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Word count: 3241

Word count abstract: 242

Title characters: 57

Combined total of Tables and Figures: 5

Total number of references: 36

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Competing Interest: None declared.

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Abstract

Background. Different ALS phenotypes have been recognized, marked by a varying involvement of spinal and bulbar upper and lower motor neurons. However, the differential characteristics of these phenotypes are still largely unknown.

Objective. To define epidemiology and outcome of amyotrophic lateral sclerosis (ALS) phenotypes in a population-based setting.

Methods. All ALS cases incident in two Italian regions have been prospectively collected from 1995 through 2004 in an epidemiological register. Cases have been classified according to established ALS phenotypes: classic, bulbar, flail arm, flail leg, pyramidal, respiratory, pure lower motor neuron (PLMN) and pure upper motor neuron (PUMN).

Results. ALS phenotype were determined in 1332 out of the 1351 incident patients (98.6%). Classic and bulbar phenotypes had similar mean annual incidence rates. Gender-specific incidence rates showed a male preponderance in respiratory, flail arm, classic and PLMN phenotypes; in all other phenotypes, men and women had similar incidence rates. Age at onset was significantly lower in pyramidal, PLMN and PUMN phenotypes and higher in the bulbar phenotype. The best outcomes were observed in PUMN, pyramidal, PLMN and flail arm phenotypes and the worst in respiratory and bulbar phenotypes.

Conclusions. Our epidemiological findings suggest that ALS phenotypes carry distinctive and easily distinguishable clinical and prognostic characteristics, strongly related to a complex interplay between gender and age. The categorization of ALS patients according to more homogenous clinical groups is relevant to identify biological markers for ALS and should be considered for the design of clinical trials.

Introduction

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder of adult life characterized by the progressive involvement of lower and upper motor neurons at bulbar and spinal level. In 5-10% of patients a positive family history for ALS can be detected. However, in most patients the cause of ALS remains unknown. It is a generally accepted notion that the clinical spectrum of ALS includes different phenotypes marked by a varying involvement of spinal and bulbar upper and lower motor neurons.[1,2] Accordingly, eight distinctive clinical phenotypes are recognized in literature – classic, bulbar, flail arm, flail leg, pyramidal, respiratory, pure lower motor neuron (PLMN) and pure upper motor neuron (PUMN).[3-9]

A discussion has recently arisen concerning the possibility that different ALS presentations have different aetiologies or underlying factors - whether genetic, environmental or both - that modify the phenotype.[10] However, at present no studies have been carried out to assess and compare all ALS phenotypes using an epidemiological approach.

The aim of this study was to evaluate the clinical characteristics and the outcome of different ALS phenotypes in a large population-based setting.

Methods

The Piemonte and Valle d'Aosta Register for ALS (PARALS) is a prospective register collecting all cases of ALS in the Piemonte and Valle d'Aosta regions of Italy (total population at the 2001 national census, 4,332,842; total area 28,692 sqkm). The register was established in 1995 and is still in operation. Epidemiological data regarding the 1995–2004 period have recently been published.[11]

Case collection. The main sources of cases were the neurology departments of the two regions. Investigators used an ad hoc questionnaire to collect patients' demographic data, disease history, neurological and laboratory findings, and treatments. Diagnostic EMG examination was performed in all patients according to standard procedures. The secondary sources for case

collection were: the Piemonte and Valle d'Aosta Central Regional Archives; and the mortality coding from the Italian Bureau of Statistics. Clinical records of cases found through secondary sources were obtained, and relevant clinical information for each case was analyzed in order to verify if the patient met the eligibility criteria; all living patients were contacted by phone and visited by one of the neurologists involved in the study.

Diagnostic criteria. The diagnosis of ALS was based on the original El Escorial diagnostic criteria (EEC)[12] although from 2000 cases were also classified according to El Escorial revised criteria.[13] Patients with PUMN[4,5] and PLMN[6] were prospectively included in the register; they were not considered in the original epidemiological paper,[11] but are included in this study. A clinical follow-up of each patient was performed at regular intervals (2 to 4 months). A standard form was used for collecting clinical information at each follow-up visit. The presence of frontotemporal dementia (FTD) was determined using an internally generated questionnaire administered to caregivers during the follow-up visits and was based on Neary's criteria.[14,15]

Phenotypic classification. We classified the patients into the eight recognized phenotypes of ALS - classic, bulbar, flail arm, flail leg, pyramidal, respiratory, PLMN and PUMN. The classification was based on clinical data gleaned from all available sources (clinical charts, clinical notes of the collaborating centres, including the standard forms used for the register) which were prospectively collected during the patients' follow-up. It was established according to the clinical and EMG picture at diagnosis and revised during the follow-up.

Classic (Charcot's) 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 and/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 evident thereafter.

Flail arm phenotype.[3] 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 pathological 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.[3] Patients were characterized by progressive distal onset of weakness and wasting in the lower limbs. In this category we also included patients with pathological 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.

Pyramidal phenotype (predominant-upper motor neuron ALS).[7,8] 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, pseudobulbar affect. Spastic paresis could be present at the beginning or in the fully developed stage of the disease. These patients showed at the same time clear-cut 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 two different sites.

