Neurobiology of Aging xxx (2014) 1–6

Contents lists available at ScienceDirect

Neurobiology of Aging

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

Genetic architecture of ALS in Sardinia

Giuseppe Borghero [a], Maura Pugliatti [b], Francesco Marrosu [a], Maria Giovanna Marrosu [c], Maria Rita Murru [c], Gianluca Floris [a], Antonino Cannas [a], Leslie D. Parish [b], Patrizia Occhineri [b], Tea B. Cau [d], Daniela Loi [d], Anna Ticca [e], Sebastiano Traccis [f], Umberto Manera [g], Antonio Canosa [g,h,i], Cristina Moglia [g], Andrea Calvo [g,i], Marco Barberis [g,j], Maura Brunetti [g,j], Hannah A. Pliner [k], Alan E. Renton [k], Mike A. Nalls [l], Bryan J. Traynor [k,m], Gabriella Restagno [j], Adriano Chiò [g,h,i,n,*], for the ITALSGEN and SARDINALS Consortia [1]

[a] Department of Neurology, Azienda Universitario Ospedaliera di Cagliari and University of Cagliari, Cagliari, Italy
[b] Department of Clinical and Experimental Medicine, University of Sassari, Sassari, Italy
[c] Multiple Sclerosis Center Laboratory, University of Cagliari, Cagliari, Italy
[d] Azienda Sanitaria Locale n. 2, Olbia-Tempio, Italy
[e] Department of Neurology, Azienda Ospedaliera San Francesco, Nuoro, Italy
[f] Department of Neurology, Ospedale Antonio Segni, Ozieri, Italy
[g] Amyotrophic Lateral Sclerosis Center, "Rita Levi Montalcini" Department of Neuroscience, University of Turin, Turin, Italy
[h] Department of Neurosciences, Ophthalmology, Genetics, Rehabilitation and Child Health, University of Genoa, Genoa, Italy
[i] Azienda Ospedaliero Universitaria Città della Salute e della Scienza, Turin, Italy
[j] Laboratory of Molecular Genetics, Azienda Ospedaliero Universitaria Città della Salute e della Scienza, Torino, Italy
[k] Neuromuscular Diseases Research Section, Laboratory of Neurogenetics, National Institute of Aging, Bethesda, MD, USA
[l] Molecular Genetics Section, Laboratory of Neurogenetics, National Institute of Aging, Bethesda, MD, USA
[m] Department of Neurology, Brain Science Institute, Johns Hopkins University, Baltimore, MD, USA
[n] Neuroscience Institute of Torino (NIT), Turin, Italy

ARTICLE INFO

Article history:
Received 4 July 2014
Accepted 12 July 2014

Keywords:
Amyotrophic lateral sclerosis
Sardinia
Phenotype
Genetics
Penetrance
Prognosis

ABSTRACT

Conserved populations, such as Sardinians, displaying elevated rates of familial or sporadic amyotrophic lateral sclerosis (ALS) provide unique information on the genetics of the disease. Our aim was to describe the genetic profile of a consecutive series of ALS patients of Sardinian ancestry. All ALS patients of Sardinian ancestry, identified between 2008 and 2013 through the Italian ALS Genetic Consortium, were eligible to be included in the study. Patients and controls underwent the analysis of *TARDBP*, *C9ORF72*, *SOD1*, and *FUS* genes. Genetic mutations were identified in 155 out of 375 Sardinian ALS cases (41.3%), more commonly the p.A382T and p.G295S mutations of *TARDBP* and the GGGGCC hexanucleotide repeat expansion of *C9ORF72*. One patient had both p.G295S and p.A382T mutations of *TARDBP* and 8 carried both the heterozygous p.A382T mutation of *TARDBP* and a repeat expansion of *C9ORF72*. Patients carrying the p.A382T and the p.G295S mutations of *TARDBP* and the *C9ORF72* repeat expansion shared distinct haplotypes across these loci. Patients with cooccurrence of *C9ORF72* and *TARDBP* p.A382T missense mutation had a significantly lower age at onset and shorter survival. More than 40% of all cases on the island of Sardinia carry a mutation of an ALS-related gene, representing the highest percentage of ALS cases genetically explained outside of Scandinavia. Clinical phenotypes associated with different genetic mutations show some distinctive characteristics, but the heterogeneity between and among families carrying the same mutations implies that ALS manifestation is influenced by other genetic and nongenetic factors.

1. Introduction

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder of the adult life, characterized by a progressive deterioration of motor function, causing death because of respiratory failure within

GB and MP contributed equally to this work.

* Corresponding author at: ALS Center, "Rita Levi Montalcini" Department of Neuroscience, Via Cherasco 15, I-10126 Torino, Italy. Tel.: +39 011 633439; fax: +39 011 6963487.
E-mail address: achio@usa.net (A. Chiò).

[1] See Supplementary Appendix for the other members of ITALSGEN and SARDINIALS.

0197-4580/$ – see front matter © 2014 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.neurobiolaging.2014.07.012

2–4 years after onset. In Caucasian population, 90% of patients appear sporadically (sporadic ALS), whereas ~10% of patients have a family history positive for ALS or frontotemporal dementia (FTD) (familial ALS, FALS). The commonest genes implied in ALS so far are *C9ORF72*, *SOD1*, *TARDBP*, and *FUS* (Renton et al., 2014), with marked differences between ethnic groups and geographical regions. Examples of this diversity include the virtual absence of *SOD1* mutations in Ireland and the Netherlands (Kenna et al., 2013; van Blitterswijk et al., 2012), the extremely high frequency of *C9ORF72* repeat expansions in Scandinavia (Majounie et al., 2012; Smith et al., 2013), and the high frequency of *OPTN* mutations combined with the relative scarcity of *C9ORF72* repeat expansions observed in Japan (Konno et al., 2013; Maruyama et al., 2010).

