ARTICLE

ALS phenotype is influenced by age, sex, and genetics

A population-based study

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Abstract

Objective

To assess the determinants of amyotrophic lateral sclerosis (ALS) phenotypes in a population-based cohort.

Methods

The study population included 2,839 patients with ALS diagnosed in Piemonte, Italy (1995–2015). Patients were classified according to motor (classic, bulbar, flail arm, flail leg, predominantly upper motor neuron [PUMN], respiratory) and cognitive phenotypes (normal, ALS with cognitive impairment [ALSci], ALS with behavioral impairment [ALSbi], ALSci and ALSbi combined [ALScbi], ALS–frontotemporal dementia [FTD]). Binary logistic regression analysis was adjusted for sex, age, and genetics.

Results

Bulbar phenotype correlated with older age ($p < 0.0001$), women were more affected than men at increasing age ($p < 0.0001$), classic with younger age ($p = 0.029$), men were more affected than women at increasing age ($p < 0.0001$), PUMN with younger age ($p < 0.0001$), flail arm with male sex ($p < 0.0001$) and younger age ($p = 0.04$), flail leg with male sex with increasing age ($p = 0.008$), and respiratory with male sex ($p < 0.0001$). *C9orf72* expansions correlated with bulbar phenotype ($p < 0.0001$), and were less frequent in PUMN ($p = 0.041$); *SOD1* mutations correlated with flail leg phenotype ($p < 0.0001$), and were less frequent in bulbar ($p < 0.0001$). ALS-FTD correlated with *C9orf72* ($p < 0.0001$) and bulbar phenotype ($p = 0.008$), ALScbi with PUMN ($p = 0.014$), and ALSci with older age ($p = 0.008$).

Conclusions

Our data suggest that the spatial–temporal combination of motor and cognitive events leading to the onset and progression of ALS is characterized by a differential susceptibility to the pathologic process of motor and prefrontal cortices and lower motor neurons, and is influenced by age, sex, and gene variants. The identification of those factors that regulate ALS phenotype will allow us to reclassify patients into pathologically homogenous subgroups, responsive to targeted personalized therapies.

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Glossary

ALS = amyotrophic lateral sclerosis; **ALSbi** = amyotrophic lateral sclerosis with behavioral impairment; **ALScbi** = amyotrophic lateral sclerosis with behavioral and cognitive impairment; **ALSci** = amyotrophic lateral sclerosis with executive cognitive impairment; **CI** = confidence interval; **DTR** = deep tendon reflex; **FTD** = frontotemporal dementia; **NECI** = nonexecutive cognitive impairment; **OR** = odds ratio; **PUMN** = predominantly upper motor neuron.

Amyotrophic lateral sclerosis (ALS) a neurodegenerative disease characterized by the progressive impairment of motor function (speech, swallowing, limb, respiration) due to the degeneration of cortical, spinal, and bulbar motor neurons. Recently the clinical picture of ALS has been enriched by the recognition that at least 50% of patients develop some degree of cognitive impairment of the frontotemporal type.[1,2]

It has been proposed that the pathology in ALS spreads contiguously through the anatomy of the upper and lower motor neuron levels.[3,4] More recently, it has been hypothesized that the lesions spreading is due to the diffusion of TDP43 pathology along axonal pathways.[5] However, it remains unclear whether the onset and progression of ALS phenotype is a stochastic process or it is influenced by specific identifiable factors. In a previous epidemiologic study, we showed that the frequencies of ALS motor phenotypes are different in the 2 sexes and across different age groups.[6]

The aim of this study is to assess the factors related to the different ALS phenotypes in a population-based cohort of patients.

Methods

The study population includes patients with ALS identified through the Piemonte and Valle d'Aosta Register for ALS (PARALS), a prospective population-based register active since 1995. The characteristics of the register have been reported in detail elsewhere.[7] For the present article, we considered all ALS cases diagnosed (incident) from January 1, 1995, to December 31, 2015 (n = 2,839).

