Science of the Total Environment 957 (2024) 177796

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Temporo-spatial analysis of amyotrophic lateral sclerosis in Spain: Altitude and land use as new determinants of the disease

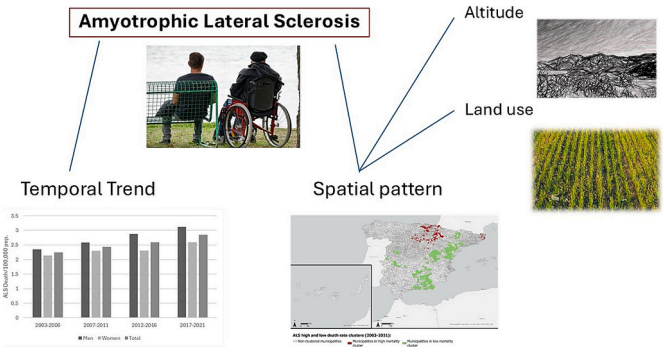
Ana Santurtún [a,b,*,1], Pablo Medín [a,1], José Antonio Riancho [b,c,d,e], Marina Santiago-Setién [e], Fernando Ortiz [e], Adolfo López de Munain [f,g,h,i], Ricardo Almendra [j,k,2], Javier Riancho [b,c,h,l,**,2]

[a] Unit of Legal Medicine, Department of Physiology and Pharmacology, University of Cantabria, Santander, Spain
[b] Valdecilla Research Institute (IDIVAL), Santander, Spain
[c] Department of Medicine and Psychiatry, University of Cantabria, Santander, Spain
[d] Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER, ISCIII), Madrid, Spain
[e] Hospital U. M. Valdecilla, University of Cantabria, Santander, Spain
[f] Neurology Department, Donostia University Hospital, Spain
[g] University of the Basque Country-Osakidetza, Spain
[h] CIBERNED, Barcelona, Spain
[i] Biodonostia, 20014 Donostia-San Sebastián, Spain
[j] Centre of Studies on Geography and Spatial Planning (CEGOT), Coimbra, Portugal
[k] Department of Geography and Tourism, Faculty of Arts and Humanities, Colégio de São Jerónimo, University of Coimbra, Coimbra, Portugal
[l] Hospital Sierrallana, Torrelavega, Spain

HIGHLIGHTS

- ALS is the most common neurodegenerative disease affecting motor neurons.
- ALS mortality showed a rising trend over time for both sexes.
- High mortality clusters were identified in the North and Northeast regions of Spain.
- Low altitude and agricultural land may influence ALS distribution.

GRAPHICAL ABSTRACT



* Correspondence to: A. Santurtún, Unit of Legal Medicine, Department of Physiology and Pharmacology, University of Cantabria, IDIVAL, Cardenal Herrera Oria s/n, 39011 Santander, Spain.
** Correspondence to: J. Riancho, Service of Neurology, Hospital Sierrallana, IDIVAL, Barrio Ganzo s/n, 39300 Torrelavega, Spain.
E-mail addresses: ana.santurtun@unican.es (A. Santurtún), javier.riancho86@gmail.com (J. Riancho).
[1] Ana Santurtún and Pablo Medin share first authorship.
[2] Ricardo Almendra and Javier Riancho share last authorship.

https://doi.org/10.1016/j.scitotenv.2024.177796
Received 16 October 2024; Received in revised form 23 November 2024; Accepted 26 November 2024
Available online 30 November 2024

ARTICLE INFO

Editor: SCOTT SHERIDAN

Keywords:
Amyotrophic lateral sclerosis
Altitude
Cluster analysis
Land cover
Agriculture
Spain

ABSTRACT

Introduction: Amyotrophic lateral sclerosis (ALS) is the most common neurodegenerative disease affecting motor neurons. Currently, ALS is conceived as the result of the interaction between genetics, environmental factors, and aging. This study analyzed the spatial and temporal patterns of ALS in Spain, delving into the potential relationships between altitude, land cover, and this disease.

Methodology: ALS death data were collected over a 19-year period, including information on sex, age and municipality of residence. The standardized mortality rate was calculated for each municipality of residencia, and Anselin's local Moran's I statistic was used to identify clusters of high and low incidence. Altitude data were sourced from the Copernicus Land Monitoring Services, while land cover data came from CORINE satellite images and national agricultural statistics.

Results: The average annual incidence of ALS deaths among adults was 2.5 per 100,000 people. Higher mortality rates were noted in males (2.8) than in females (2.3), with both sexes exhibiting a rising mortality trend in a temporal analysis. Cluster analysis revealed that high mortality areas were mostly located in the North and Northeast of the country. Municipalities in these clusters had significantly lower median altitudes and larger areas of Permanently Irrigated Arable Land and Broad-Leaved Forest.

Conclusion: This study provides new evidence about the increase in ALS cases in European countries during the last decades, reporting for the first time altitude and certain agricultural land uses as potential geographic determinants of the disease.

1. Introduction

Amyotrophic Lateral Sclerosis (ALS) is the most common neurodegenerative disease affecting motor neurons (MNs). The annual incidence of ALS worldwide ranges from 0.6 to 3.8 cases per 100,000 inhabitants, with Europe reporting the highest incidence (2.1–3.8 cases/100,000) (Feldman et al., 2022; Riva et al., 2024). Despite differences in incidence across various regions, numerous authors have reported a global increase in disease incidence over the past decade, predicting that it will continue rising in the coming decades (Arthur et al., 2016). Specifically, in Cantabria (a small Spanish province in the Cantabric Coast), we recently described a 70 % increase in incidence over the past 40 years (Riancho et al., 2016), and it is projected that worldwide, the incidence will increase by around 60 %, mainly due to increases in developing countries (Arthur et al., 2016). A small proportion of ALS cases have a familial origin (fALS), secondary to mutations in specific genes. In contrast, approximately 90 % of cases are classified as sporadic ALS (Al-Chalabi et al., 2024; Riancho et al., 2019). Sporadic ALS cases are according to multistep hypothesis (Al-Chalabi et al., 2014) presumed to result from the consequences of, a probably variable in each case, interaction of genetic risk factors, environmental factors, personal lifestyle patterns and aging (Zufiría et al., 2016). Thus, motor neuron degeneration will occur when the sum of an individual's genetic risk factors and a combination of environmental risk factors, favored by aging, reaches a certain threshold, beyond which self-perpetuating mechanisms will be established.