Respiratory phenotype.[9] These patients had prevalent respiratory impairment at onset, defined as orthopnoea or dyspnoea at rest or during exertion, with only mild spinal or bulbar signs in the first 6 months after onset. These patients showed signs of upper motor neuron involvement.

PLMN.[6] These patient had clinical and electrophysiological evidence of progressive LMN involvement. From this category we excluded patients with motor conduction block(s) on extensive standardized nerve conduction studies, clinical UMN signs, history of disease that mimic motor

neuron disease, family history of inherited SMA and deletion in the SMN1 gene or expansion of CAG repeat in the androgen receptor gene. Neuroimaging studies were performed to rule out structural lesions.

PUMN.[4,5] These patients had clinical signs of UMN involvement, i.e. severe spastic para/tetraparesis, Babinski or Hoffmann sign, hyperactive reflexes, clonic jaw jerk, dysarthric speech, pseudobulbar affect. From this category we excluded patients with clinical or electromyographical signs of LMN involvement, according to El Escorial criteria, during the follow-up, history of disease that mimic motor neuron disease, family history of spastic paraparesis/tetraparesis and mutation of genes related to hereditary spastic paraplegia (SPG3A, SPG4, SPG6, SPG7 and SPG20).

Statistical Methods. Ninety-five percent confidence intervals (CIs) were calculated assuming a Poisson distribution.[16] Comparison between means were made with the analysis of variance (ANOVA). The effect of age and gender on ALS phenotypes was assessed with multivariate analysis of variance (MANOVA), including age, gender and the interaction between age and gender as dependent variables. Survival was calculated to death/tracheostomy or censoring date (December 31st, 2009), using the Kaplan-Meier method and compared with the log-rank test. Multivariable analysis was performed with Cox's proportional hazards model (stepwise forward) (see E-Table 2 for a list of the variables included in the model).

A p level <0.05 was considered significant. Data were processed using SAS statistical package (Cary, NC; version 8.2). No patients were lost to follow-up.

Standard Protocol Approvals, Registrations, and Patient Consents. The study design was approved by the Ethical Committee of the coordinating centre. Patients' consent was not required since this study did not modify the routine clinical practice. However, databases were managed according to the Italian law for the protection of privacy.

Results

A total of 1351 patients were diagnosed with ALS in the period 1995-2004 in Piemonte and Valle d'Aosta.[11] Clinical phenotype was established in 1332 patients (98.6%). Clinical phenotype was established blindly by three of the authors (AC, LM, GM). When the classification differed, a discussion was performed to reach a consensus. In 19 patients (1.4%) no consensus was reached mainly due to lack of full clinical details; these cases were excluded from the analysis. These patients did not differ in terms of demographic or clinical characteristics from those with full clinical details.

Epidemiological and clinical characteristics of ALS phenotypes. The clinical features of ALS phenotypes are reported in Table 1 and their mean annual incidence rates and gender rate ratios are shown in Table 2.

1. ***Classic phenotype***. This is the commonest ALS phenotype in men and the second commonest in women, with a men to women rate ratio of 1.65:1. Its age at onset is 62.8 years (SD 11.3), with a peak of the age-specific incidence rate in the 7th decade in both genders (Figure 1A). Four percent of cases with this phenotype have FTD. Its median survival time is 2.6 years (Supplemental Table 1), with a 10-year survival rate of 13.0%.
2. ***Bulbar phenotype***. Bulbar ALS has the same incidence in the two genders (1/100,000 population), with a men to women rate ratio of 0.98:1. The peak of the age-specific incidence rate is in the 8th decade in both genders (Figure 1B). Nine percent of bulbar patients have FTD, the highest figure among ALS phenotype. The median survival time of bulbar ALS is the second worst (2.0 years); only 3.4% of patients survived up to 10 years.
3. ***Flail arm phenotype***. This phenotype is relative rare and more common in men (incidence rates, 0.28 in men and 0.07 in women), with a men to women rate ratio of 4.00:1. Its mean age at onset is 62.6 years (SD 11.8). In this phenotype FTD is very rare (1.4%). Flail arm phenotype is relatively benign, with a median survival time of 4.0 years and a 10-year

survival rate of 17.4%. In these patients, ALS remains restricted to upper limbs for a mean of 20 months after the onset.

