative mutation (nmALS group) yielded similar sex distribution, significantly higher age at ALS onset in the nmALS group, non-significant differences in the time elapsing from disease onset to diagnosis (King's stage 1 to 2A) among groups, and a greater frequency of family history of ALS in the TARDBP and C9orf72 groups (Table 1). With regard to phenotype at onset, classic limb onset was more frequent in the C9orf72 and nmALS group than in the TARDBP group; flail arm/leg was more frequent in the TARDBP group, whereas the frequency of bulbar onset, predominant upper motor neuron phenotype, and respiratory onset did not significantly differed among groups (Table1).

Survival analysis

Over the follow-up, 133 patients died (42/76 TARDBP patients, 9/28 C9orf72 patients, and 82/158 nmALS patients), $p=0.1$) and 97 were tracheostomized (23/76 TARDBP patients, 17/28 C9orf72 patients, and 57/158 nmALS, $p=0.02$), and 32 were still alive. The time elapsed from involvement of the first region to death/tracheostomy was significantly longer in the TARDBP group than in the C9orf72 and nmALS groups (45.2 + 29.3 vs. 30.8 + 22.5 vs. 32.8 + 20.8; one-way ANOVA, $F=4.9$, $p=0.008$; post hoc test: TARDBP different from the other groups, $p<0.05$).

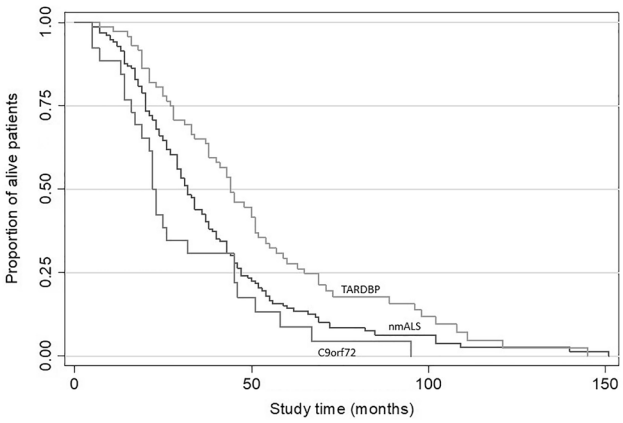

Fig. 1 Kaplan-Meyer curves for survival in TARDBP mutation carriers vs. C9orf72 mutation carriers vs. non-mutated patients (nmALS)

Kaplan–Meyer survival curves indicated that patients carrying TARDBP mutation were less likely to experience death/tracheostomy than the other groups (Fig. 1). Univariable Cox regression analysis confirmed a significantly lower risk of death/tracheostomy in patients carrying the TARDBP mutation as compared to the nmALS group as the reference (Table 2), while no different risk of death/tracheostomy was observed in the C9orf72 group (Table 2). On multivariable analysis adjusted for sex, age at onset, diagnostic delay, phenotype at ALS onset, and family history of ALS, however, even the C9orf72 estimate reached significance, with the main confounding being exerted by the variable age at onset (Table 2). Among adjustment variables, only age at ALS onset (adjusted HR 1.04; 95% CI 1.02–1.05; $p<0.0001$) and diagnostic delay (adjusted HR 0.95; 95% CI 0.93–0.96); $p<0.0001$) reached significance on multivariable modelling.

Table 1 Demographic and clinical features of patients who carried TARDBP or C9orf72 mutation and those who did not

	TARDBP ($n=76$)	C9orf72 ($n=28$)	Non-mutated ALS ($n=158$)	p by Chi-square test or one-way ANOVA
Sex (men/women)	54/22	17-Nov	93/65	0.2
Mean age (years) at disease onset ± SD	61.7 + 12.5	60.6 + 8.4	65.6 + 11.1*	$F=4.45$, $p=0.01$
Mean time (months) elapsed from onset to diagnosis ± SD	17.2 + 17.8	10.3 + 7.8	14.9 + 14.3	$F=2.2$, $p=0.1$
N. patients with family history of amyotrophic lateral sclerosis (%)	25 (33%)	8 (28%)	16 (10%)	< 0.0001
Phenotype at onset:				
Classic limb onset	29 (38%)	13 (46%)	90 (57%)	0.02
Bulbar onset	13 (17%)	9 (32%)	27(17%)	0.15
Flail arm/legs	26 (34%)	4 (14%)	33 (21%)	0.04
Predominant upper motor neuron	8 (10%)	1 (4%)	6 (4%)	0.1
Respiratory onset	0	1 (4%)	2 (1.3%)	0.3

