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A population based study to analyse amyotrophic lateral sclerosis as a multi-step process

Anna D'Amico, Roberta Cucunato, Giuseppe Salemi, Vincenzo La Bella & Paolo Aridon✉

Recent studies suggest that Amyotrophic Lateral Sclerosis (ALS) follows a multistep process. We evaluated this hypothesis in a well-defined ALS population in Palermo, Sicily, almost entirely followed by our ALS Clinical Center. Incident data from the ALS Center (2014–2023) were analyzed, including both sporadic and familial ALS forms of the disease. To evaluate the multistep process, we regressed the natural log of age-specific incidence against the natural log of patient age We identified 216 ALS patients. We obtained a slope of 5 ($r^2 = 0.93$); the 95% CI ranged from 2.51 to 7.60, remaining relatively wide due to the small sample size, with a p-value of 0.008. The slope estimate was consistent with a 6-step process. In the Palermo ALS population, the multistep analysis confirms a process consistent with a 6-step model. This data, obtained in a relatively homogeneous population, further highlights the probability of strict interaction between environmental and genetic variables in the disease. Our data offer insights into the complexity of the mechanisms involved in the pathogenesis of the disease, particularly during its asymptomatic phase. This study supports the hypothesis that a single therapeutic silver bullet would probably be insufficient to arrest or slow the disease's progression.

Keywords ALS, incidence, multistep process.

Amyotrophic Lateral Sclerosis (ALS) is a rare neurodegenerative disease that affects upper and lower motoneurons, leading to progressive voluntary muscle paralysis and death[1,2]. Like most neurodegenerative diseases, its clinical presentation is generally subtle, often leading to a late diagnosis[3].

Despite the countless discoveries on its etiopathogenesis, which support the extreme complexity of the disease, finding drugs to treat the pathology has been a difficult challenge. Hundreds of clinical trials with drugs supposed to affect the disease course have ended unsuccessfully, and now there is a growing consensus that a single drug cannot be the therapeutic solution for ALS[4,5].

Currently, the only treatment options available for ALS are Riluzole and, most recently, Edaravone and phenylbutyrate/taurursodiol, the latter two licensed for use in only a few countries, including the USA, Canada, and Japan. None of them can, however, stop or slow down, in a clinically appreciable fashion, the progression of the disease.

ALS is, in fact, a complex multifactorial disease where genetic and environmental variables give substantial contributions to its etiopathogenesis[6,7]. The aging process, by sharing critical molecular pathways with ALS, is a significant risk factor for the disease[8].

The complex epidemiology of ALS overlaps with cancer: in both diseases, environmental and genetic factors interplay is crucial to etiopathogenesis. Furthermore, it is a long-established acquisition that carcinogenesis and the risk of ALS increase with age.

Working on cancer some seventy years ago, researchers provided solid mathematical support for the fact that in the disorder, there is a linear relationship between the natural log incidence and age, leading to the suggestion that carcinogenesis follows a multi-stage process[9,10]. The hypothesis was based on the mutational load and the importance of age in increasing risk[9]. In particular, in a 1953 work, C.O. Nordling proposed that cancer cells result from seven successive genetic and/or somatic mutations[11].

If the occurrence of each mutation is held constant throughout life, the cancer incidence rate at a given age *t*years should be proportional to the probability of manifestation of each mutation per unit of time, and the age of cancer onset rises to a power of 6[10]. In other words, according to the model, each mutation represents a single

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step, and cancer develops when all steps are completed. A formula was therefore derived showing that the log incidence rate of cancer is directly proportional to the log of age and will rapidly increase six times.

$$log\,incidence = 6log\,\mathrm{t} + \mathrm{c}$$

*c*is a constant representing the number of steps (i.e., mutations at a given age) necessary for carcinogenesis[10].

