C9ORF72 hexanucleotide repeat expansions in the Italian sporadic ALS population

Mario Sabatelli*a,1, Francesca Luisa Confortib,1, Marcella Zollinc, Gabriele Morad,1, Maria Rosaria Monsurro*e, Paolo Volantid, Kalliopi Marinoud, Fabrizio Salvide, Massimo Corboh, Fabio Gianninii, Stefania Battistini, Silvana Pencol, Christian Lunettah, Aldo Quattroneb, Antonio Gambardellab, Giancarlo Logroscinok, Isabella Simonek, Ilaria Bartolomeik, Fabrizio Pisanok, Gioacchino Tedeschik, Amelia Contek, Rossella Spataram, Vincenzo La Bellam, Claudia Caponnetton, Gianluigi Mancardif, Paola Mandichn, Patrizia Solak, Jessica Mandraio, Alan E. Rentonp, Elisa Majounier, Yevgeniya Abramzonp, Francesco Marrosur, Maria Giovanna Marrosus, Maria Rita Murrus, Maria Alessandra Sotgiut, Maura Pugliattit, Carmelo Rodolico, the ITALSGEN consortium2, Cristina Mogliaw, Andrea Calvow, Irene Ossolaa, Maura Brunettii, Bryan J. Traynot, Giuseppe Borgheroint, Gabriella Restagnoxt, and Adriano Chiòvar,1

aNeurological Institute, Catholic University and I.CO.M.M. Association for ALS Research, Rome, Italy
bInstitute of Neurological Sciences, National Research Council, Mangone, Cosenza, and University of Magna Grecia, Catanzaro, Italy
cMolecular Genetics Laboratory, Catholic University of Rome, Italy
dSalvatore Maugeri Foundation IRCSS, Scientific Institute of Milan, Italy
eDepartment of Neurological Sciences, Second University of Naples, Naples, Italy
fSalvatore Maugeri Foundation IRCSS, Scientific Institute of Mistretta, Italy
gCentre for Diagnosis and Cure of Rare Diseases, Department of Neurology, Bellaria Hospital, Bologna, Italy
hNeuromuscular OnmiCenter, Serena Foundation, Milan, Italy
iDepartment of Neuroscience, Neurology Section, University of Siena, Italy
jDepartment of Laboratory Medicine, Medical Genetics, Niguarda Ca’ Granda Hospital, Milan, Italy
kDepartment of Neuroscience, University of Bari, Italy
lSalvatore Maugeri Foundation IRCSS, Scientific Institute of Veruno, Italy
mALS Clinical Research Center, Bio.Ne.C., University of Palermo, Palermo, Italy

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*Corresponding author. Tel.: +39 0116335439; fax: +39 0116963487. achio@usa.net (A. Chiò).
1These authors contributed equally to this work.
2See Appendix or the other members of ITALSGEN

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Disclosure statement
The authors disclose no conflicts. Appropriate Ethical Committee approvals were in place for this work.
Abstract

It has been recently reported that a large proportion of patients with familial amyotrophic lateral sclerosis (familial ALS) and frontotemporal dementia (FTD) are associated with a hexanucleotide \((\text{GGGGCC})\) repeat expansion in the first intron of \textit{C9ORF72}. We have assessed 1,757 Italian sporadic ALS cases, 133 from Sardinia, 101 from Sicily, and 1,523 from mainland Italy. Sixty (3.7\%) of 1,624 mainland Italians and Sicilians and 9 (6.8\%) of the 133 Sardinian sporadic ALS cases carried the pathogenic repeat expansion. None of the 619 regionally-matched control samples (1,238 chromosomes) carried the expansion. Twenty-five cases (36.2\%) had behavioral FTD in addition to ALS. FTD or unspecified dementia was also detected in 19 pedigrees (27.5\%) in first-degree relatives of ALS patients. Cases carrying the \textit{C9ORF72} hexanucleotide expansion survived one year less than cases who did not carry this mutation. In conclusion, we found that \textit{C9ORF72} hexanucleotide repeat expansions represents a sizeable proportion of apparent sporadic ALS in the Italian and Sardinian population, representing by far the commonest mutation in Italy and the second more common in Sardinia.

Keywords

Amyotrophic lateral sclerosis; \textit{C9ORF72}; frontotemporal dementia; survival

1. Introduction

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder of the adult life characterized by a progressive loss of lower motor neurons at spinal and bulbar level and of upper motor neurons. Its course is invariably fatal within 3 to 5 years from onset. While 5 to 10\% of cases is familial in nature (familial ALS), the majority of patients present as apparently sporadic (sporadic ALS).