*Post hoc test: different from other groups, $p<0.05$

Table 2 Univariable and multivariable Cox regression analysis of the influence exerted by TARDBP and C9orf72 mutation on survival rate

	Univariable analysis Hazard ratio (95% confidence interval), *p*	Multivariable analysis Hazard ratio (95% confidence interval), *p*
Non-mutated ALS	1 (reference)	1 (reference)
C9orf72 group	1.45 (0.95–2.23), 0.1	1.68 (1.06–2.66), 0.027
TARDBP group	0.66 (0.49–0.89), 0.007	0.72 (0.53–0.99), 0.045

In the multivariable model, estimates were adjusted for sex, age at disease onset, diagnostic delay, phenotype at disease onset, and family history of amyotrophic lateral sclerosis

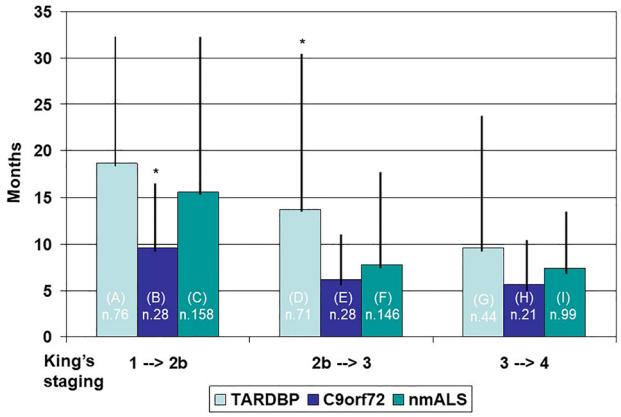

Fig. 2 Time elapsed between stages of the King's staging system in patients who carried TARDBP or C9orf72 mutation and in those who did not. Data are mean months ± SD. Number of patients from each group is in the columns. One-way ANOVA. ABC: $F = 3.5$, $p = 0.03$; *post hoc test: different from other groups, $p < 0.05$. DEF: $F = 7.6$, $p = 0.0006$; *post hoc test: different from other groups, $p < 0.05$. GHI: $F = 1.41$, $p = 0.25$

King's staging analysis

All the 262 patients who participated in the study reached King's stage 2B, 245 reached stage 3, and 164 reached stage 4 (Fig. 2, Supplemental Table 2). Analysis of the time taken from disease onset (King's stage 1) to stages 2B to 4 yielded the following results: (i) mean cumulative time taken to reach every next stage tend to increase in the overall population and in each subgroup (Supplemental Table 2); (ii) the time elapsed from symptom onset to involvement of a second region (King's stage 1- to 2B) was significantly longer in the TARDPB and nmALS group than in the C9orf72 group (Fig. 2); (iii) the time elapsed from involvement of the first to the third region (King's stage 1–3) and to need for gastrostomy and/or non-invasive ventilation (King's stage 1 to 4) were significantly longer in the TARDBP group than in the other groups (of note, the three study groups did not differ for frequency of gastrostomy and/or NIV [44/76 TARDBP patients, 20/28 C9orf72 patients, and 100/158 nmALS patients; $p = 0.4$]) (Supplemental Table 2); (iv) the time elapsed from King's stages 2B to 3 was significantly longer in the TARDPB group than in the other groups (Fig. 2); and (v) the time elapsed from King's stages 3 to 4 did not significantly differ among the three groups (Fig. 2).

Discussion

In this study, we analysed the prognostic profile of ALS patients carrying the A382T and the G295S TARDPB mutations as compared to nmALS and C9orf72 patients. To assess prognosis, we used both survival analysis that points to the end stage of the disease and King's clinical staging system that provided more information on the early to mid-disease clinical and anatomical progression. On survival analysis, patients carrying TARDBP mutations reached death/tracheostomy later than C9orf72 and nmALS patients. The finding was independent of potentially confounding factors, i.e. possible prognostic indicators like male sex, age and/or phenotype at onset, and diagnostic delay. On King's staging system, the time elapsed between disease onset (King's stage 1) and involvement of the second region (King's stage 2B) was similar in TARDBP and nmALS patients but longer in TARDBP than in C9orf72-positive patients. Moreover, TARDBP patients reached involvement of a third region (King's stage 3), and PEG/ NIV (King's stage 4) later than C9orf72 and nmALS patients. Overall, these findings indicated that TARDBP-positive patients have a better prognosis than C9orf72-positive and nmALS patients. King's staging analysis also suggested that the higher survival rate and the slower progression associated with the TARDBP mutation could mainly be attributed to the longer time elapsed between King's stages 2B to 3.