Recently, the multistep hypothesis has been transferred to ALS. The first large study on this subject was done in Europe, and the authors used four population registries to generate incidence data adjusted by age and sex[12]. Cumulative and single-registry data confirmed for ALS a linear relationship between log incidence and log age at onset, strongly supporting a multistep model for the disease with a six-step process. Each step could be genetic or environmental for sporadic ALS; at least one should trigger the disease onset[12]. These data were subsequently confirmed in a large Asian/Oceanian study involving Australia, South Korea, and Japan, suggesting that the multistep process in ALS may not depend on the geographical/ethnic background of the patients[13,14].

The multistep hypothesis was also used for ALS patients with a pathogenic mutation of one of the four primary gene-related diseases, i.e., cu/znSOD1, C9orf72, TARDBP, and FUS. A population-based study showed that ALS patients with genetic mutations had five steps, a reduced number compared to sporadic ALS[15].

The study led to two important conclusions: first, fewer steps are necessary for disease onset in genetic ALS; second, environmental factors still play a significant role, contributing to the variability in age at onset and, importantly, in the probability of actual onset of the disease[12,15].

In summary, the multistep hypothesis of ALS pathogenesis has been studied in large patient cohorts, demonstrating that, for this neurodegenerative disorder, the process leading to the disease mainly involves six steps.

To verify the consistency of the multistep process in smaller ALS populations, in the present work, we applied the model to a relatively small population of incident ALS patients (2014–2023) living in a defined geographic area, i.e., the metropolitan city of Palermo, Sicily Island, Italy.

2. PATIENTS AND METHODS

2.1 Patients

The study was performed province of Palermo, Sicily Island, Southern Italy (Fig. 1a-b), with a geographical extension of 4.992 km^2 and a population of 1.204.189, according to the Italian Institute of Statistics (ISTAT) database for the years 2014–2023. We used incident data by age and sex for ALS patients referred or diagnosed at our ALS Clinical Center in the University Hospital of Palermo between January, 1, 2014 and September, 30, 2023. We included those ALS patients fulfilling the revised *El-Escorial* criteria integrated with the more recent *Gold Coast*criteria for diagnosis[16,17].

ALS patients from the Metropolitan Province of Palermo are referred to our center in Palermo for either diagnosis or follow-up soon after diagnosis (i.e., within one or two months). Since 2012, we have performed regular genetic screening on *all* ALS patients, both sporadic and familial.

In the Metropolitan Province of Palermo, the ALS Clinical Center of Palermo is the only certified prescriber of riluzole tablets, the only drug formally approved in Italy for the therapy of ALS (either as tablets or suspension)[18]. Using the combination of patients' ALS Center database and the riluzole's new prescriptions/year as a drug tracer[19], we capture almost every incident patient over the years. Therefore, following this analysis, we could estimate the disease's age- and sex-adjusted incidence province of Palermo.

Incident data by age and sex stored in the ALS Center database were extracted from 1 Jan 2014 through 30 Sep 2023 and cross-verified with the new prescription/year of Riluzole. This process led to the identification of 216 incident ALS patients in the study.

This study was conducted in accordance with the tenets of the Declaration of Helsinki. All patients in our database have signed the official informed consent of the University Hospital Policlinico "Paolo Giaccone" of Palermo, to process their sensitive data for research purposes. The scientific use of the collected hospital data was approved by the local Ethics Committee, despite the fact that, according to Italian law, observational, retrospective, non-interventional studies do not require the approval by an ethics committee in Italy.

2.2. The model

The Armitage and Doll model[10]is the methodology recently adapted to ALS to demonstrate that the disease follows a multistep process[12–15].

As outlined in the introduction, this model, borrowed from oncology, uses a formula showing that the logarithm of incidence is directly proportional to the logarithm of age and will rapidly increase six times (log incidence = 6log t + c). If the disease requires more than one step (each of them with risk *u*i), the probability of undergoing the first step at age *t* is *u*1*t*, then for the second step, is *u*2*t*, and so on for subsequent steps. The state is reached after n-1 steps, during which the subject is prepared so that the subsequent and final step, which has a risk u, causes a disease[12].

In those published studies, mortality data of relatively large population-based registries were used. In the present study, we implemented the same model in the relatively small ALS population of the Metropolitan City of Palermo, Sicily Island.