The importance of environmental factors in the genesis of the disease is highlighted by the increased incidence of ALS in recent decades (Goutman et al., 2023). Given the low probability of significant changes in the genetic background in recent years, the aging population, along with changes in exposure to environmental factors, seem to be responsible for the higher frequency of cases (Riancho et al., 2018). The term environmental factors encompasses a wide range of situations, including both external factors (exposure to pollutants [aerial, dissolved in water or contaminating the food], residence in different geographical areas, exposure to infectious agents, etc.) and internal factors (physical activity, nutritional habits, exposure to recreational toxic and drugs, presence of other comorbidities, etc.) (Goutman et al., 2023; Riancho et al., 2021; Castanedo-Vazquez et al., 2018). One of the first pieces of evidence of the importance of environmental factors and the possibility of establishing preventive strategies from their study was the identification of a disproportionate occurrence of an ALS-like syndrome in the indigenous population of Guam Island, related to a neurotoxin present in the roots of the Cycad plant (Reed et al., 1966; Murch et al., 2004). Since then, the existence of other high-incidence ALS clusters has been investigated to identify environmental factors that may favor the onset of the disease. In this regard, increased incidence rates associated with a high number of factors, such as the consumption of selenium-rich waters exposures, and exposures to other metals, have been documented (Riancho et al., 2018). Recently, Spencer et al., aiming to delve into the role of environmental factors, reviewed the spatial clusters described worldwide. In this regard, while they describe the scarcity of studies and their limitations, they indicate that in most cases, toxic agents and neurotoxins could be implicated behind high-incidence clusters (Spencer et al., 2019). Additionally, clusters of disease incidence lower than expected have been described, but without any significant correlation reported by researchers (Rooney et al., 2015a). To improve knowledge about the possible toxins involved, some authors have focused on analyzing occupational exposures to chemical agents (solvents, heavy metals, and pesticides), and some studies have described an association with agricultural pesticides (Kang et al., 2014; Kamel et al., 2012; Capozzella et al., 2014). By contrast, other studies found no occupational relationship but did find a link with living in rural environments where there may be greater indirect exposure to certain agricultural products (Korner et al., 2019).

In this regard, the present study aims to analyze the spatial pattern in Spain at the municipal level, seeking possible high-incidence clusters to discuss the role of potential risk factors involved. Secondly, the relationship between ALS and the type of land cover (with particular attention to agricultural land use) will be evaluated.

2. Methodology

An ecological study of the incidence of ALS in Spain was conducted over a 19-year period using a spatial analysis methodology.

2.1. Study area

Spain is the second largest country in the EU, covering an area of 506,000 km^2 with >48.5 million inhabitants. Administratively, Spain is divided into 50 provinces and two autonomous cities (NUTS 3 level). Additionally, the provinces are subdivided into municipalities (currently, there are 8131 municipalities in the country).

2.2. Mortality data

Annual data on deaths due to ALS (ICD-10 G12.2 Motor Neuron Disease) were collected from 2003 to 2021, including sex, age and municipality of residence. The data source was the National Institute of Statistics (INE). Non-adult cases (age below 20 years) and those with no

recorded municipality of residence were excluded (Fig. 1).

2.3. Altitude and land cover

The altitude of the municipalities located in peninsular Spain and the Balearic Islands was derived from the Copernicus Land Monitoring Services (EU-DEM dataset); data corresponding to the Canary Islands and the autonomous cities of Ceuta and Melilla were not available (Fig. 1).

Land cover was analyzed using data from both satellite images (from the CORINE [Coordination of Information on the Environment] program) and national statistics (from the INE). The CORINE program was launched by the European Commission to develop a standardized methodology for producing continent-scale land cover, biotope, and air quality maps. Since 1990 (when the first CORINE Land Cover dataset was produced) it has become a flagship component of the European Environment Agency's Copernicus Land Monitoring Service, providing essential information on European land cover/land use for over three decades. The present study works with data from the last update, 2018, in which 44 thematic classes of land cover are differentiated. The dataset has a Minimum Mapping Unit (MMU) of 25 ha (ha) for areal phenomena and a Minimum Mapping Width (MMW) of 100 m for linear phenomena and is available both as vector and as 100 m raster data. Of the 44 thematic classes, those that covered at least 5 % of the country's territory were included (Table 1). To work at the municipal scale and compare with ALS incidence rates, a filter on the Municipalities layer with QGIS was created.

Subsequently, from national statistics, the land area used for agriculture was collected, differentiating permanent outdoor CROPS (CROP 1) and greenhouse or accessible high shelter (CROP 2) from all municipalities in Spain. The source of the data was the INE, which collects it from the census of agricultural holdings.

2.4. Statistical analysis

We divided the analysis period into two stages to separately analyze the last 10 years and the prior period (2003–2011 and 2012–2021). The Kendall rank correlation coefficient with a 95 % CI was used for the trend calculations of rates.

The number of ALS deaths in each municipality was standardized using the Curtin and Klein (1995) direct method, with the average population distribution by five-year age groups in the year 2012 in the Spanish municipalities as the reference. Subsequently, the average standardized rate was calculated and the mean for the whole period was obtained from the standardized rates over the 19-year period.

The geographic patterns of an event may range from being fully clustered at one extreme (i.e. Positive spatial autocorrelation) to completely dispersed at the other (i.e. negative spatial autocorrelation). In the absence of significant spatial pattern the event spatial pattern is often described as random. Moran's I spatial autocorrelation analysis was conducted to simultaneously analyze the spatial distribution of ALS mortality rate and to explore the spatial dependence of this event across the different municipalities of Spain. Anselin local Moran's I statistic was applied to identify clusters of municipalities with significantly higher and lower mortality rates of ALS. The models applied considered distances as Euclidean through inverse distance method of conceptualization of spatial relationships combined with a row standardization.

To analyze the relationship between municipalities where ALS incidence clusters were detected and the thematic classes of land cover and land use from the CORINE Land Cover dataset, the percentage of the total area of each municipality allocated to each class was calculated. The differences in land cover between municipality categories (Non cluster area, Low incidence cluster area, High incidence cluster area) were assessed using the non-parametric Kruskal-Wallis test. The Mann-Whitney test was employed to compare the cluster categories for the thematic classes of land cover. This same method was used to analyze the relationship with the altitude.

To assess the statistical association between ALS mortality standardized rate and the percentage of CROP 1 and CROP 2, we attributed a value to each municipality corresponding to the average density. Then, we performed a global and stratified linear correlation analysis, at NUT III level.

3. Results

Over the 19-year study period, 17,956 adults (aged 20 and over) died from ALS in Spain. Of these deaths, 52.7 % occurred in males. The median age of individuals who died from this cause was 71 years (53 [P10]- 83[P90]); 70 years in males (51[P10]- 82[P90]), and 72 years in females (55[P10]- 84[P90]).