Classification of motor phenotypes

Clinical phenotypes were classified for all 2,839 patients as follows: classic, bulbar, flail arm, flail leg, predominantly upper motor neuron, and respiratory.[6] The phenotypic classification was based on clinical data obtained from available sources (clinical charts and clinical notes). For 2,347 patients (82.7%), clinical data were prospectively collected (mean number of visits for patient, 6.9). Therefore, the phenotype was initially established according to the clinical and neurophysiologic picture at diagnosis but ultimately revised during the follow-up.

Classic (Charcot) phenotype

Classic ALS was characterized by onset of symptoms in upper or lower limbs, with clear but not predominant pyramidal signs.

Bulbar phenotype

These patients had a bulbar onset with dysarthria or dysphagia, tongue wasting, fasciculation, and no peripheral spinal involvement for the first 6 months after symptoms onset. Pyramidal signs were not required to be evident in the first 6 months, but needed to be present thereafter.

Flail arm phenotype

Patients in this group were characterized by progressive predominantly proximal weakness and wasting in the upper limbs. In this category we also included patients with pathologic deep tendon reflexes (DTRs) or Hoffman sign in the upper limbs at some point during the disease, but without hypertonia or clonus. Functional involvement had to be confined to the flail limbs for at least 12 months after symptoms onset.

Flail leg phenotype

Patients were characterized by progressive distal onset of weakness and wasting in the lower limbs. In this category we also included patients with pathologic DTRs or Babinski sign in the lower limbs at some point during the disease, but without hypertonia or clonus. Patients with wasting and weakness beginning proximally in the legs without distal involvement at presentation were classified as classic ALS. Functional involvement had to be confined to the flail limbs for at least 12 months after symptoms onset.

Predominantly upper motor neuron (PUMN) ALS

These patients had clinical manifestations dominated by pyramidal signs, mainly severe spastic para/tetraparesis, associated with one or more of the following signs: Babinski or Hoffmann sign, hyperactive reflexes, clonic jaw jerk, dysarthric speech, or pseudobulbar affect. Spastic paresis could be present at the beginning or in the fully developed stage of the disease. These patients showed clear signs of lower motor neuron impairment from onset of the disease, as indicated by muscle weakness and wasting and by the presence of chronic and active denervation at the EMG examination in at least 2 different muscles.

Respiratory phenotype

These patients had prevalent respiratory impairment at onset, defined as orthopnea or dyspnea at rest or during exertion, with only mild spinal or bulbar signs in the first 6 months after onset. These patients showed mild signs of upper motor neuron involvement.

Classification of cognitive phenotypes

From June 2007, patients with ALS underwent an extensive cognitive battery according to the consensus criteria for the diagnosis of frontotemporal cognitive and behavioral syndromes in ALS.[8,9] Originally, participants were classified in 4 categories based on neurobehavioral and cognitive testing: participants with normal cognition, participants with executive cognitive impairment (ALSci), participants with behavioral impairment (ALSbi), participants with ALS comorbid with frontotemporal dementia (FTD), and participants with nonexecutive cognitive impairment (NECI).[2] With the publication of the revised criteria,[9] participants were reclassified according to 5 categories, that is, participants with normal cognition, patients with ALSci, patients with ALSbi, patients meeting the criteria for both ALSci and ALSbi (ALScbi), and patients with ALS-FTD.[10] The category of ALS-NECI was therefore dropped. The characteristics of patients with ALS who underwent cognitive testing are reported in detail elsewhere.[11]

Genetic analysis

After 2005, most patients underwent genetic evaluation. All the coding exons and 50 bp of the flanking intron–exon boundaries of *SOD1*, of exon 6 of *TARDBP*, and of exons 14 and 15 of *FUS* have been PCR amplified, sequenced using the Big-Dye Terminator v3.1 sequencing kit (Applied Biosystems, Foster City, CA), and run on an ABIPrism 3130 genetic analyzer. These exons were selected as the vast majority of known pathogenic variants are known to lie within these mutational hotspots. A repeat-primed PCR assay was used to screen for the presence of the GGGGCC hexanucleotide expansion in the first intron of *C9ORF72*.[12]