The natural log of the incidence *per*100,000 inhabitants in the ten years of data collection was plotted against the natural log of the patient's age, excluding the patients younger than 26 years due to insufficient data and the patients older than 75 years since the model presented seems to be associated with lower linearity in the older groups[13,15]. The slope of the linear relationship between the natural log of incidence and age equals $n-1$, which is one step less than the number of those required to develop the disease.

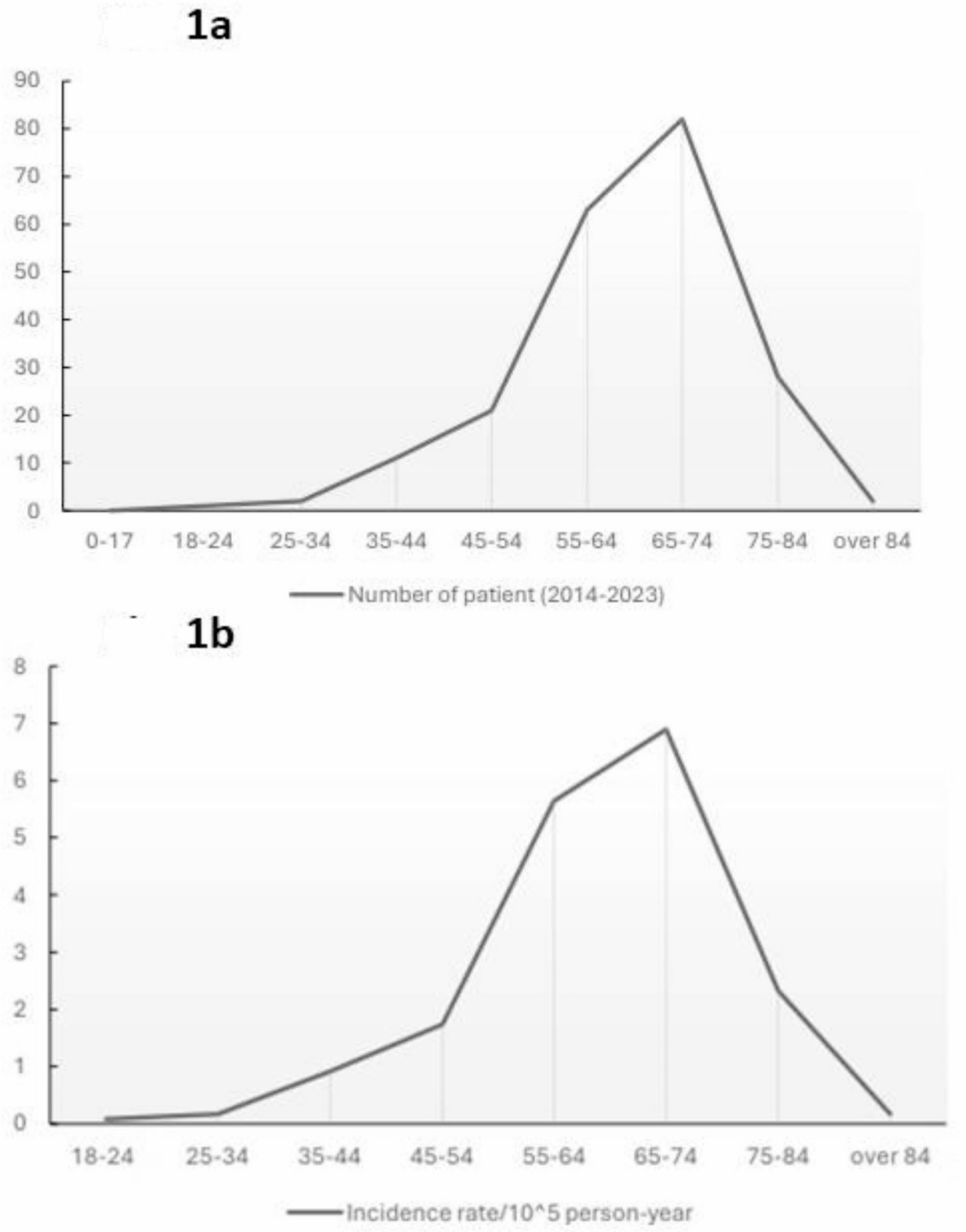

Fig. 1. ALS patients diagnosed/referred at the ALS Clinical Research Center in Palermo in 2014–2023. **a)** Number of patients by age. **b).** Crude incidence rates by age.