4. ***Flail leg phenotype***. This phenotype has a similar incidence in the two genders, with a men to women rate ratio of 1.03:1. The mean age at onset is the second highest (65.0 years) and the peak of age-specific incidence rate is in the 8th decade (Figure 1D). FTD is present in 4% of patients with this phenotype. The median survival time (3.0 years) and the 10-year survival rate (12.8%) of this phenotype are similar to those of classic ALS. In these patients, ALS remains restricted to upper limbs for a mean of 16 months after the onset.
5. ***Pyramidal phenotype***. Patients with this phenotype have a quite young age at onset (58.3 years). Gender are equally represented, with a men to women rate ratio of 1.04:1. The peak of the age-specific incidence rate is in the 7th decade (Figure 1E). FTD is rather uncommon (2.5%). The median survival time is 6.3 years and the 10-year survival rate is 31.9% (E-Table 1).
6. ***Respiratory phenotype***. This is the rarest phenotype (annual incidence rate: men, 0.06/100,000; women, 0,01/100,000). Its median survival time is 1.4 years and no patient with this phenotype survived up to 10 years.
7. ***PLMN***. PLMN has a quite low incidence rate and is twice more frequent in male gender (men to women rate ratio, 2.04:1). Patients with PLMN are younger than those with any other ALS phenotype (56.2 years) with a peak of the age-specific incidence rate in the 7th decade among men and in the 6th among women (Figure 1F). No patient with PLMN phenotype developed FTD. PLMN patients have a longer survival than any other phenotype besides PUMN (median survival time, 7.3 years).
8. ***PUMN***. PUMN has a relatively low incidence rate (0.12 in both genders) and mean age at onset (58.9 years). The peak of age-specific incidence rate is in the 6th decade in both genders (Figure 1G). The median survival time of PUMN is the longest among ALS phenotypes (13.1 years), with a 10-year survival rate of 71.1%.

Relative frequency of ALS phenotypes through the age-groups. The relative frequency of ALS phenotypes showed an intriguing trend with age: among men (Figure 2A) there was a decline in frequency of flail arm, pyramidal and classic phenotypes with increasing age, and an increase of bulbar (from 10% to 51%) and flail leg (from 0 to 12%) phenotypes; among women (Figure 2B), the decline in frequency of pyramidal (from 37.5% to 6%) and classic (from 37.5% to 12%) phenotypes was even more pronounced, as well as the rise in frequency of the bulbar phenotype (from 6% to 72%).

Influence of age and gender on ALS phenotypes. According to the results of the multivariate analysis of variance (MANOVA), 54% of total variance of ALS phenotypes was explained by age and gender.

ALS phenotypes survival. The survival curves of ALS phenotypes are compared in Figure 3. The outcome of the different phenotypes differed significantly ($p<0.0001$). At multivariable analysis (Cox model) (Supplemental Table 2), the variables independently related to outcome were age at onset ($p<0.0001$), bulbar phenotype ($p<0.0001$), PUMN ($p<0.0001$), pyramidal phenotype ($p<0.0001$), respiratory phenotype ($p<0.0001$), FTD ($p=0.0008$), and flail arm phenotype ($p=0.008$).

Discussion

This is the first comprehensive survey directly comparing all ALS phenotypes in a large epidemiological setting with a prospective design. The advantage of this large population-based study is that it includes detailed and standardized phenotypic information, thus avoiding the referral bias that is inherent to clinic-based cohorts.[17]

The main finding of our study is that ALS clinical phenotypes carry highly distinctive clinical, demographic and prognostic characteristics. In brief, bulbar phenotype is typical of older

patients, has similar incidence rates in the two genders, and carries the worst survival. At the opposite extreme, pyramidal phenotype and PUMN are typical of younger patients and have the most benign outcome. Flail arm phenotype is rare, more frequent in men and has a relatively good prognosis, while flail leg phenotype, which is the third more common phenotype, has an equal incidence in both genders and a slightly worse outcome. Classic phenotype, at last, has the highest incidence among men, and carries an intermediate outcome.

The different clinical and demographic characteristics of ALS phenotypes translates in different probabilities of having a presentation with a specific phenotype at different ages. As shown in Figure 2, bulbar phenotype represented less than 10% of cases under 39, to increase up to more than 50% over 80, with a more marked trend among women, whereas classic phenotype steadily decreased going from the younger to the older decades. The influence of age on ALS clinical features had been also reported in a clinical-based series of ALS patients in Japan.[18]

The male predominance generally reported in ALS[17,19] is true for only four phenotypes - classic, flail arm, respiratory and PLMN. The two genders were almost equally represented among the other phenotypes. Therefore, when considering incidence rates and not the absolute figures, the predominance of women in the bulbar phenotype described in clinical series appears to be simply related to the older age at onset of this phenotype.

ALS phenotype bears a strong influence on disease outcome. Bulbar and respiratory phenotypes carried the worst prognosis,[20] while pyramidal phenotype had by far the longest survival.[7] Interestingly, the survival time of the pyramidal phenotype of ALS was shorter than that of PUMN, but longer than all other ALS phenotypes, supporting the notion that it represents an intermediate form between classic ALS and PUMN.[8,21] Also flail arm phenotype had a relatively good outcome, with a median survival time of four years.[3,22] The outcome of classic and flail leg phenotypes were similar, at variance with the findings of a previous paper reporting a better prognosis for flail leg phenotype.[3] Cox's multivariable analysis confirmed the independent role of

ALS phenotype on survival and suggests that this classification should be used for patients' stratification in clinical trials, for example utilizing the minimization method.[23]

FTD was present in about 5% of patients, mainly those with bulbar phenotype. However, many ALS patients have evidence of frontotemporal behavioural dysfunction that may not satisfy Neary criteria for FTD and subclinical syndromes have not been considered in the present paper.[24]

Our findings support the idea that ALS is a clinically heterogeneous disease. The heterogeneity of motor phenotypes in ALS has been recently analyzed considering three symptom dimensions - body region of onset, relative mix of upper motor neuron and lower motor neuron involvement, and rate of progression,[2] and hypothesizing that the initial trigger of ALS is a stochastic process, with an apparently random but focal initiation. According to our findings, however, the focal initiation of ALS is not entirely random, since it is strongly determined by patients' age and gender, as indicated by the fact that these two factors explained about 50% of the clinical variance.