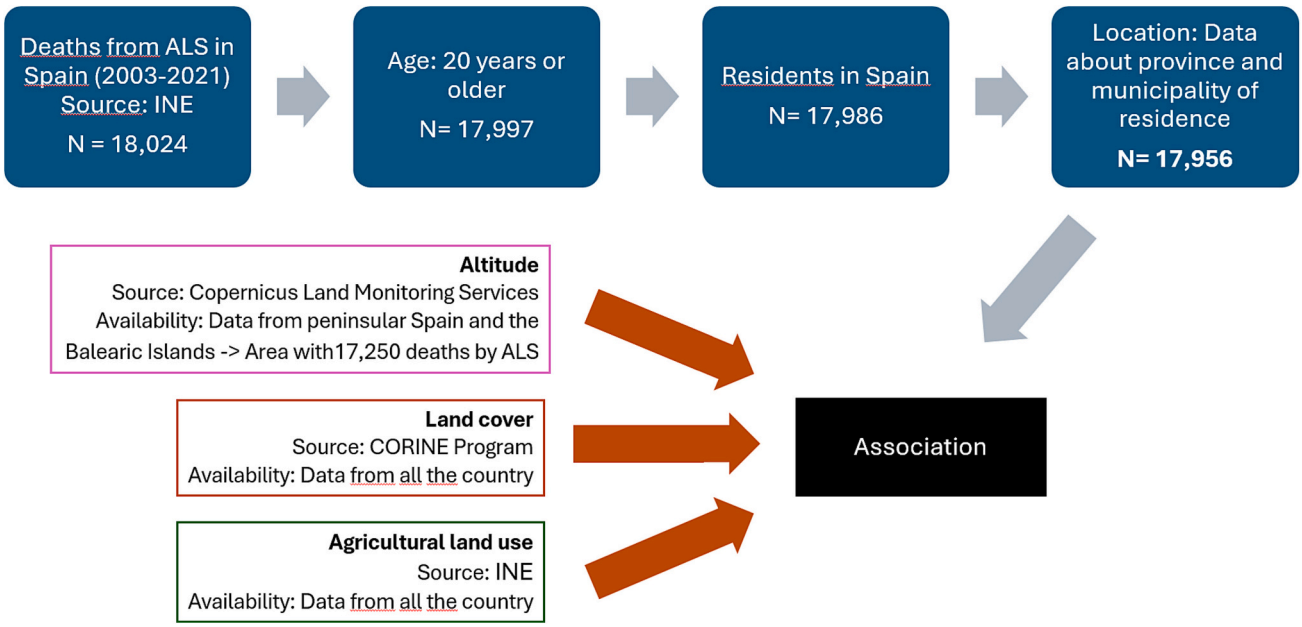


Fig. 1. Flowchart for the selection of ALS mortality data and variables for association analysis.

Table 1
Descriptive analysis of the CORINE Land Cover Classes at least covering a 5 % of the country's territory by ALS Clusters categories, values (%).

Code	Type	Non-cluster areas			Low incidence cluster areas			High incidence cluster areas		
		Average	SD	Median (P10-P90)	Average	SD	Median (P10-P90)	Average	SD	Median (P10-P90)
211	Non-irrigated arable land	24.5	29.5	9.7 (0.0–73.7)	11.9	18.7	2.8 (0.0–38.8)	26.2	26.7	21.5 (0.0–68.2)
212	Permanently irrigated land	6.1	15.3	0.0 (0.0–19.3)	3.1	9.4	0.0 (0.0–7.5)	6.8	13.5	0.0 (0.0–23.2)
311	Broad-leaved forest	10.3	15	3.4 (0.0–30.7)	5.8	9.5	1.4 (0.0–17.9)	18.1	20.1	11.6 (0.0–49.0)
312	Coniferous forest	8.8	14.8	1.5 (0.0–29.1)	14.6	18.5	6.7 (0.0–42.2)	14.4	17.8	7.5 (0.0–43.3)
321	Natural grassland	5.6	9.7	1.3 (0.0–17.0)	8.7	12.1	3.8 (0.0–25.4)	2.8	5.8	0.7 (0.0–6.8)
323	Sclerophyllous vegetation	8.6	13.2	2.4 (0.0–26.4)	16.8	18.1	11.1 (0.2–44.3)	6.1	10.1	1.6 (0.0–17.2)

The average annual incidence rate of death due to ALS among adults over the 19-year study period was 2.5 cases per 100,000 inhabitants (2.8 cases per 100,000 inhabitants in males; 2.3 cases per 100,000 inhabitants in females). During the first period of the study (2003−2011), the average incidence rate among adults was 2.4 deaths per 100,000 inhabitants (2.5 in males and 2.2 in females). By 2012 and 2021, it increased up to 2.7 deaths per 100,000 inhabitants (3.0 in males and 2.5 in females) (Supplementary Fig. 1).

In the analysis of trends in annual rates at the provincial level by sex among the adult population, we observed that the trend was statistically significantly increasing in 16 (Albacete, Alicante, Burgos, Cádiz, Castellón, A Coruña, Granada, Guipúzcoa, León, Murcia, Ourense, Asturias, Santa Cruz de Tenerife, Sevilla, Valencia and Vizcaya) and 6 (Cádiz, Guipúzcoa, Las Palmas, Salamanca, Santa Cruz de Tenerife and Vizcaya) out of the 52 provinces studied for males and females, respectively (Fig. 1A, Supplementary Table 1). By contrast, we observed that in 1 province, the trend was significantly decreasing among females (Fig. 2A, Supplementary Table 1). When assessing the latest 10 years of the study period, the increasing trend in men remained exclusively in 3 of the provinces (A Coruña, Asturias and Vizcaya) while in women it became statistically significant in up to 8 provinces (Barcelona, Cádiz, Málaga, Cantabria, Segovia, Sevilla, and Valladolid). In addition, we observed that the trend was significantly decreasing in men in the province of Zamora (Fig. 2B, Supplementary Table 1).

The ALS standardized mortality rate at the municipal level of residence varied considerably across the country. While in the majority of the municipalities (5194 there was no record of deaths, the municipality with the highest rate reached values of 307 deaths by 100.000 habitants (Supplementary Fig. 2). The spatial autocorrelation test showed a significant, yet low, positive association (Global Moran's I: 0.003; *p*-value: <0.001). The cluster analysis identified clusters of high mortality located between people living in the North and Northeast of the country and of low mortality without a specific spatial pattern (Fig. 3).

3.1. Association between ALS mortality and altitude

The 8131 municipalities were classified into 3 categories depending on whether they were located within a cluster with a high incidence rate of mortality due to ALS, a low one, or had any cluster association. The results of the Kruskal–Wallis test showed that there existed highly significant differences ($p < 0.001$) in median altitude across cluster categories. The assessment between municipalities coinciding with clusters of high incidence and those outside these clusters confirmed that the formers had a significantly lower median altitude than the others (High incidence cluster areas [Median = 568.78, 130.19[P10]-1058.72[P90]], Low incidence cluster areas [Median = 848.96, 312.93[P10]-1361.69 [P90]]; Non-cluster areas [Median = 667.66; 148.39[P10]-1109.22 [P90]]).

3.2. Association between ALS mortality and land cover

The average percentage of surface area in the municipalities for the 3 categories and for each thematic class of land cover is represented in Table 1. The results of the Kruskal-Wallis test showed that in the 6 classes of land cover that represented at least 5 % of the country's territory there were statistically significant differences ($p < 0.001$) with the municipality's incidence clusters (Table 1).

The results of the Mann Whitney pairwise comparisons between the cluster categories for the thematic classes of land cover showed that Permanently irrigated arable land (212) and Broad-leaved forest (311) were significantly higher for those municipalities coinciding with clusters of high incidence ($p < 0.001$). On the other hand, Natural grassland (321) was significantly higher for those municipalities located in clusters of low incidence ($p < 0.001$).