Statistical methods

Binary logistic regression analysis (backward) for each phenotype was performed, adjusting for sex and age (classified in 10-year age classes). Each phenotype was classified with a binary (dummy) variable: 0, absent; and 1, present. The following 10-year age classes were considered: 20–49, 50–59, 60–69, 70–79, 80–89. The 20–49 age group and male sex were considered as references. The interaction between age (A) and sex (S) was also included (A × S) in the analysis. In all analyses, we considered age at onset. In the analysis of cognitive phenotypes, normal cognition was considered as reference. Statistical analyses were carried out using the SPSS 25.0 statistical package (SPSS, Chicago, IL).

Ethical considerations

The study was approved by the Ethical Committees of the 2 ALS centers involved in the study (Comitato Etico Azienda Ospedaliero-Universitaria Città della Salute e della Scienza, Torino, and Comitato Etico AOU Maggiore della Carità, Novara). All patients provided written informed consent before enrollment. The databases were anonymized according to the Italian law for the protection of privacy.

Data availability

Data will be available upon request by interested researchers.

Results

A total of 2,839 ALS incident patients were included in the study, 1,529 men and 1,310 women, with a median age at onset of 67.1 years (interquartile range 59.6–73.5). A flow chart reporting the different steps of the study is reported in figure 1.

Figure 1 Flow chart reporting the enrollment of cases

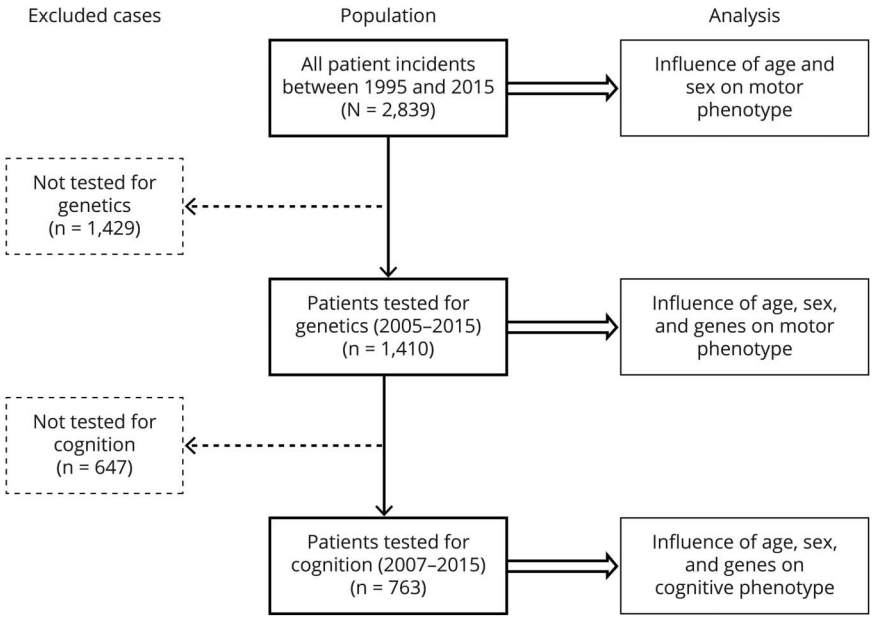


Table 1 Correlation of motor phenotypes with age and sex: binary logistic regression analysis