A previous study analyzed ALS prognostic groups using a latent class clustering.[25] The authors identified five classes, of which the first included most patients with classic, flail arm and flail leg phenotypes, the second, which carries the worst prognosis, includes most bulbar onset patients, and the fourth and fifth include patients with mixed phenotypes but with a milder clinical course.

The reasons of the influence of these two factors on ALS clinical presentation remain presently unknown. In the pre-clinical model of ALS, the SOD1 transgenic mouse, male rodents carrying the human mutated gene have an earlier age at onset, an early progress of locomotor rating scores, and a shorter survival than their female counterparts, with a clear gender-related phenotypic dimorphism.[26,27] This dimorphism has been related to gonadal hormones.[28] However, in human ALS, data on reproductive factors and sexual hormones are scarce and contradictory,[29,30] not allowing to draw any definitive conclusion.

A strong effect of age and gender on clinical phenotype has been reported also in Parkinson's disease (PD),[31] where the later age at onset, the more frequent presentation with tremor and the milder clinical course in female have been explained with the higher initial dopamine levels in the substantia nigra, which delay the moment of reaching a critical threshold of striatal dopamine depletion. Unfortunately, in ALS there are no studies assessing the age- and gender-related diversities in motor neuron pool and the receptor function at upper and lower motor neuron level.

Genetic factors may play a role in determining the range of ALS phenotypes, although to date no genes have been shown to have a definite effect on phenotype. Patients with SOD1 mutations show a phenotypic heterogeneity even within the same mutation,[32] although some specific missense mutations carry a consistently worse (i.e. A4V, G41S) or better prognosis (i.e. H46R, G93C). Patients with FUS mutations are also quite heterogeneous, but some mutations seem to carry more defined phenotypes: the R514S and R521C missense mutations are characterized by a predominantly proximal and axial phenotype[33,34] and the P525L missense mutation is characterized by a very young age at onset (<30 years), with a bulbar presentation and a short duration.[33,35]

A role of environmental factors on ALS phenotypic expression cannot be ruled out. The overall higher frequency of ALS in men could be related to occupational exposures to metals and toxins, while the increase of cigarette smoking, an established exogenous risk factor for ALS,[36] could explain the progressive reduction of the gender gap reported in recent ALS epidemiological studies.[17]

We conclude that our epidemiological data strongly support that recognized ALS phenotypes have different clinical, demographic and outcome characteristics, and can be recognizable even in a population-based setting. The pathophysiological bases of the varying pattern of motor neuron degeneration, partly related to age and gender, are still largely unknown. A better understanding of the factors that influence the phenotypic expression of ALS would be important for identifying the underlying biochemical, genetic and environmental mechanisms of the disease. Moreover, the categorization of ALS patients according to clinical phenotypes should be

considered for improving the design of clinical trials and to better focus the communication of diagnosis and outcome with the patients and their families.

Acknowledgements

Adriano Chiò, Letizia Mazzini, and Gabriele Mora had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. This paper was supported in part by grants from Regione Piemonte (Ricerca Finalizzata 2002, grant 12944; Ricerca Scientifica Applicata 2004, grant A317), and Compagnia di San Paolo (grant 2003.0078).

References

1. Ravits J, Paul P, Jorg C. Focality of upper and lower motor neuron degeneration at the clinical onset of ALS. Neurology 2007; 68:1571-1575.

2. Ravits JM, La Spada AR. ALS motor phenotype heterogeneity, focality and spread. Deconstructing motor neuron degeneration. Neurology 2009; 73:805-811.

3. Wijesekera LC, Mathers S, Talman P, et al. Natural history and clinical features of the flail arm and flail leg ALS variants. Neurology 2009; 72:1087-1094.

4. Pringle CE, Hudson AJ, Munoz DG, Kiernan JA, Brown WF, Ebers GC. Primary lateral sclerosis: clinical features, neuropathology and diagnostic criteria. Brain 1992; 115 (2):495-520.

5. Tartaglia MC, Rowe A, Findlater K, Orange JB, Grace G, Strong MJ. Differentiation between primary lateral sclerosis and amyotrophic lateral sclerosis: examination of symptoms and signs at disease onset and during follow-up. Arch Neurol 2007;64:232-236.

6. Visser J, van den Berg-Vos RM, Franssen H, et al. Disease course and prognostic factors of progressive muscular atrophy. Arch Neurol 2007; 64:522-528.

7. Sabatelli M, Madia F, Conte A, et al. Natural history of young-adult amyotrophic lateral sclerosis. Neurology 2009; 71: 876-881.

8. Gordon PH, Cheng B, Katz IB, Mitsumoto H, Rowland LP. Clinical features that distinguish PUMN, upper motor neuron– dominant ALS, and typical ALS. Neurology 2009; 72:1948-1952.