3.3. Association between ALS mortality and farming

The percentage of utilized agricultural area of permanent outdoor crops (Supplementary Fig. 3) and the percentage of utilized agricultural

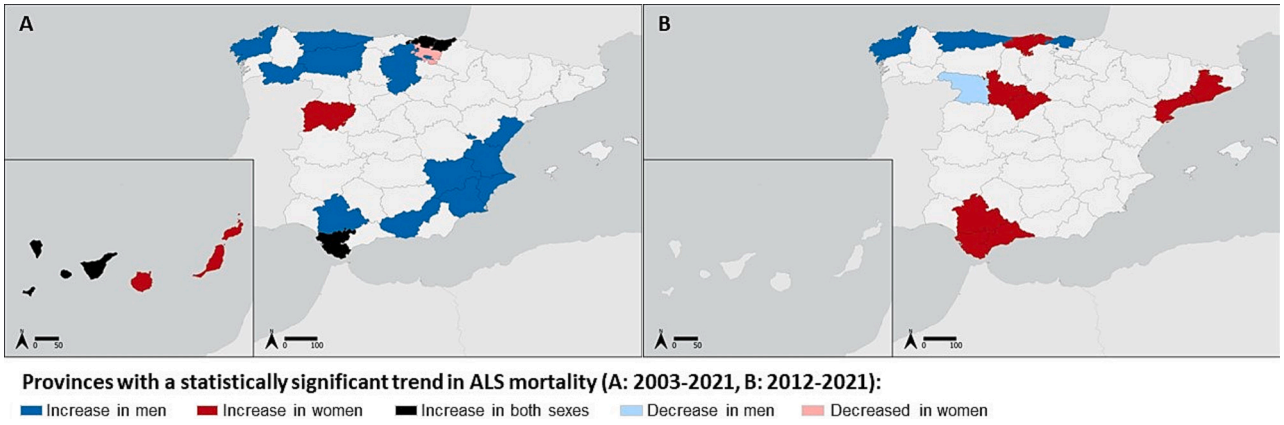


Fig. 2. ALS mortality rate by sex and period of the study.
Provinces with a statistically significant trend in ALS mortality rate by sex are represented. A: Period 2003–2021; B: Period 2012–2021.

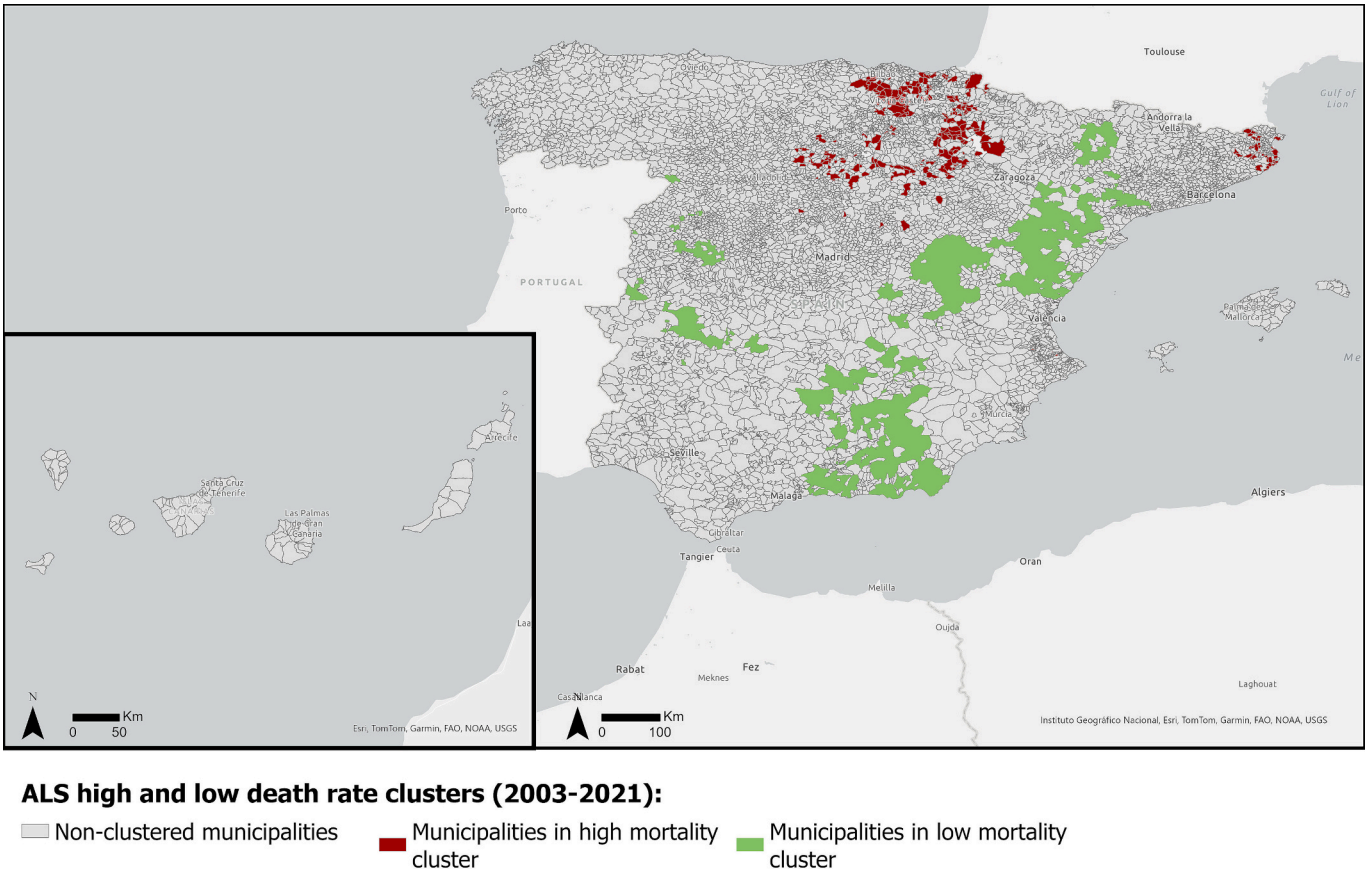


Fig. 3. ALS clusters.
ALS high and low rate clusters across Spain. Non clustered-municipalities, high-clustered municipalities and low-clustered municipalities are represented in grey, red and green, respectively.

area in greenhouse or accessible high shelter (Supplementary Fig. 4) exhibited important cross-country disparities. While the former tended to be higher in the municipalities of the center and the south of the country, the presence of greenhouse or accessible high shelter agriculture was more frequent in municipalities near the coastline.

Overall, there was not a linear correlation between ALS death rate and the percentage of utilized agricultural area of permanent outdoor crops (correlation coefficient: 0.001; *p*-value: 0.943). However, significant coefficients were found at the NUT III level, where two regions (Huesca and Las Palmas) exhibited a significant positive association and one (Cáceres) a significant negative association (Fig. 4A).