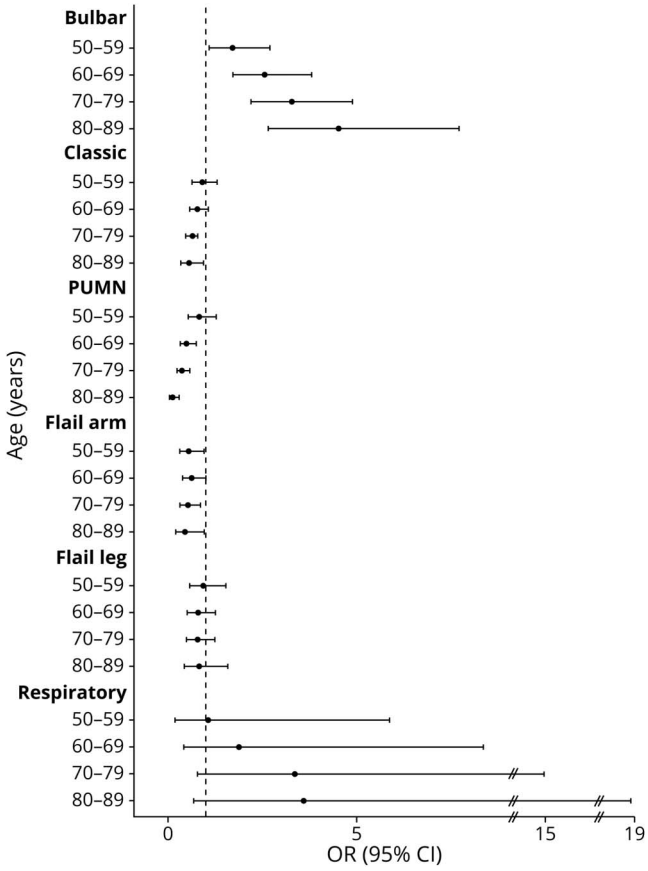
Motor phenotypes	Factors	Levels	OR (95% CI)	*p* Value
Bulbar (n = 999)	Age ($p = 0.0001$)	20–49	1	
		50–59	1.71 (1.09–2.70)	0.020
		60–69	2.56 (1.72–3.81)	0.0001
		70–79	3.28 (2.20–4.89)	0.0001
		80–89	4.52 (2.65–7.71)	0.0001
	Age × sex ($p = 0.0001$)[a]	20–49	1	
		50–59	1.37 (0.91–2.07)	0.136
		60–69	1.78 (1.37–2.32)	0.0001
		70–79	1.73 (1.32–2.26)	0.0001
		80–89	2.70 (1.54–4.74)	0.0001
	Sex			0.55
Classic (n = 835)	Age ($p = 0.029$)	20–49	1	
		50–59	0.91 (0.64–1.30)	0.60
		60–69	0.78 (0.58–1.07)	0.12
		70–79	0.65 (0.47–0.79)	0.008
		80–89	0.56 (0.34–0.94)	0.03
	Age × sex ($p = 0.0001$)[b]	20–49	1	
		50–59	0.83 (0.57–1.21)	0.34
		60–69	0.63 (0.47–0.83)	0.001
		70–79	0.61 (0.44–0.82)	0.001
		80–89	0.80 (0.42–1.50)	0.48
	Sex			0.65
Predominantly UMN (n = 240)	Age ($p = 0.0001$)	20–49	1	
		50–59	0.83 (0.54–1.28)	0.001
		60–69	0.49 (0.33–0.75)	0.0001
		70–79	0.37 (0.24–0.58)	0.0001
		80–89	0.11 (0.04–0.30)	0.0001
	Age × sex			0.84
	Sex			0.18
Flail arm (n = 187)	Age ($p = 0.04$)	20–49	1	
		50–59	0.55 (0.32–0.96)	0.03
		60–69	0.63 (0.39–1.01)	0.05
		70–79	0.53 (0.32–0.86)	0.01
		80–89	0.45 (0.21–0.96)	0.04
	Sex ($p < 0.0001$)	Female	0.42 (0.30–0.58)	0.0001
	Age × sex			0.99
Flail leg (n = 531)	Age × sex ($p = 0.008$)[c]	20–49	1	
		50–59	1.13 (0.80–1.62)	0.49
		60–69	1.06 (0.81–1.38)	0.68
		70–79	1.15 (0.88–1.51)	0.30
		80–89	0.21 (0.08–0.51)	0.001
	Age			0.78
	Sex			0.67
Respiratory (n = 47)	Sex (0.0001)	Female	0.16 (0.07–0.38)	0.0001
	Age			0.09
	Age × sex			0.98