Sardinia, the second largest Mediterranean island, represents a genetic isolate, characterized by a high frequency of autoimmune disorders (such as multiple sclerosis and diabetes mellitus type 1) and monogenic diseases (such a Wilson disease). As might be expected, this population displays decreased genetic and allelic heterogeneity. We and others have reported that ALS patients of Sardinian ancestry have a higher frequency than expected of the *TARDBP* p.A382T missense mutation and of the rate of familial ALS (Chiò et al., 2011; Orrù et al., 2012). Despite this, the incidence of ALS in Sardinians retrospectively investigated seems to be within the range of European studies (Pugliatti et al., 2013).

In this report, we describe the genetic profile of a larger series of ALS patients of Sardinian ancestry and extend our analysis to include other ALS genes (*C9ORF72*, *SOD1*, *TARDBP*, and *FUS*).

2. Methods

2.1. Patients

All ALS patients of Sardinian ancestry (i.e., defined as subjects with both parents of Sardinian origin) were eligible to be included in the study. Patients were identified between 2008 and 2013 through the Italian ALS Genetic Consortium, which includes 16 ALS centers in Italy (Chiò et al., 2012a). Clinical information, including cognitive status, were collected on all patients. ALS patients met the El Escorial–revised criteria for definite, probable, probable laboratory-supported, or possible ALS (Brooks et al., 2000). The genetics of *TARDBP* and *SOD1* for 135 cases has been published elsewhere (Chiò et al., 2011).

2.2. Controls

DNA samples obtained from 700 subjects of Sardinian ancestry not affected by neurodegenerative disorders were screened for *TARDBP* mutations. This cohort included 604 control samples that were previously reported (Cannas et al., 2013). DNA samples obtained from 262 subjects of Sardinian ancestry not affected by neurodegenerative disorders were screened for *SOD1*, *FUS*, and *C9ORF72* mutations. This cohort included 166 control samples that were previously reported (Chiò et al., 2012a). Control subjects were collected at the Department of Neurology, University of Cagliari, and were spouses or non-blood relatives of patients diagnosed with ALS or multiple sclerosis.

2.3. Classification of familial ALS

Patients were classified according to the current revised classification of familial ALS (Byrne et al., 2011; Chiò et al., 2014).

2.4. Mutational screening

The following exons and 50-base pair flanking intron-exon boundaries were screened for mutations by polymerase chain reaction amplification, sequencing using the Big-Dye Terminator v3.1 kit (Applied Biosystems Inc), and analysis on an ABIPrism 3130 genetic analyzer: (1) all 5 coding exons of *SOD1*, (2) exon 6 of *TARDBP*, and (3) exons 14 and 15 of *FUS*. These exons were selected as the vast majority of known pathogenic variants lie within these mutational hot spots. A repeat-primed polymerase chain reaction assay was used to screen for the presence of the GGGGCC hexanucleotide expansion in the first intron of *C9ORF72* (Dejesus-Hernandez et al., 2011; Renton et al., 2011). A cutoff of $\geq$30 repeats combined with a typical sawtooth pattern was considered pathologic.

2.5. Haplotype analysis

For haplotype analysis, we analyzed genome-wide single-nucleotide polymorphism data from patients carrying the same mutation. A custom PERL software script was used to compare unphased sample genotype data.

2.6. Statistical analysis

Differences between groups were analyzed using *t* test for continuous variables (such as age at symptom onset) and chi-square test for discrete variable (such as gender, site of onset, and presence of FTD). Comparison between series of means was performed with analysis of variance. Survival was calculated using Kaplan-Meier curves, and the log-rank test was used to compare survival across groups. The last day of follow-up was March 31, 2014, and none of the patients were lost to follow-up. Significance was set at $p < 0.05$, 2-tail test. Statistical Package for the Social Sciences (SPSS) version 21 (SPSS Inc, IBM, Somers, New York, USA) was used.

2.7. Standard protocol approvals, registrations, and patient consents

The study design was approved by the ethical committees of all the involved centers. Patients and controls signed written informed consent. The study was conducted in line with the Italian ethical

Table 1
ALS in Sardinia: frequency of mutations according to presence or absence of positive family history for ALS or FTD

Mutation	FALS ($n = 100$)	SALS ($n = 275$)	FALS + SALS ($n = 375$)
Wild type	26 (26.0%)	194 (70.5%)	220 (58.7%)
TARDBP (p.A382T, heterozygous and homozygous)	25 (25.0%)	53 (19.3%)	88 (23.5%)
TARDBP (p.G295S, heterozygous and homozygous)	3 (3.0%)	8 (2.9%)	11 (2.9%)
TARDBP (p.A382T and p.G295S double mutation)	1 (1.0%)	—	1 (0.3%)
C9ORF72	33 (33.0%)	18 (6.5%)	51 (13.6%)
C9ORF72 and *TARDBP* (p.A382T)	6 (6.0%)	2 (0.7%)	8 (2.1%)
SOD1	4 (4.0%)	—	4 (1.1%)
MATR3	2 (2.0%)	—	2 (0.5%)

Key: ALS, amyotrophic lateral sclerosis; FALS, familial ALS; FTD, frontotemporal dementia; SALS, sporadic ALS.