While at the national level the linear correlation between ALS death rate and the percentage of utilized agricultural area of greenhouse or accessible high shelters was also non-significant (correlation coefficient:

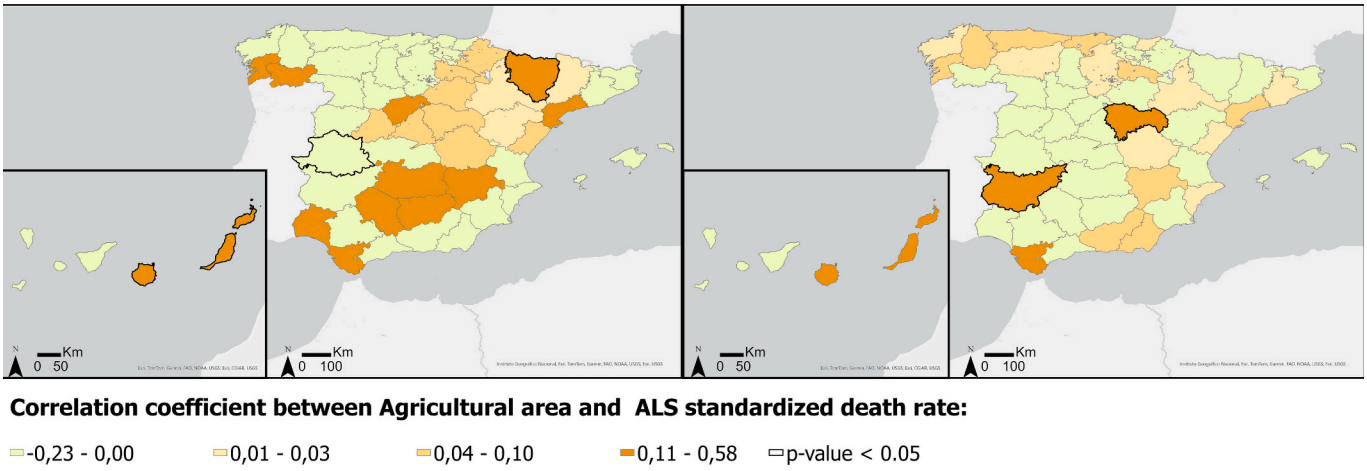


Fig. 4. Association between ALS mortality and farming at a provincial level.
A. CROP 1 – Association with agricultural area of permanent outdoor crops. B. CROP 2 – Association with agricultural area in greenhouse or accessible high shelter. Those provinces showing negative or positive correlation are colored in green and orange scale respectively. Provinces exhibiting a statistically significant correlation have been highlighted.

0.001; p-value: 0.966), two NUT III regions (Badajoz and Guadalajara) presented positive significant associations (Fig. 4B).

4. Discussion

Our study analyzes the pattern of ALS in Spain from both a spatial and temporal perspective. Additionally, it delves into the relationship between the presence of ALS, the altitude, and the type of land and its agricultural use.

Regarding disease incidence, our results are consistent with the increase in disease frequency reported by other groups in recent decades (Arthur et al., 2016; McFarlane et al., 2024). These findings align with other studies from our group at both regional and national levels conducted just a decade ago (Riancho et al., 2016; Santurtún et al., 2016). Due to the methodology used, the crude incidence figure cannot be compared with other previously published studies, as in this study it was considered the number of cases occurring in individuals over 18 years old and the total adult population. Recently, various studies have pointed to an increase in cases in the age group above 75–80 years (Aragones et al., 2016). Characteristically, bulbar-onset ALS, more common in women, tends to appear at older ages than spinal-onset forms [6,7]. This fact could explain, at least partially, the significant trend in the increase of cases in the female population observed in this study. Additionally, it could be speculated that women's habits and lifestyles have been changing in recent years. In this regard, factors such as increasing smoking habits and women's entry into industrial work with relevant professional exposures over the past two decades could also contribute to these findings (Menson and Coleman, 2024).

Importantly, in the present study, both bulbar-onset and spinal-onset forms of ALS were analyzed together because the data source did not differentiated between them. Globally, spinal-onset forms are significantly more frequent than bulbar-onset ones, with the latter representing around 25–30 % of total cases (Ludolph et al., 2024; Rizea et al., 2024). Despite the existence of subtle differences between these disease subtypes (Grassano et al., 2024; Canosa et al., 2023), both bulbar and spinal variants share the main pathogenic milestones (Rizea et al., 2024; Mead et al., 2023). Future studies, stratifying not only by onset form but also differentiating between genetic and sporadic forms, will provide new evidences about the role of distinct environmental factors, including altitude and land use, in the different forms of the disease.

Regarding the identification of high and low incidence clusters of ALS, it is described clusters of higher-than-expected incidence in some provinces mainly located in the northern and northeastern regions. Similar results with a higher proportion of cases in territories further away from the equatorial axis were described by our group a few years ago (Santurtún et al., 2016). This has also been reported in other countries (Valenzuela et al., 2015), although other studies in the literature are discordant regarding the potential role that latitude might play in the appearance of ALS (Logroscino et al., 2005). On the other hand, we identified a series of Administrative units with lower-than-expected disease incidence. Although they showed a preferential geolocation along the Spanish east coast, no particular geographic feature was detected. In this line, other epidemiological cohort studies have also described other low-incidence clusters in Europe. Specifically, Rooney et al. reported two low-incidence territories in Ireland without identifying a clear demographic pattern or differential environmental exposure, leading them to hypothesize that the observed differences might be related geographic-associated genetic variants (Rooney et al., 2015b).

Given the importance of acquired factors in sporadic ALS, we analyzed the association with altitude. To the best of our knowledge this geographic factor had not been previously associated to ALS. Interestingly, we found that municipalities located in high-incidence clusters had significantly lower altitudes. That novel fact migh have distinct potential explanations. First, people living in higher-altitude areas are exposed to lower oxygen pressure and to different degrees of celular hypoxia. Under these situations it has been described to increase the expression of hypoxia-inducible factor (HIF)-1α (West, 2012). Notably, one of the most relevant functions of HIF is the regulation of several mitochondrial gene pathways related to energy utilization (West, 2012). In this regard, mitochondrial dysfunction and disorders in energy supply have been described as key drivers for both ALS onset and progression (Jankovic et al., 2021). In addition, HIFs also modulate nitric oxide metabolism genes (West, 2012). Nitric oxide has been reported to play a critical role in the age-related cascade of neurodegeneration in neurodegenerative diseases, and particularly in ALS (Drechsel et al., 2012; Iova et al., 2023). On the other hand, low-altitude areas are characterized by worsened air quality due to several facts including i) higher population density, ii) higher rates of urbanization, iii) large number of vehicles, and, in general, iv) increased human activity. Specifically, higher levels of particulate matter (PM) have been reported (Ning et al., 2018) in low-altitude areas, and, intriguingly some previous studies have pointed a potential link between exposure to fine particulate matter (PM2.5) and ALS (Nunez et al., 2022; Nunez et al., 2023; Seelen et al., 2017).