Abbreviations: CI = confidence interval; OR = odds ratio; UMN = upper motor neuron.
The 20- to 49-year age group and male sex were used as references. For nonsignificant factors, only *p* values are reported.
[a] Age × sex interaction indicates that women are more affected than men at increasing age.
[b] Age × sex interaction indicates that men are more affected than women at increasing age.
[c] Age × sex interaction indicates that men are more affected than women at increasing age.

Motor phenotypes: influence of age and sex

Bulbar phenotype was correlated with older age ($p < 0.0001$) (table 1 and figure 2) and showed an A × S interaction, where women were more affected than men at increasing ages ($p < 0.0001$). Classic phenotype, on the contrary, was associated with younger age ($p = 0.029$) (figure 2) and showed an A × S interaction, where the risk in men increased with age

Figure 2 Graphic representation of odds ratios (ORs) of age classes for the phenotypes of amyotrophic lateral sclerosis



The 20- to 49-year age group served as reference. 95% confidence intervals (CIs) are reported. PUMN = predominantly upper motor neuron phenotype.

($p < 0.0001$). PUMN was correlated with younger age ($p < 0.0001$) (figure 2). Flail arm phenotype was associated with male sex (odds ratio [OR] 0.42 for women, 95% confidence interval [CI] 0.30–0.58; $p < 0.0001$), and less strongly with younger age ($p = 0.04$) (figure 2). Flail leg phenotype showed an A × S interaction, where the risk in men increased with age ($p = 0.008$); however, this association was due to the increased OR for men in the 80–89 age group. Finally, respiratory phenotype was correlated with male sex (OR 0.16%, 95% CI 0.07–0.38; for women, $p < 0.0001$) and showed a trend toward a higher frequency in older age ($p = 0.09$).

Genetics and motor phenotype

A total of 1,410 patients diagnosed after 2005 underwent genetic analysis (table 2). Overall, we found *C9orf72* expansions in 99 cases (7.0%), *SOD1* mutations in 37 cases (2.6%), *TARDBP* mutations in 21 cases (1.6%), and *FUS* mutations in 15 cases (1.1%). Genetic mutations did not modify the effect of age and sex on motor phenotypes. *C9orf72* expansions were related to bulbar phenotype (OR 2.39, 95% CI 1.54–3.69; $p < 0.0001$), and were less frequent in PUMN phenotype (OR 0.33, 95% CI 0.12–0.92; $p = 0.03$) (figure 3). *SOD1* mutations were more frequently related to flail leg phenotype (OR 3.75, 95% CI 1.92–7.33; $p < 0.0001$) and less frequent in bulbar phenotype (OR 0.27, 95% CI 0.09–0.80; $p < 0.0001$) (figure 3); *TARDBP* mutations were related to PUMN phenotype (OR 2.65, 95% CI 1.01–7.54; $p = 0.049$); finally, *FUS* mutations were not related to any specific phenotype.

Cognitive phenotypes: influence of age, sex, motor phenotypes, and genetics

A total of 763 patients were also evaluated for cognition (table 3). Among them, 390 (51.1%) were cognitively normal (including 16 patients with nonexecutive impairment), 126 (16.5%) were classified as ALSci, 62 (8.1%) as ALSbi, 38 (5.0%) as ALScbi, and 146 as ALS-FTD (19.2%). When assessing the determinants of ALS-FTD, we found that *C9orf72* was the strongest determinant of ALS-FTD (OR 6.88, 95% CI 3.86–12.25; $p = 0.0001$); also the A × S interaction ($p = 0.0001$), such that women were more affected than men at increasing ages, and bulbar phenotype (OR 1.74, 95% CI 1.16–2.61; $p = 0.008$) were correlated to ALS-FTD. ALScbi was related to PUMN (OR 2.64, 95% CI 1.11–6.29; $p = 0.014$). ALSci was only related to older age ($p = 0.008$). Finally, for ALSbi, we did not find any correlation with age, sex, genetic mutations, or motor phenotypes.