Genetic analysis

All incident ALS patients identified (both sporadic and with a positive familial history of the disease) had been tested for mutations in disease-related genes using a panel of 19 genes which include, among others, cu/znSOD1, TARDBP, FUS, and C9orf72. Due to the relatively low number of cases identified, it was not possible to reproduce the same model for the various genetic mutations.

Data Analysis and statistics

All analyses were performed with SIGMAPLOT 8.0 (Systat Software Inc., San José, CA, USA) software package, and Microsoft Excel Version 16.89.

The adopted Armitage-Doll model assumes that the plot of the log of ALS incidence vs. the log of age should be linear, with a slope n – 1; therefore, adding 1 to this value identifies the number of steps needed for the disease onset[10].

The incidence by age was calculated using five 10-year age groups, i.e., from 25 to 34 through 65–74. The younger and older age groups were omitted from the Armitage-Doll model because of the risk of imprecise measurements, similar to what has been seen in cancer. The upper limit of each age group was used for the regression analysis, which was underweighted to avoid the risk of a slope biased towards older age groups.

Variables were expressed as Mean ± Standard Deviation or Median with interquartile intervals (IQR) where appropriate.

RESULTS

The Italian Institute of Statistics reports that the population of the Metropolitan City of Palermo has been stable over the years 2014–2023, with a mean of 1.204.189 inhabitants and an annual percent variation of −0.91 (range between − 1.39 and − 0.28). The retrospective analysis covering ten years allowed us to identify 216 incident ALS patients (96.3% sporadic and 3.7% with a positive family history for the disease) stably living in Palermo province (Table 1). The low percentage of patients with a family history of ALS may be due to the lack of awareness of the disease in the local area in the past decades. As a result, disorders attributable to the disease were often unrecognized and misdiagnosed. Thus, there is often a lack of awareness in families, and it is not uncommon for family histories of neurological diseases to be discovered later after careful analysis by some affected family members.

Regarding the site of onset, 74.1% of the patients were spinal ($n = 160$), 23.1% were bulbar ($n = 50$), and 2.8% respiratory ($n = 6$), with a median age-at-onset of 65 (IQR = 59–70). The Men/Women ratio was 1.18 (Table 1).

As we routinely screen all consecutive ALS patients with ECAS, a widely-used tool for frontal-lobe dysfunction in this disease, we found in our population six patients (2,8%) meeting clinical and neuropsychological criteria for ALS and behavioral-variant FrontoTemporal Dementia, ALS-bvFTD[20,21], and 41 (19%) with frontal-lobe dysfunction but not dementia[20]. Since this study did not involve cognitive variables, these cases were included in the analysis. The Edinburgh Cognitive and Behavioural ALS Screen (ECAS) was first developed to identify cognitive and behavioral changes in ALS patients. The ECAS is a brief, multi-domain, neuropsychological screening test that evaluates executive function, social cognition, verbal fluency, and language (ALS-specific domain), as well as memory and visuospatial skills (non-ALS-specific domain). It also provides an option for patients to choose between oral and written answers to minimize the effect of physical impairment on performance measures[22].

The screening for gene mutations using a 19-gene NGS panel in our incident ALS population led to the identification of 8 patients (4.5%) with known mutations in TARDBP (G294V and two brothers with A382T, $n = 3$), FUS (R521G, $n = 1$) and C9orf72 pathological GGGGCC expansions ($n = 4$). No cases with cu/znSOD1 mutations were identified. Single gene variants of uncertain significance (VUS) were detected in 22 ALS patients with no other mutations (three with a familial history of the disease and the others sporadic, accounting for 10,2% of all population).