Finally, given the results related to altitude, and based on previous investigations in different countries pointing to pesticide exposure as a possible etiological factor (Povedano et al., 2018; Andrew et al., 2021), we conducted a correlation study between land use and high and low incidence clusters. For this analysis, data from CORINE Land Cover [CLC]—acquired predominantly by visual interpretation of Landsat satellite imagery—for monitoring changes in settlement development and land use were used (https://land.copernicus.eu/en/products/corine-land-cover/clc2018, n.d.). Importantly, this methodology has some limitations as it may overestimate/underestimate some land categories (Śleszyński et al., 2020). Specifically, a recent study conducted in Spain reported that the land use categories most prone to biased data were transitional woodland-shrub, complex cultivation patterns, artificial, grasslands, and forests (Martínez-Fernández et al., 2019).

We found a significant relationship with permanently irrigated arable land and broad-leaved forest. Permanently irrigated arable land consists of cultivated land parcels under agricultural use for arable crops that are permanently or periodically irrigated, using a permanent infrastructure (irrigation channels, drainage network, and additional irrigation facilities). Most of these crops cannot be cultivated without artificial water supply. On the other hand, broad-leaved forest is defined as a vegetation formation composed principally of trees, including shrub and bush understorey, where broad-leaved species predominate. Conversely, we found an inverse and statistically significant relationship between the percentage of natural grassland [grasslands under no or moderate human influence], often situated in areas of rough, uneven ground and frequently including rocky areas or patches of other (semi)-natural vegetation and ALS cases (https://land.copernicus.eu/content/corine-land-cover-nomenclature-guidelines/html/index.html, n.d.). These findings could be explained by the fact that natural environments, free from external human intervention, are likely to be associated with a lower degree of exposure to potentially deleterious external agents (heavy metals, herbicides, and other toxins, pollution associated with industrial activity, and other human activities, etc.).

Delving into agricultural land use, we analyzed the relationship between disease frequency and the use of outdoor agriculture versus that associated with greenhouses. Regarding the former, it is noteworthy that in some communities (Huesca and Las Palmas), we found a direct relationship, while in another (Cáceres), we found an inverse relationship with ALS cases. Although the results may initially appear contradictory, it is important to consider the diversity of crops across the national geography. In this context, agricultural activities in regions with a higher-than-expected prevalence of ALS cases are predominantly centered on crops such as barley, wheat, corn, fruit trees, and vineyards, which typically involve intensive herbicide use, supplemented to a lesser extent by fungicides and insecticides (García et al., 2022). For instance, Huesca is a province specializing in cereal and forage crop production, both characterized by the extensive use of herbicides such as glyphosate

and 2,4-dichlorophenoxyacetic acid (García et al., 2022) —two compounds that have been suggested as toxins associated with ALS and neurodegeneration (Anderson, 2022; Freitas et al., 2019). Conversely, open-field agriculture in Cáceres, a province where an inverse correlation is described, is characterized by the cultivation of species that do not require such intensive use of herbicides/pesticides, such as large areas of cork oak forests for cork production.

Regarding the study of the correlation between ALS cases and greenhouse use, we describe a significant positive correlation in two Spanish provinces, Guadalajara and Badajoz. These provinces are characterized by high greenhouse cultivation of vegetables, such as tomatoes or peppers, which also require intensive use of pesticides, entre ellos el ya mencionado glifosfato and fungicides (García et al., 2022), like folpet.

Exposure to pesticides as a risk factor for ALS had already been described (Saucier et al., 2023), and it is worth noting that the heterogeneous pattern of association we found in Spain is not unique to our country. Similar findings were reported by Vinceti et al. (2017) in Italy, where certain crops, such as citrus plantations and olive groves, were identified in the southern part of the country as being associated with a higher risk of the disease (Vinceti et al., 2017).

In recent years, numerous studies have delved into the relationship between herbicide and pesticide exposure and ALS with varying results (Andrew et al., 2021; Bermudo Fuenmayor et al., 2023) The relationship between herbicide use and ALS development may be explained by the interference with various biological pathways (Anderson, 2022), Firstly, at an astroglial level, herbicide exposure, particularly glyphosate, is associated to the suppression of the melatonergic pathway with secondary activation of the NFKB and YY1 pathways, leading to pro-inflammatory states and to the consequent loss of homeostasis at a motor neuron level (Anderson, 2022). Additionally, glyphosate exposure has been associated with peripheral damage, with reduction and degeneration of muscle fibers and loss of neuromuscular junctions (Barbosa et al., 2022).

This study, based on mortality data from ALS over the past two decades in Spain, showed a clear upward trend in disease frequency in both men and women. The geospatial analysis at the municipal level identified clusters of high ALS frequency in the northern and northeastern regions of the country. In subsequent independent analyses, we described for the first time in the literature altitude and certain agricultural land uses as geographic determinants of the disease.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2024.177796.

CRediT authorship contribution statement

Ana Santurtún: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Pablo Medín:** Writing – review & editing, Writing – original draft, Visualization, Software, Methodology, Investigation, Formal analysis, Data curation. **José Antonio Riancho:** Writing – review & editing, Validation, Supervision, Methodology, Funding acquisition, Data curation, Conceptualization. **Marina Santiago-Setién:** Writing – original draft, Resources, Investigation. **Fernando Ortiz:** Writing – review & editing, Resources, Investigation. **Adolfo López de Munain:** Writing – review & editing, Supervision, Methodology, Investigation, Conceptualization. **Ricardo Almendra:** Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Javier Riancho:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was supported by the Instituto de Salud Carlos III, Madrid, Spain (PI23/00905). Javier Riancho received support from the Instituto de Investigación Valdecilla, IDIVAL, Santander, Spain (INT/A23/07). Ricardo Almendra received support from the Centre of Studies in Geography and Spatial Planning (CEGOT), funded by national funds through the Foundation for Science and Technology (FCT) under the reference UIDB/04084/2020.

Data availability

The authors do not have permission to share data.

References

Al-Chalabi A, Calvo A, Chio A, Colville S, Ellis CM, Hardiman O, et al. Analysis of amyotrophic lateral sclerosis as a multistep process: a population-based modelling study. Lancet Neurol. 2014 Nov;13(11):1108–13.

Al-Chalabi, A., Andrews, J., Farhan, S., 2024. Recent advances in the genetics of familial and sporadic ALS. Int. Rev. Neurobiol. 176, 49–74.

Anderson, G., 2022 Dec 29. Amyotrophic lateral sclerosis Pathoetiology and pathophysiology: roles of astrocytes, gut microbiome, and muscle interactions via the mitochondrial Melatonergic pathway, with disruption by glyphosate-based herbicides. Int. J. Mol. Sci. 24 (1), 587.

Andrew, A., Zhou, J., Gui, J., Harrison, A., Shi, X., Li, M., et al., 2021 Dec. Pesticides applied to crops and amyotrophic lateral sclerosis risk in the U.S. Neurotoxicology 87, 128–135.

Aragones, J.M., Altimiras, J., Roura-Poch, P., Homs, E., Bajo, L., Povedano, M., et al., 2016. Amyotrophic lateral sclerosis: A higher than expected incidence in people over 80 years of age. Amyotroph Lateral Scler Frontotemporal Degener. 17 (7–8), 522–527.