Discussion

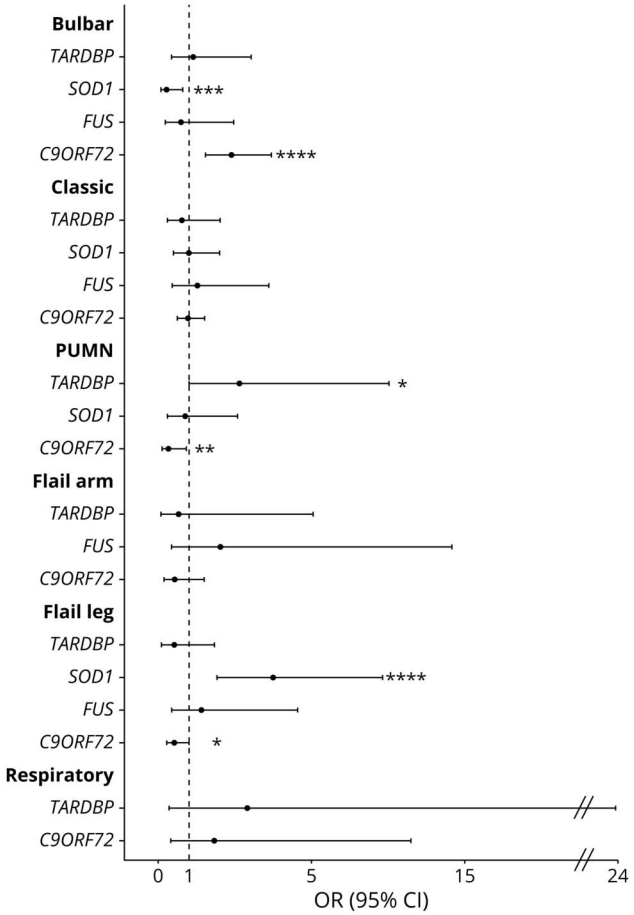
We found that the onset and early progression of motor and cognitive manifestations of ALS is strongly determined by at least 2 demographic factors, age and sex, and, to some extent, also by genetic variations. In particular, the frequency of bulbar phenotype increases with aging, while the frequency of PUMN and of classic phenotypes decreases. Male sex is associated with flail arm and respiratory phenotypes, female sex with bulbar phenotype, but only through an interaction with age. *C9orf72* expansions are related to a 2-fold increase of bulbar phenotype and *SOD1* mutations to a 3.5-fold increased

Table 2 Correlation between motor phenotypes and genetics: binary logistic regression analysis corrected for age, sex, and age × sex interaction

Motor phenotypes	Genes	OR (95% CI)	*p* Value
Bulbar (n = 442)	*SOD1*	0.27 (0.09–0.80)	0.018
	C9ORF72	2.39 (1.54–3.69)	0.0001
Predominantly UMN (n = 130)	*C9ORF72*	0.33 (0.12–0.92)	0.03
	TARDBP	2.65 (1.01–7.54)	0.049
Flail leg (n = 270)	*SOD1*	3.75 (1.92–7.33)	0.0001
	C9ORF72	0.52 (0.28–0.99)	0.049

Abbreviations: CI = confidence interval; OR = odds ratio; UMN = upper motor neuron.
Classic, flail arm, and respiratory phenotypes did not show any correlation with the examined genes and are therefore omitted.