The accuracy of our findings is strengthened by the use of incident cases. Incident cohorts tend to better reflect the clinical phenomenology of ALS while prevalent cohorts tend to be younger, live longer, have a higher proportion of male patients and fewer with bulbar onset [19] all factors that may variably affect prognosis. Further supporting the validity of our observation, the overall study population was characterized by demographic and clinical features that resembled the general population of cases [2, 19]. Likewise, known prognostic indicators like age at ALS onset and diagnostic delay also affected the outcome in our sample, though independently from the genetic profile. Finally, in our sample

times to milestones from the King's staging system were consistent with those from previous observations.

In Sardinia, the A382T mutation almost certainly has arisen from a single common founder [23]. Thus, it is possible that other genetic protective factors that offset the effect of this mutation have been acquired through multiple generations, hence leading to some homozygous mutant cases. However, all patients in the present study were heterozygous for the mutation. Owing to the rarity of TARDBP mutations out of Sardinia, only a few recent studies explored the contribution of the TARDBP gene to disease progression [5, 24, 25]. In these studies, however, the sample size was smaller than in our study, and the results were inconclusive. By contrast, mutations in the C9orf72 gene featured a faster progression rate compared to non-mutated patients [11, 12]. Consistent with previous studies in other populations from Italy and Germany [5, 7, 8], data from our multivariable Cox analysis also suggested a shorter survival in Sardinian patients with C9orf72 mutation. However, we could not observe any significant differences in C9orf72 and nmALS estimates on King's staging analysis, even though duration estimates from C9orf72-positive patients tend to be lower than those from nmALS patients. Probably, low statistical power due to the small size of the C9orf72 sample may have contributed to the finding.

We could not provide any inference about the mechanisms underlying the effect of the two genes on disease progression and survival. Nevertheless, analysis of King's staging allowed us a few speculations. In the C9orf72 group, the worse prognosis as compared to the TARDBP and nmALS groups was mainly due to a faster progression in the initial stage of the disease (King's stage 1–2B). In the TARDBP group, the better prognosis likely reflected a slower progression in the intermediate stage of the disease (King's stage 2B–3). Thus, the variable effect of C9orf72 and TARDBP mutations on ALS progression would rely on a differential influence of the two genes on different disease stages. This also raises the possibility that mechanisms of disease progression vary along the disease course, representing different targets for potential disease-modifying treatments.

The main limitations of this study are represented by the retrospective nature of our analysis, the small sample size of C9orf72-positive ALS subgroup, and the exclusion of those patients who did not undergo genetic testing. However, the clinical phenomenology of the patients missing genetic information did not differ from that of patients who participated in the study. Therefore, their exclusion probably did not affect the difference in prognostic estimates between TARDPB and nmALS patients. If genetic information could have been available even for the 77 patients who did not participate in the study, then an increasing number of C9orf72 patients could have increased study power and differences between C9orf72 and nmALS groups could have possibly reached significance. The great majority of TARDBP patients (69/76) carried the A382T mutation and therefore our results refer to this mutation. Owing to the small number of patients carrying the G295S mutation (*n.* 7/76) of the TARDBP gene, we could not explore whether there were prognostic differences between the A382T and the G295S TARDBP mutations. An analysis of other TARDBP mutations discovered in other parts of the world has not carried out and, therefore, our observations mainly refer to the Sardinia population. Moreover, DNA analysis was conducted with Sanger sequencing, which did not allow us to exclude the copresence of other genes that may have modified the phenotype and the rate of progression in individual patients [10]. Finally, we could not have data on the cognitive assessment in a significant number of patients.

In conclusion, our results confirm that genes implicated in the development of ALS may also contribute to progression and survival. The present study provides new information indicating that in Sardinian ALS patients the carriers of TARDBP mutations have a better prognosis than C9orf72-positive and nmALS patients. Patients carrying TARDBP mutation reached milestones along the ALS course, i.e. involvement of third region, need to gastrostomy/non-invasive ventilation and death/tracheostomy, later than nmALS and C9orf72 patients. The better survival in TARDBP patients was independent of other known prognostic indicators like age at ALS onset and diagnostic delay and was probably to be charged on the extension of the interval elapsing between King's stage 2B and stage 3 (involvement of the second to the third region). This information may have relevance for the design and analysis of clinical trials and the proper allocation of strategies aiming to slow down the disease progression.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00415-023-12037-x.

Data availability The data that support the findings of this study are available from author G.B., upon reasonable request.

Declarations

Conflicts of interest None.

Ethical standard statement Study approved by the local Institutional Review Board and conducted in line with the ethical rules for data collection.

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