9. Shoesmith CL, Findlater K, Rowe A, Strong MJ. Prognosis of amyotrophic lateral sclerosis with respiratory onset. J Neurol Neurosurg Psychiatry 2007;78 (6):629-631.

10. Rosenfeld J, Swash M. What's in a name? Lumping or splitting ALS, PLS, PMA, and the other motor neuron diseases. Neurology 2006; 66:624-625.

11. Chiò A, Mora G, Calvo A, Mazzini L, Bottacchi E, Mutani R. Epidemiology of ALS in Italy: a 10-year prospective population-based study. Neurology 2009; 72:725-731.

12. Brooks BR. El Escorial World Federation of Neurology criteria for the diagnosis of amyotrophic lateral sclerosis: Subcommittee on Motor Neuron Diseases/Amyotrophic Lateral Sclerosis of the World Federation of Neurology Research Group on Neuromuscular Diseases and the El Escorial Clinical Limits of Amyotrophic Lateral Sclerosis workshop contributors. J Neurol Sci 1994; 124(suppl):96–107.

13. Brooks BR, Miller RG, Swash M, Munsat TL, World Federation of Neurology Research Group on Motor Neuron Diseases. El Escorial revisited: revised criteria for the diagnosis of amyotrophic lateral sclerosis. Amyotroph Lateral Scler Other Motor Neuron Disord 2000; 1:293–299.

14. The Lund and Manchester Group. Clinical and neuropathological criteria for frontotemporal dementia. J Neurol Neurosurg Psychiatry 1994; 57:416-418.

15. Neary D, Snowden JS, Gustafson L, et al. Frontotemporal lobar degeneration: a consensus on clinical diagnostic criteria. Neurology 1998; 51:1546–1554.

16. Schoenberg BS. Calculating confidence intervals for rates and ratios. Neuroepidemiology 1983; 2:257–265.

17. Logroscino G, Traynor BJ, Hardiman O, et al. Incidence of amyotrophic lateral sclerosis in Europe. J Neurol Neurosurg Psychiatry 2010; 81:385-390.

18. Atsuta N, Watanabe H, Ito M, et al. Age at onset influences on wide-ranged clinical features of sporadic amyotrophic lateral sclerosis. J Neurol Sci 2008; 276:163-169.

19. Talman P, Forbes A, Mathers S. Clinical phenotypes and natural progression for motor neuron disease: analysis from an Australian database. Amyotroph Lateral Scler 2009; 10:79-84.

20. Chiò A, Logroscino G, Hardiman O, et al. Prognostic factors in ALS: A critical review. Amyotroph Lateral Scler. 2009;10:310-323.

21. Gordon PH, Cheng B, Katz IB, et al. The natural history of primary lateral sclerosis. Neurology 2006; 66:647–653.

22. Katz JS, Wolfe GI, Andersson PB, et al. Brachial amyotrophic diplegia: a slowly progressive motor neuron disorder. Neurology 1999; 53:1071-1076.

23. Altman DG, Bland JM. Treatment allocation by minimization. Br Med J. 2005;330:853.

24. Strong MJ. The syndromes of frontotemporal dysfunction in amyotrophic lateral sclerosis. Amyotroph Lateral Scler 2008; 9:323-38.

25. Ganesalingam J, Stahl D, Wijesekera L, et al. Latent cluster analysis of ALS phenotypes identifies prognostically differing groups. PLoS One. 2009 Sep 22;4(9):e7107.

26. Veldink JH, Bar PR, Joosten EA, Otten M, Wokke JH, van den Berg LH. Sexual differences in onset of disease and response to exercise in a transgenic model of ALS. Neuromuscul Disord 2003; 13:737–743.

27. Suzuki M, Tork C, Shelley B, et al. Sexual dimorphism in disease onset and progression of a rat model of ALS. Amyotroph Lateral Scler 2007; 8:20-25.

28. Groeneveld GJ, van Muiswinkel FL, Sturkenboom JM, Wokke JH, Bar PR, van den Berg LH. Ovariectomy and 17beta-estradiol modulate disease progression of a mouse model of ALS. Brain Res 2004; 1021:128–131.

29. Rudnicki SA. Estrogen replacement therapy in women with amyotrophic lateral sclerosis. J Neurol Sci 1999; 169:126-7.

30. Popat RA, Van Den Eeden SK, Tanner CM, Bernstein AL, Bloch DA, Leimpeter A, McGuire V, Nelson LM. Effect of reproductive factors and postmenopausal hormone use on the risk of amyotrophic lateral sclerosis. Neuroepidemiology 2006; 27:117-21.

31. Haaxma CA, Bloem BR, Borm GF, Oyen WJ, Leenders KL, Eshuis S, Booij J, Dluzen DE, Horstink MW. Gender differences in Parkinson's disease. J Neurol Neurosurg Psychiatry 2007; 78:819-24.

32. Andersen PM, Nilsson P, Keränen ML, et al. Phenotypic heterogeneity in motor neuron disease patients with CuZn superoxide dismutase mutations in Scandinavia. Brain 1997; 120:1723–1737.