	ALS patients (tot.216)	ALS patients (%)	
Family History			
sALS	208	96,3%	
fALS	8	3,7%	
Site of onset			
Spinal-onset	160	74,10%	
Bulbar-onset	50	23,10%	
Respiratory-onset	6	2,80%	
M/W ratio	1,18		
Mean age at onset	65 (59–70)		
Cognitive Features			
ALS-FTD	6	2,8%	
Frontal-lobe dysfunction (no dementia)	41	19%	
Genetics			
Causative mutation	8	3,7%	TARDBP : 3 (1 = G294V; 2 = A382T); FUS:1 (R521G); C9orf72:4 (GGGGCC expansions)
VUS	22	10,2%	

Table 1. The clinical and demographical characteristics of the patients under study. M, men; W, women; sALS: Sporadic ALS. fALS: Familial ALS. VUS: variant of uncertain significant.

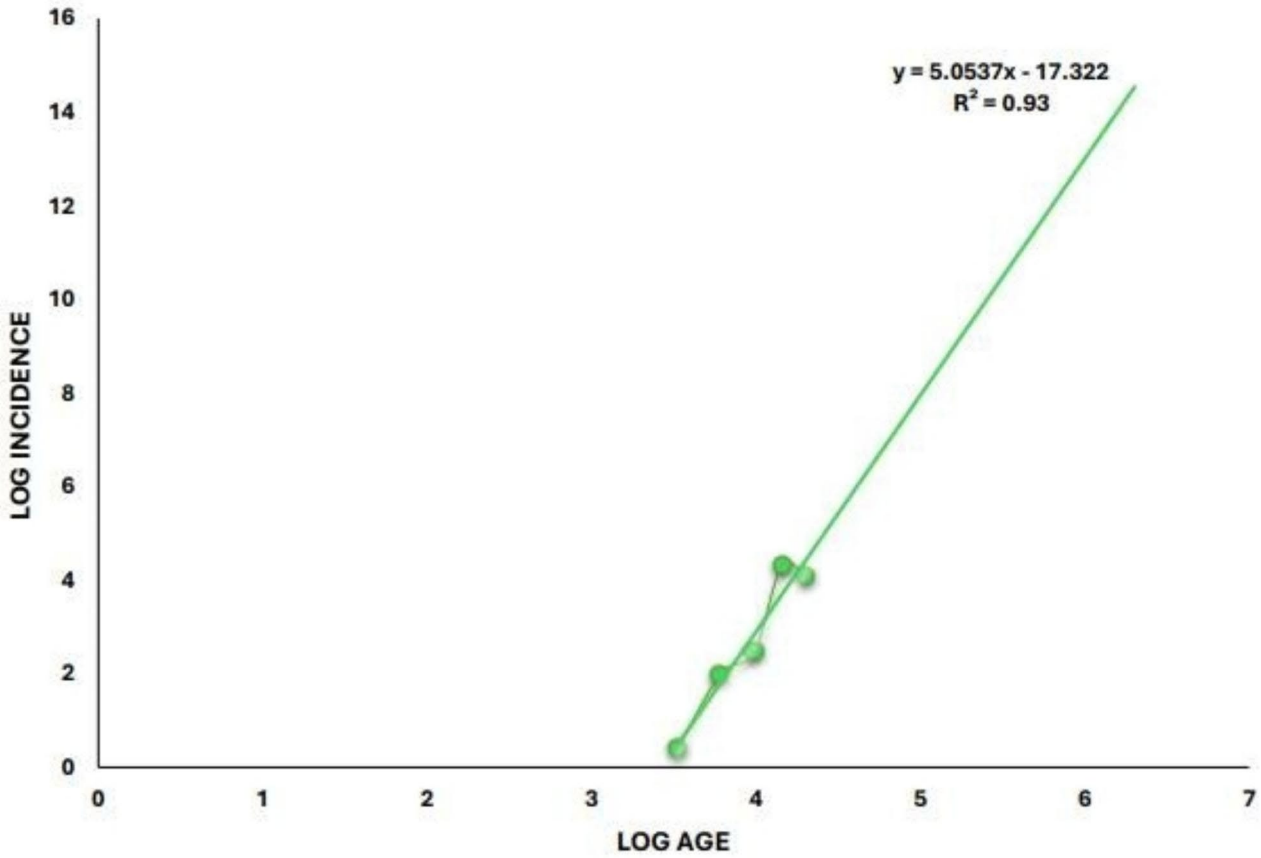

Fig. 2. Log incidence vs. log age in the ALS population province of Palermo, Sicily Island geographical area. The graph shows a good fit with a straight line with r2 = 0.93 consistent with the multistep model. The slope estimate is 5 (CI 95% 2.51–7.60; $p = 0.008$), suggesting that six steps are needed to trigger the onset of the disease.

Figure 1a shows the number of ALS cases identified between 2014 and 2023 according to age-at-onset. In the graph, we plotted the age in 10-year intervals, except the first range, which was 0–24 (because of the extreme rarity of ALS cases in the age range), and, for the same reason, the last range included all cases diagnosed/identified after the age 74. As reported in most population-based studies[23,24], the age range at higher risk for ALS is roughly 50–75 years, and our data align with this trend, with a good overlap between men and women. Accordingly, the incidence data in our population show higher values in the age range 55–75 years (Fig. 1b). The median incidence in 2014–2023 was $1.8/10^5$ person-years (range = 1.69–2.25), with slightly higher values in men than in women (Table 1). Incidence data, of course, are biased by the statistical analysis performed on a single center (although it collects the majority of patients in western Sicily).

The incidence data concerning the ALS patients (both sexes), retrospectively identified in the ten years of analysis, represented the basis for the subsequent multistep analysis.

The multistep model, applied to the incident ALS population of the Metropolitan City of Palermo, demonstrated a logarithmic increase in incidence with age and resulting in a straight line that fit the data perfectly ($r^2 = 0.93$) (Fig. 2). The slope estimate was 5 with with a 95% confidence interval of 2.51–7.60 ($p = 0.008$). Since the multistep process implies a linear graph with a slope equal to n-1 (10,11), our estimate indicates a 6-step model for our Sicilian ALS population.