Figure 3 Graphic representation of odds ratios (ORs) of gene mutations for the phenotypes of amyotrophic lateral sclerosis



Wild-type patients served as reference. 95% confidence intervals (CIs) are reported. PUMN = predominantly upper motor neuron phenotype.

frequency of flail leg phenotype and a 3.5-fold reduced frequency of bulbar phenotype. Finally, we found a strong interrelation between motor and cognitive phenotypes, with a higher propensity of patients with bulbar phenotype to develop FTD, and an increased risk of developing cognitive impairment with increasing age.

ALS is a complex disease, and such complexity hampers the design of clinical trials.[13] ALS complexity is partly due to the heterogeneity of its phenotype, which has motor and cognitive components, variously blended, reflecting the diverse involvement in the pathologic process of primary motor and prefrontal cortices, bulbar motor nuclei, and ventral horns in the spinal cord. As such, ALS phenotype may be considered a multidimensional space–time process, being determined by the different spatial extent of anatomical lesions as well by the varied diffusion of these lesions over time in each patient (table 4). The spread of lesions across the CNS in ALS has been explained either as a neuroanatomical contiguous propagation in the upper and lower motor neurons, with random onset in discrete body regions,[3] or as a prion-like propagation, with either contiguous or noncontiguous diffusion,[4,14] or, finally, as a sequential pattern of spread of TDP43 pathology with a corticofugal mechanism.[5,15,16] Independently from the modality of spreading of lesions across the CNS, we have found that the resulting clinical phenotype is determined by detectable factors and therefore can be in part predicted.

Age is the strongest risk factor of ALS.[7] In the present study, we have shown that age is also a strong modifier of ALS phenotype. Since the phenotype is a combination of upper and lower motor neurons impairment, we can hypothesize that the vulnerability of these different neurons to ALS changes with age. Supporting this hypothesis, the *SOD1* preclinical models reported an age-related conversion of astrocytes to a senescent phenotype that leads to a reduction of their support to motor neurons, which can differentially affect cortical, brainstem, and spinal cord motor neurons.[17] Similarly, the ^{A315T}TARDBP transgenic mice show different age-related vulnerability of cortical and spinal motor neurons.[18] There are no biological studies in humans on the differential susceptibility of spinal and cortical motor neurons with aging in ALS.

ALS is characterized by a sexual dimorphism, the most obvious being the higher risk of developing the disease in men.[7] More subtle differences between the 2 sexes have been reported both in ALS preclinical models and in humans. Female *SOD1* transgenic mice and rats experience extended lifespan and delayed onset compared to their male counterparts, with notable differences related to their genetic background.[19,20] However, this difference in rats is not modified by gonadectomy or chronic treatment of neuroprotective neurosteroids such as dehydroepiandrosterone,[21] indicating that the sexual dimorphism is not totally explained by hormonal influences. Data about humans are more scattered. A Dutch case–control study found that female patients with ALS did not differ compared to controls in the duration of reproductive timespan, but among female patients with ALS a longer exposition to estrogens was significantly correlated with a longer survival.[22] A large international case–control study demonstrated a negative association between ALS and hormonal contraception use in women, indicating a possible protective role of estrogens and progestogens.[23] In an MRI study, significant sex differences in the anatomical patterns of cortical and subcortical pathology in ALS have been found, mostly localized to extramotor, frontotemporal, and cerebellar regions.[24]

In this study we have assessed the influence on ALS phenotype of 2 demographic factors (age and sex) and 4 main ALS-related genes (*C9orf72*, *SOD1*, *TARDBP*, and *FUS*). However, there are indications that other factors, either genetic or environmental, may modify the ALS phenotype. First, several other genes besides *C9orf72*, *SOD1*, and *TARDBP* may have an appreciable influence on ALS phenotype.[25] Second, there are indications that genes influence phenotype also through oligogenic mechanisms, that is, through gene–gene (or protein–protein) interactions.[26,27] Third, ALS phenotype may be

Table 3 Correlation between cognitive phenotypes and motor phenotypes, age, sex, and genetics: binary logistic regression analysis