33. Chiò A, Restagno G, Brunetti M, et al. Two Italian kindreds with familial amyotrophic lateral sclerosis due to FUS mutation. Neurobiol Aging 2009; 30:1272-1275.

34. Lai SL, Abramzon Y, Schymick JC, et al. FUS mutations in sporadic amyotrophic lateral sclerosis. Neurobiol Aging 2010 Feb 4. [Epub ahead of print]

35. Kwiatkowski TJ Jr, Bosco DA, Leclerc AL, et al Mutations in the FUS/TLS gene on chromosome 16 cause familial amyotrophic lateral sclerosis. Science 2009; 323:1205-1208.

36. Armon C. Smoking may be considered an established risk factor for sporadic ALS. Neurology 2009; 73:1693-1698.

Table 1. Mean age at onset, mean time delay from onset to diagnosis and frequency of frontotemporal dementia

Phenotype	Number of cases (%)	Mean age at onset (SD), years	Median age at onset (interquartile range) § (SD)	Mean diagnostic delay (SD), months	Median diagnostic delay (interquartile range) § (SD)	Cases with FTD (%)
Classic	404 (30.3%)	62.8 (11.3)	64.6 (56.1-70.6)	10.9 (9.6)	8 (5-13)	16 (4.0%)
Bulbar	456 (34.2%)	68.8 (9.7)	69.9 (62.9-75.0)	9.8 (7.0)	8 (5-12)	41 (9.0%)
Flail arm	74 (5.5%)	62.6 (11.8)	63.3 (54.8-72.2)	12.8 (11.0)	9 (5-15)	1 (1.4%)
Flail leg	173 (13.0%)	65.0 (9.6)	65.6 (58.5-71.2)	13.1 (10.1)	11 (7-17)	7 (4.1%)
Pyramidal	120 (9.1%)	58.3 (13.5)	60.1 (49.2-68.3)	15.9 (13.4)	12 (6-22)	3 (2.5%)
Respiratory	14 (1.1%)	62.2 (8.6)	62.0 (58.3-65.3)	6.4 (4.3)	5 (3-9)	-
PLMN	38 (2.9%)	56.2 (11.3)	55.2 (45.7-61.3)	15.5 (12.4)	14 (10-19)	-
PUMN	53 (4.0%)	58.9 (10.9)	56.5 (48.3-62.6)	15.9 (14.3)	15 (10-19)	2 (3.8%)
Overall ALS	**1332**	**64.3 (11.3)**	**65.3 (59.7-71.8)**	**10.8 (10.4)**	**9 (5-14)**	**70 (5.4%)**
		p=0.0001 *		p=0.0001 *		p=0.0001 **

* ANOVA; ** chi square; § Q1-Q3

Table 2. ALS phenotypes. Overall and men vs. women mean annual crude incidence rates (/100,000 population), 95% confidence intervals (CIs), and gender incidence rate ratios

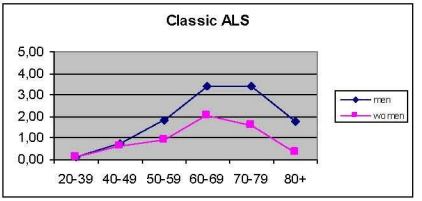
Phenotype	*Overall, incidence rate (CI)*	*Men, incidence rate (CI)*	*Women, incidence rate (CI)*	*Men to Women incidence rate ratio*
Classic	0.94 (0.85-1.04)	1.17 (1.03-1.32)	0.71 (0.61-0.83)	1.65:1
Bulbar	1.05 (0.96-1.15)	1.04 (0.91-1.19)	1.06 (0.94-1.20)	0.98:1
Flail arm	0.17 (0.13-0.21)	0.28 (0.21-0.36)	0.07 (0.04-0.12)	4.00:1
Flail leg	0.40 (0.34-0.47)	0.40 (0.32-0.50)	0.39 (0.31-0.48)	1.03:1
Pyramidal	0.28 (0.23-0.34)	0.28 (0.21-0.36)	0.27 (0.21-0.35)	1.04:1
Respiratory	0.03 (0.02-0.05)	0.06 (0.03-0.10)	0.01 (0-0.03)	6.00:1
PLMN	0.08 (0.06-0.11)	0.11 (0.07-0.17)	0.05 (0.03-0.08)	2.04:1
PUMN	0.12 (0.09-0.16)	0.12 (0.08-0.18)	0.12 (0.08-0.17)	0.98:1
Overall ALS	**3.07 (2.89-3.25)**	**3.46 (3.23-3.71)**	**2.68 (2.44-2.90)**	**1.29:1**

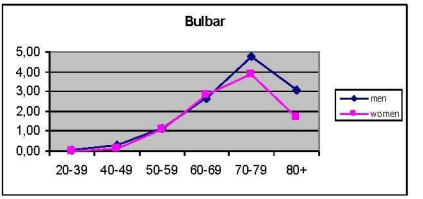
Figure legends

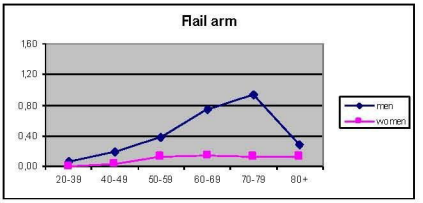
Figure 1. ALS phenotypes: incidence rates according to age group for males vs. females. Respiratory phenotype is not shown due to the low number of cases. PUMN, pure upper motor neuron phenotype; PLMN, pure upper motor neuron phenotype.