DISCUSSION

In this study, we demonstrate that the multistep process applied to the incident ALS population in a relatively small geographical area, i.e., the Metropolitan City of Palermo, appears to be consistent with a 6-step process, being similar to the data obtained with large, often nationwide, ALS registries[12,13,15]. The steps found are consistent with an interplay between environmental and genetic variables in the pathogenesis of this neurodegenerative complex disease.

The multistep model was initially established to explain the sequential mutational steps leading to the increased incidence of cancer with age in adulthood and to quickly understand the latency time between exposure to the carcinogen and the manifestation of the neoplasm[10,11]. Furthermore, it allows a better understanding of many of

the epidemiological features recorded in the field of oncology: the increase in mortality with increasing age in many forms of cancer, the irregularity in the increase of cancer at some sites, the latency time, the finding that the incidence of neoplasia increases as the concentration of the applied carcinogen increases[10].

The multistep process model has been recently successfully adapted to Amyotrophic Lateral Sclerosis[12–15]. This progressive neurodegenerative disease shares several factors with cancer, including the incidence increases with age. It also exhibits a clinical progression with a complex inheritance pattern that rarely follows a rigorous Mendelian canon[25,26]. Furthermore, like the pathological process in oncology, ALS involves a well-defined cell population, i.e., the motor neurons. However, it appears to be a critical difference between the two pathological conditions: while in cancer, the sequential environmental and/or genetic insults induce aberrant cell de-differentiation and proliferation, in the neurodegenerative process leading to ALS, the inability of neurons to divide and proliferate leads to cell abiotrophy and death[27–30].

In our study, we enrolled all patients with suspected or already diagnosed ALS residents in province of Palermo who are referred to our ALS Center for management and the disease-specific Riluzole prescription. Therefore, we could identify roughly 96–99% of the incident ALS patients during the established 2014–2023 interval.

The genetic screening of the incident ALS population identified a small proportion of patients with mutations in significant ALS-associated genes and VUS, totaling some 10.2% of the population. This proportion is relatively small, and we could not perform an independent multistep analysis of the cases with gene mutations and VUS.