Cognitive phenotypes	Factors/covariates	Levels	OR (95% CI)	*p* Value
ALS-FTD (n = 146)	Age × sex ($p = 0.0001$)[a]	20–49	1	
		50–59	4.03 (0.44–37.28)	0.22
		60–69	12.47 (1.53–102.07)	0.02
		70–79	22.11 (2.70–180.99)	0.004
		80–89	31.96 (3.34–305.81)	0.003
	Bulbar phenotype		1.74 (1.16–2.61)	0.008
	C9ORF72		6.88 (3.86–12.25)	0.0001
ALScbi (n = 38)	PUMN		2.64 (1.11–6.29)	0.028
ALSci (n = 126)	Age ($p = 0.008$)	20–49	1	
		50–59	2.05 (0.66–6.42)	0.22
		60–69	2.80 (0.96–8.13)	0.06
		70–79	4.57 (1.54–13.18)	0.005
		80–89	3.34 (0.89–12.47)	0.07

Abbreviations: ALS = amyotrophic lateral sclerosis; ALScbi = amyotrophic lateral sclerosis with behavioral and cognitive impairment; ALSci = amyotrophic lateral sclerosis with executive cognitive impairment; CI = confidence interval; FTD = frontotemporal dementia; OR = odds ratio; PUMN = predominantly upper motor neuron.
Nonsignificant variables/covariates are omitted.
[a] Age × sex interaction indicates that women are more affected than men at increasing age.

influenced by environmental factors, which can have long-standing negative effects through an accumulation of toxicity, but also can have protective effects, as is the case of cognitive reserve for Alzheimer disease[28] and FTD.[29] Fourth, a gene–environment (i.e., interactomics) effect cannot be ruled out, acting through methylation or other epigenetic mechanisms.[30]

This study is not without weaknesses. First, cognitive phenotypes were classified at diagnosis; therefore, we cannot exclude that the cognitive phenotype may have varied over time. Second, we examined only 4 ALS-related genes, although several other genes may modify ALS phenotype. Third, in the assessment of factors related to cognitive phenotypes, the number of patients in each category, in particular in the reference category (20–49 age group), is relatively low; therefore, even if ORs resulted significant, they have quite large CIs, limiting the possibility to interpret the results.

Our data indicate that ALS motor and cognitive phenotypes do not develop randomly but are associated with demographic and genetic factors. The apparent focal onset of ALS may point toward a specific trigger of the disease, while the spatial-temporal combination of motor and cognitive events leading to the clinical onset and progression of ALS may represent the failure of the cortico–motorneuronal system to compensate a decline already started during the preclinical phase of the

Table 4 Clinical phenotypes and underlying anatomical regions

	Anatomical region			Somatic region		
Clinical phenotype	**UMN**	**LMN (bulbar)**	**LMN (spinal)**	**Bulbar**	**Upper limbs**	**Lower limbs**
Classic	++	+/− (late)	+++	+/− (late)	+++	+++
Bulbar	++	+++	+/− (late)	+++	+/− (late)	+/− (late)
Predominantly UMN	+++	+/−	+/−	++ (pseudobulbar palsy)	++	+++
Flail arm	+/−	−	+++	−	+++	−
Flail leg	+/−	−	+++	−	−	+++
Respiratory	+/−	+/− (late)	+/− (late)	−	++ (diaphragm)	−

Abbreviations: LMN = lower motor neuron; UMN = upper motor neuron.

disease, characterized by a differential susceptibility of the motor and prefrontal cortices and bulbar and spinal motor neurons to the pathologic process influenced by aging, sex, gene variants, and other, still unexplored, factors. The identification of those factors that regulate ALS phenotype over time and space may help reclassify patients into more homogenous and pathogenically meaningful subgroups ideally responsive to targeted personalized therapies. Further studies on different cohorts of patients and including environmental factors will allow identification of other mechanisms on the basis of the heterogeneity of ALS phenotype.

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Disclosure

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Continued

Appendix *(continued)*

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