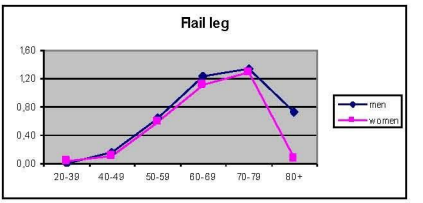
Figure 2. Relative frequency of ALS phenotypes according to age. A, men; B, women. The percentage is calculated on the incidence rates. PUMN, pure upper motor neuron phenotype; PLMN, pure upper motor neuron phenotype.

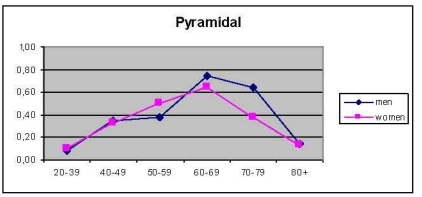
Figure 3. Tracheostomy-free survival, according to ALS phenotypes.
Yellow, PUMN; red, PLMN; light blue, pyramidal ALS; grey, flail arm; violet, classic ALS; green, flail leg; blue, bulbar; cyan, respiratory. Crosses are censored patients.

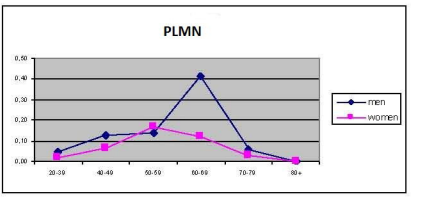

Classic ALS
5,00
4,00
3,00
2,00
1,00
0,00
20-39 40-49 50-59 60-69 70-79 80+
men
women