It is known, however, that gene mutations affect the 6-step model suggested for the disease[12,13]. It has been indeed demonstrated that in cu/znSOD1 mutation carriers, the process likely takes two steps; in TARDBP mutation carriers, the steps are most probably four, and in patients with C9orf72 GGGGCC pathological expansion, the process follows three steps, well below the 6-steps necessary to allow ALS onset in patients without germ-line mutations[15]. The number of steps, therefore, decreases in those patients with genetic mutations, and this evidence strongly supports the preeminent role of ALS-associated genes in the onset and pathogenesis of the disease.

Most familial cases of our ALS population (4/6 subjects, 66.6%) had mutations in the major ALS-associated gene or VUS. Though the proportion of familial ALS seems low in the present study, it further confirms that gene mutations/VUS are prevalent in this subgroup.

In this context, the model definitively confirms the long-held hypothesis that people with a family history of ALS have a greater genetic predisposition towards ALS, as they may need fewer steps to reach the level of molecular damage leading to the onset of the disease[15]. However, the transmission pattern in these cases is rarely with complete penetrance. Rare exceptions are, for instance, FUS mutations at the C-terminal (e.g., FUS^{P525L}) or C9orf72 with more than 100/150 GGGGCC repeats. The variable penetrance of most ALS-related gene mutations/expansions indicates that, in most familial cases, co-exposure to environmental risk factors is necessary to drive disease onset.

The multistep model applied to ALS in different studies[12–15], including the present one, confirms that similar environmental and genetic factors probably play a role in the onset of the disease and pathogenesis, regardless of the geographical area studied and its wideness. Because of the expected important role of genetic variables in all forms of ALS, we routinely screen all consecutive ALS patients with multigene NGS panels, whether they are sporadic or familial. These panels are continually expanded to include new ALS-associated pathogenic/likely pathogenic gene variants. The collection of the data should therefore lead to the identification of at least a few of the steps necessary for the disease to develop, allowing a better knowledge of its complex pathogenesis.

Our study has some limitations. First and foremost, the small patient population covered by the statistical analysis leading to a wider confidence interval. In addition, although our outpatient clinic for motor neuron disease collects almost all patients in western Sicily, we cannot ignore the presence of other centers in Sicily Island dealing with this disease.

It would, also, be very useful to further investigate our model by evaluating the differences in patients with genetic mutations (especially for the 4 pathogenic mutations C9orf72, SOD1, FUS, TARDBP). Indeed, as previously mentioned, the number of steps decreases depending on the genetic mutation detected. This finding could be correlated with environmental factors to understand which steps the patient was exposed to before the disease onset and the impact of each factor.

CONCLUSIONS

In conclusion, we have demonstrated that the multistep model, even when applied to an ALS population in a small geographic area, i.e., the metropolitan city of Palermo, Sicily Island, allows the disease to be explained by a 6-step process. Environmental (i.e. occupational activity and premorbid stress) and genetic variables are constitutive elements of the different steps.

Identifying the steps leading to the ALS onset may be of great help in dissecting the disease's pathogenic mechanisms and finding novel therapeutic pathways.

Data availability

The data supporting this study's findings are available from the corresponding author upon reasonable request.

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Author contributions

A D: acquisition of the data, analysis, and interpretation of the data, drafting the manuscript and figures, revising the manuscript for important intellectual content; V.B.: conception and design of the study, analysis, and interpretation of the data, revising the manuscript for important intellectual content and approval of the final version. G.S.: revising the manuscript for important intellectual content and approval of the final version; R.C.: analysis, and interpretation of the data, revising the manuscript for important intellectual content and approval of the final version. P.A.: analysis and interpretation of the data, drafting the manuscript and figures , revising the manuscript for important intellectual content.

Declarations

Competing interests

Dr. D'Amico reports no conflict of interest related to this work; Dr. Cucunato reports no conflict of interest related to this work; Prof. Salemi reports no conflict of interest related to this work; Prof. Aridon reports no conflict of interest related to this work.

Additional information

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