Arthur, K.C., Calvo, A., Price, T.R., Geiger, J.T., Chiò, A., Traynor, B.J., 2016 Aug. Projected increase in amyotrophic lateral sclerosis from 2015 to 2040. Nat. Commun. 11 (7), 12408.

Barbosa A, Zazula MF, Oliveira MC de, Teleken JL, Costa RM, Bonfleur ML, et al. Maternal exposure to glyphosate-based herbicide promotes changes in the muscle structure of C57BL/6 mice offspring. Anat Rec (Hoboken). 2022 Nov;305(11): 3307–16.

Bermudo Fuenmayor, S., Serrano Castro, P.J., Quiroga Subirana, P., López Palmero, S., Requena Mullor, M., Parrón, Carreño T., 2023 Sep. Environmental exposure to pesticides and amyotrophic lateral sclerosis in the south of Spain. Neurologia 38 (7), 447–452.

Canosa, A., Martino, A., Giuliani, A., Moglia, C., Vasta, R., Grassano, M., Palumbo, F., Cabras, S., Di Pede, F., De Mattei, F., Matteoni, E., Polverari, G., Manera, U., Calvo, A., Pagani, M., Chiò, A., 2023 Feb. Brain metabolic differences between pure bulbar and pure spinal ALS: a 2-[18F]FDG-PET study. J. Neurol. 270 (2), 953–959. https://doi.org/10.1007/s00415-022-11445-9.

Capozzella, A., Sacco, C., Chighine, A., Loreti, B., Scala, B., Casale, T., et al., 2014. Work related etiology of amyotrophic lateral sclerosis (ALS): a meta-analysis. Ann. Ig. 26 (5), 456–472.

Castanedo-Vazquez, D., Bosque-Varela, P., Sainz-Pelayo, A., Riancho, J., 2018. Infectious agents and amyotrophic lateral sclerosis: another piece of the puzzle of motor neuron degeneration. J. Neurol. 266 (1), 27–36.

Drechsel, D.A., Estévez, A.G., Barbeito, L., Beckman, J.S., 2012 Nov 10. Nitric oxide-mediated oxidative damage and the progressive demise of motor neurons in ALS. Neurotox. Res. 22 (4), 251–264.

Feldman, E.L., Goutman, S.A., Petri, S., Mazzini, L., Savelieff, M.G., Shaw, P.J., et al., 2022 Oct 15. Amyotrophic lateral sclerosis. Lancet 400 (10360), 1363–1380.

Freitas L, Valadares L de A, Camozzi M, de Oliveira P, Ferreira Machado M, Lima F. Animal models in the neurotoxicology of 2,4-D. Hum. Exp. Toxicol. 2019 Oct 30;38 (10):1178–82.

García, M.G., Sánchez, J.I.L., Bravo, K.A.S., Cabal, M.D.C., Pérez-Santín, E., 2022 Nov. Review: presence, distribution and current pesticides used in Spanish agricultural practices. Sci. Total Environ. 845, 157291.

Goutman SA, Savelieff MG, Jang DG, Hur J, Feldman EL. The amyotrophic lateral sclerosis exposome: recent advances and future directions. Nat. Rev. Neurol. 2023 Oct;19(10):617–34.

Grassano, M., Moglia, C., Palumbo, F., Koumantakis, E., Cugnasco, P., Callegaro, S., Canosa, A., Manera, U., Vasta, R., De Mattei, F., Matteoni, E., Fuda, G., Salamone, P., Marchese, G., Casale, F., De Marchi, F., Mazzini, L., Mora, G., Calvo, A., Chiò, A., 2024 Jul. Sex differences in amyotrophic lateral sclerosis survival and progression: A