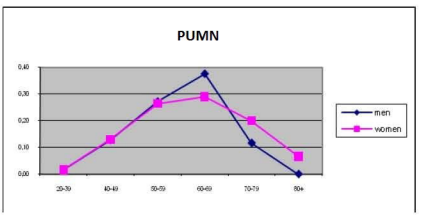

Bulbar
5,00
4,00
3,00
2,00
1,00
0,00
20-39 40-49 50-59 60-69 70-79 80+
men
women

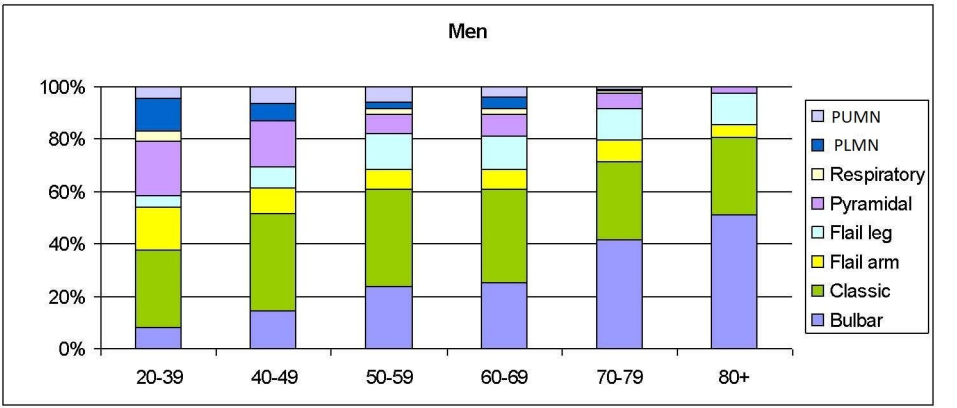

Flail arm
1,60
1,20
0,80
0,40
0,00
20-39 40-49 50-59 60-69 70-79 80+
men
women

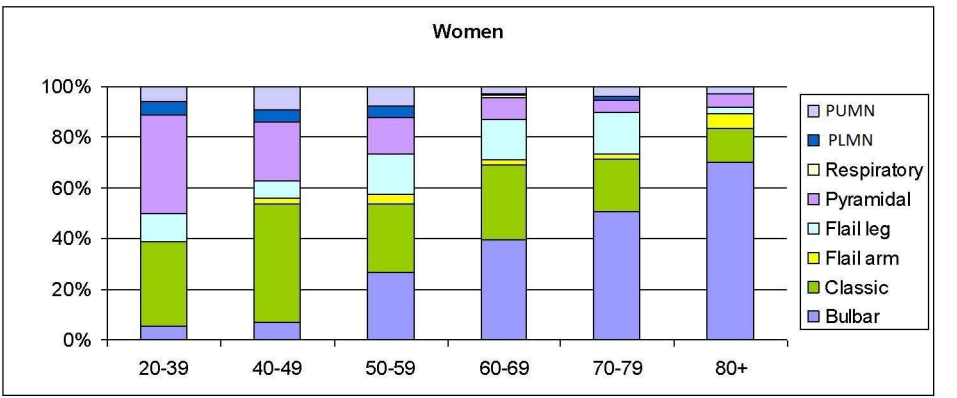

Flail leg
1,60
1,20
0,80
0,40
0,00
20-39 40-49 50-59 60-69 70-79 80+
men
women

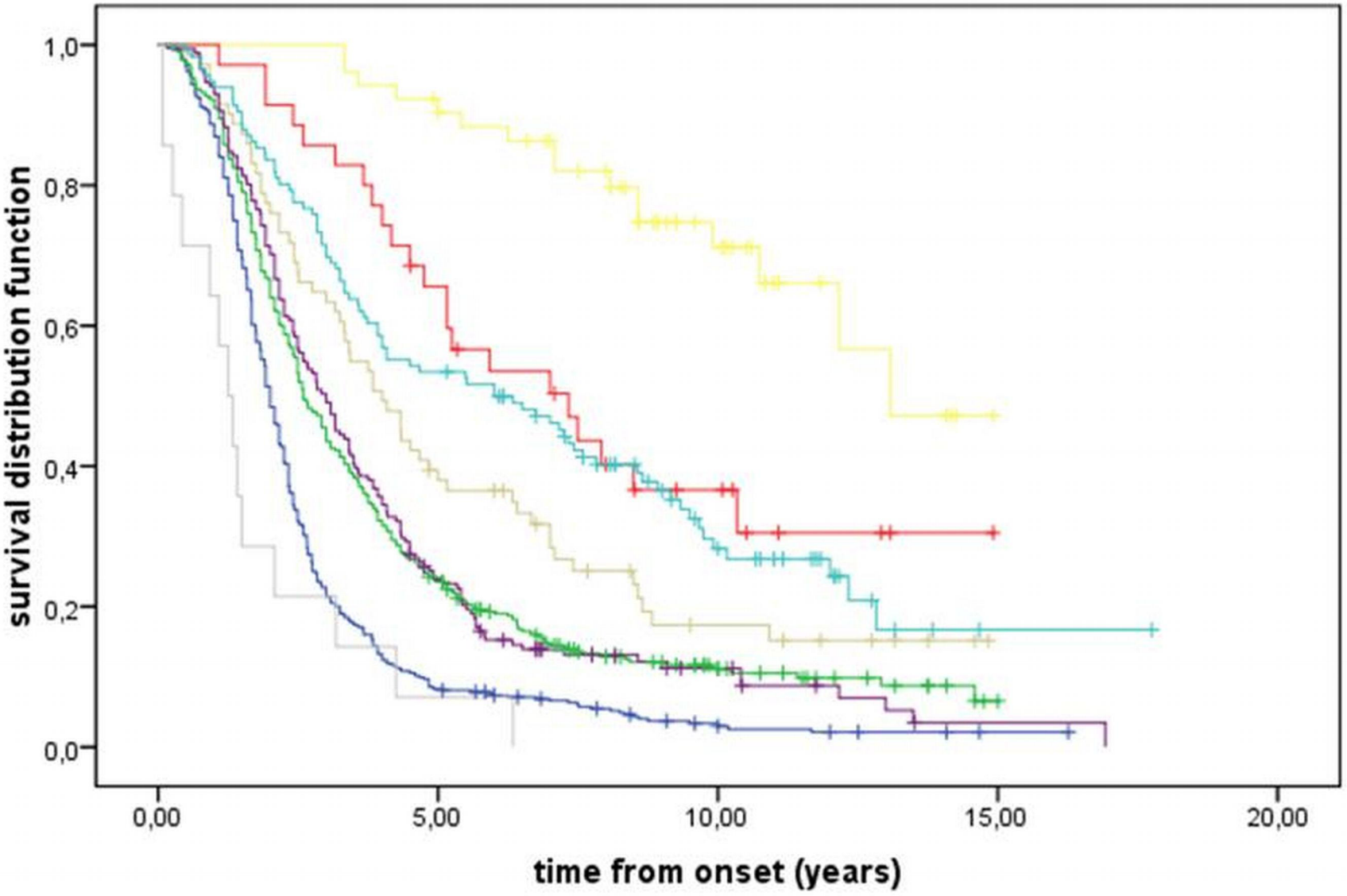

Pyramidal
1,00
0,80
0,60
0,40
0,20
0,00
20-39 40-49 50-59 60-69 70-79 80+
men
women


PLMN
0,50
0,40
0,30
0,20
0,10
0,00
men
women


PUMN
0,40
0,30
0,20
0,10
0,00
men
women


Men
100%
80%
60%
40%
20%
0%
20-39
40-49
50-59
60-69
70-79
80+
PUMN
PLMN
Respiratory
Pyramidal
Flail leg
Flail arm
Classic
Bulbar


Women
100%
80%
60%
40%
20%
0%
20-39
40-49
50-59
60-69
70-79
80+
PUMN
PLMN
Respiratory
Pyramidal
Flail leg
Flail arm
Classic
Bulbar


survival distribution function
1,0
0,8
0,6
0,4
0,2
0,0
0,00
5,00
10,00
15,00
20,00
time from onset (years)