multidimensional analysis. Ann. Neurol. 96 (1), 159–169. https://doi.org/10.1002/ana.26933.
https://land.copernicus.eu/content/corine-land-cover-nomenclature-guidelines/html/index.html.
https://land.copernicus.eu/en/products/corine-land-cover/clc2018.
Iova, O.M., Marin, G.E., Lazar, I., Stanescu, I., Dogaru, G., Nicula, C.A., et al., 2023 Mar 20. Nitric oxide/nitric oxide synthase system in the pathogenesis of neurodegenerative disorders-an overview. Antioxidants (Basel). 12 (3).
Jankovic, M., Novakovic, I., Gamil Anwar Dawod, P., Gamil Anwar Dawod, A., Drinic, A., Abdel Motaleb, F.I., et al., 2021 Sep. Current concepts on genetic aspects of mitochondrial dysfunction in amyotrophic lateral sclerosis. Int. J. Mol. Sci. 11;22 (18):9832.
Kamel, F., Umbach, D.M., Bedlack, R.S., Richards, M., Watson, M., Alavanja, M.C.R., et al., 2012 Jun. Pesticide exposure and amyotrophic lateral sclerosis. Neurotoxicology 33 (3), 457–462.
Kang, H., Cha, E.S., Choi, G.J., Lee, W.J., 2014. Amyotrophic lateral sclerosis and agricultural environments: A systematic review. J. Korean Med. Sci. 29 (12), 1610.
Korner, S., Kammeyer, J., Zapf, A., Kuzma-Kozakiewicz, M., Piotrkiewicz, M., Kuraszkiewicz, B., et al., 2019 Apr. Influence of environment and lifestyle on incidence and Progress of amyotrophic lateral sclerosis in A German ALS population. Aging Dis. 10 (2), 205–216.
Logroscino, G., Beghi, E., Zoccolella, S., Palagano, R., Fraddosio, A., Simone, I.L., et al., 2005 Aug. Incidence of amyotrophic lateral sclerosis in southern Italy: a population based study. J. Neurol. Neurosurg. Psychiatry 76 (8), 1094–1098.
Ludolph, A.C., Dietrich, J., Dreyhaupt, J., Kassubek, J., Del Tredici, K., Rosenbohm, A., 2024 Aug. Clinical spreading of muscle weakness in amyotrophic lateral sclerosis (ALS): a study in 910 patients. J. Neurol. 271 (8), 5357–5367. https://doi.org/10.1007/s00415-024-12408-y.
Martínez-Fernández, J., Ruiz-Benito, P., Bonet, A., Gómez, C., 2019 Dec 2. Methodological variations in the production of CORINE land cover and consequences for long-term land cover change studies. The case of Spain. Int. J. Remote Sens. 40 (23), 8914–8932.
McFarlane, R., Heverin, M., Walsh, C., Hardiman, O., 2024 Jun. Irish amyotrophic lateral sclerosis incidence: age, period, and cohort effects using a partial least squares regression model. Neurology 102 (11), e209391.
Mead, R.J., Shan, N., Reiser, H.J., Marshall, F., Shaw, P.J., 2023 Mar. Amyotrophic lateral sclerosis: a neurodegenerative disorder poised for successful therapeutic translation. Nat. Rev. Drug Discov. 22 (3), 185–212. https://doi.org/10.1038/s41573-022-00612-2.
Menson, K.E., Coleman, S.R.M., 2024 Aug. Smoking and pulmonary health in women: A narrative review and behavioral health perspective. Prev Med (Baltim). 185, 108029.
Murch, S.J., Cox, P.A., Banack, S.A., Steele, J.C., Sacks, O.W., 2004 Oct. Occurrence of beta-methylamino-l-alanine (BMAA) in ALS/PDC patients from Guam. Acta Neurol. Scand. 110 (4), 267–269.
Ning, G., Wang, S., Ma, M., Ni, C., Shang, Z., Wang, J., et al., 2018 Jan. Characteristics of air pollution in different zones of Sichuan Basin. China. Science of The Total Environment. 612, 975–984.
Nunez Y, Boehme AK, Goldsmith J, Li M, van Donkelaar A, Weisskopf MG, et al. PM2.5 composition and disease aggravation in amyotrophic lateral sclerosis: An analysis of long-term exposure to components of fine particulate matter in New York State. Environ Epidemiol. 2022 Apr;6(2):e204.
Nunez, Y., Balalian, A., Parks, R.M., He, M.Z., Hansen, J., Raaschou-Nielsen, O., et al., 2023 Sep 1. Exploring relevant time windows in the association between PM2.5 exposure and amyotrophic lateral sclerosis: A case-control study in Denmark. Am. J. Epidemiol. 192 (9), 1499–1508.
Povedano, M., Saez, M., Martínez-Matos, J.A., Barceló, M.A., 2018. Spatial assessment of the association between long-term exposure to environmental factors and the occurrence of amyotrophic lateral sclerosis in Catalonia, Spain: A population-based nested case-control study. Neuroepidemiology 51 (1–2), 33–49.
Reed, D., Plato, C., Elizan, T., Kurland, L.T., 1966 Jan. The amyotrophic lateral sclerosis/parkinsonism-dementia complex: a ten-year follow-up on Guam. I. Epidemiologic studies. Am J Epidemiol. 83 (1), 54–73.
Riancho J, Lozano-Cuesta P, Santurtún A, Sánchez-Juan P, López-Vega JM, Berciano J, et al. Amyotrophic lateral sclerosis in northern Spain 40 Years later: What has changed? Neurodegener Dis. 2016;16(5–6).
Riancho, J., Bosque-Varela, P., Perez-Pereda, S., Povedano, M., de Munaín, A.L., Santurtun, A., 2018 Aug. The increasing importance of environmental conditions in amyotrophic lateral sclerosis. Int. J. Biometeorol. 62 (8), 1361–1374.
Riancho, J., Gonzalo, I., Ruiz-Soto, M., Berciano, J., 2019. Why do motor neurons degenerate? Actualization in the pathogenesis of amyotrophic lateral sclerosis. Neurologia 34 (1), 27–37.
Riancho, J., Delgado-Alvarado, M., Andreu, M.D., Paz-Fajardo, L., Arozamena, S., Gil-Bea, F.J., et al., 2021 Mar. Amyotrophic lateral sclerosis (ALS), cancer, autoimmunity and metabolic disorders: an unsolved tantalizing challenge. Br. J. Pharmacol. 178 (6), 1269–1278.
Riva, N., Domi, T., Pozzi, L., Lunetta, C., Schito, P., Spinelli, E.G., et al., 2024 Jul 27. Update on recent advances in amyotrophic lateral sclerosis. J. Neurol. 271 (7), 4693–4723.
Rizea, R.E., Corlatescu, A.D., Costin, H.P., Dumitru, A., Ciurea, A.V., 2024 Sep 15. Understanding amyotrophic lateral sclerosis: pathophysiology, diagnosis, and therapeutic advances. Int. J. Mol. Sci. 25 (18), 9966. https://doi.org/10.3390/ijms25189966.
Rooney, J., Vajda, A., Heverin, M., Elamin, M., Crampsie, A., McLaughlin, R., et al., 2015 Apr 14. Spatial cluster analysis of population amyotrophic lateral sclerosis risk in Ireland. Neurology 84 (15), 1537–1544.
Rooney, J., Vajda, A., Heverin, M., Elamin, M., Crampsie, A., McLaughlin, R., et al., 2015 Apr 14. Spatial cluster analysis of population amyotrophic lateral sclerosis risk in Ireland. Neurology 84 (15), 1537–1544.
Santurtún, A., Villar, A., Delgado-Alvarado, M., Riancho, J., 2016. Trends in motor neuron disease: association with latitude and air lead levels in Spain. Neurol. Sci. 37 (8).
Saucier, D., Registe, P.P.W., Bélanger, M., O'Connell, C., 2023 Mar. Urbanization, air pollution, and water pollution: identification of potential environmental risk factors associated with amyotrophic lateral sclerosis using systematic reviews. Front. Neurol. 8 (14), 1108383. https://doi.org/10.3389/fneur.2023.1108383.
Seelen, M., Toro Campos, R.A., Veldink, J.H., Visser, A.E., Hoek, G., Brunekreef, B., van der Kooi, A.J., de Visser, M., Raaphorst, J., van den Berg, L.H., Vermeulen, R.C.H., 2017 Sep 27. Long-term air pollution exposure and amyotrophic lateral sclerosis in Netherlands: A population-based case-control study. Environ. Health Perspect. 125 (9), 097023. https://doi.org/10.1289/EHP1115.
Ślęszyński, P., Gibas, P., Sudra, P., 2020 Jul 14. The problem of mismatch between the CORINE land cover data classification and the development of settlement in Poland. Remote Sens. (Basel) 12 (14), 2253.
Spencer, P.S., Lagrange, E., Camu, W., 2019 Dec. ALS and environment: clues from spatial clustering? Rev. Neurol. (Paris) 175 (10), 652–663.
Valenzuela, D., Zitko, P., Lillo, P., 2015. Amyotrophic lateral sclerosis mortality rates in Chile: A population based study (1994-2010). Amyotroph Lateral Scler Frontotemporal Degener. 16 (5–6), 372–377.
Vinceti, M., Filippini, T., Violi, F., Rothman, K.J., Costanzini, S., Malagoli, C., Wise, L.A., Odone, A., Signorelli, C., Iacuzio, L., Arcolin, E., Mandrioli, J., Fini, N., Patti, F., Lo Fermo, S., Pietrini, V., Teggi, S., Ghermandi, G., Scillieri, R., Ledda, C., Mauceri, C., Sciacca, S., Fiore, M., Ferrante, M., 2017 Aug 29. Pesticide exposure assessed through agricultural crop proximity and risk of amyotrophic lateral sclerosis. Environ. Health 16 (1), 91. https://doi.org/10.1186/s12940-017-0297-2.
West JB. High-altitude medicine. Am. J. Respir. Crit. Care Med. 2012 Dec 15;186(12):1229–37.
Zufiría, M., Gil-Bea, F.J., Fernández-Torrón, R., Poza, J.J., Muñoz-Blanco, J.L., Rojas-García, R., et al., 2016. ALS: A bucket of genes, environment, metabolism and unknown ingredients. Prog. Neurobiol. 142.