Recently, we found that a large hexanucleotide \((\text{GGGGCC})\) repeat expansion in the first intron of \textit{C9ORF72}, a gene located on chromosome 9p21, is pathogenic in a large proportion of patients with familial ALS and frontotemporal dementia (FTD) of Finnish and
European ancestry (Renton et al., 2011; Dejesus-Hernandez et al., 2011). In these families, the genetic defect is dominantly transmitted; however, mutations of this gene have also been detected in a number of patients with apparently sporadic ALS or FTD (Renton et al., 2011; Dejesus-Hernandez et al., 2011). To date, little clinical information are available about the characteristics of apparently sporadic ALS patients carrying C9ORF72 hexanucleotide repeat expansions.

The aim of the present paper was to provide a detailed genotype-phenotype description of sporadic ALS cases carrying the expansion including age at onset, gender, site of onset, cognitive status and survival.

2. Methods

A total of 1,757 Italian sporadic ALS cases have been collected thorough the Italian ALS Genetic consortium (ITALSGEN), which is a collaborative effort between fifteen ALS centers throughout the Italian peninsula and the Mediterranean islands of Sicily and Sardinia. Of these, 133 were of Sardinian ancestry, 101 were from Sicily, and the remaining 1,523 were from mainland Italy. Of note, we previously reported the C9ORF72 repeat expansion status for 465 of these patients in our previous paper (Majounie et al., submitted). All cases included in the present study had a negative family history for ALS and were negative for mutations in major ALS genes (SOD1, TARDBP and FUS). All cases had a diagnosis of definite, probable, probable laboratory-supported or possible ALS according to El Escorial revised criteria (Brooks et al., 2000).

Controls consisted of 402 neurologically healthy subjects of mainland Italian ancestry, 61 of Sicilian ancestry and 156 of Sardinian ancestry.

The clinical history of patients found to carry the C9ORF72 expansion was systematically reviewed to identify details of the disease phenotype including gender, age of onset, disease duration, disease variant, details of family history and the presence of cognitive impairment. Formal cognitive evaluation was not systematically performed in this series. In 66 non-expanded cases the age at onset, the site of onset and the survival were not known.

C9ORF72 analysis

A repeat-primed PCR assay was used to screen the presence of the GGGGCC hexanucleotide expansion in the first intron of C9ORF72 as described in the original paper (Renton et al, 2011). This assay rapidly and robustly determines whether a sample carries the repeat expansion, but does not measure the actual number of repeats in the expansion. ALS-associated pathological expansions in C9ORF72 have been defined as greater than 30 repeats.

Statistical analysis

Continuous variables (i.e. age at symptom onset) were compared with t-test and discrete variable (i.e. gender, site of onset) with χ²-square. Survival was calculated with Kaplan and Meier curves and compared with log-rank test, using as end point death or tracheostomy. For censored cases, the last day of follow-up was November 1, 2011. Multivariable analysis was performed with the Cox’s proportional hazards model (stepwise backward). The following variables were included in the model: age (included as continuous variable), gender (male vs. female), site of onset (bulbar vs. spinal), ancestry (Italian vs. Sardinian), and C9ORF72 status (expanded vs. not expanded). A p-value <0.05 was considered significant. All calculation were made with SPSS (IBM corporation, version 17). The study was approved by the ethical committees of the participating centers. All patients signed a written informed consent.
3. Results

**Frequency of C9ORF72 pathological repeat expansion in sporadic ALS**

We tested 1,523 mainland Italian patients, 101 Sicilian patients, and 133 Sardinian patients diagnosed with sporadic ALS for the presence of the GGGGCC hexanucleotide repeat expansion of the C9ORF72 gene using a repeat-primed PCR assay. Fifty-five (3.6%) of the mainland Italians carried the pathogenic repeat expansion, whereas 5 (4.9%) of the 101 patients with Sicilian ancestry, and 9 (6.8%) of the 133 Sardinian sporadic ALS cases were carriers. None of the 402 mainland Italian, 61 Sicilian and 156 Sardinian control samples (1,238 chromosomes) carried the expansion. The median number of repeats in mainland Italian and Sicilian controls was 3 (range 0 to 23, interquartile range 0–5), and in Sardinian controls was 4 (range 0 to 12, interquartile range 0–5, see Supplemental Figure).

Evaluation of the pedigrees of sporadic ALS carrying the pathological expansion did not reveal other cases of ALS. However, FTD or unspecified dementia was detected in first-degree relatives of ALS patients in 19 (27.5%) pedigrees (27.5%). Moreover, we identified first degree relatives with parkinsonism in three pedigrees, and with schizophrenia in two pedigrees.

**Genotype-phenotype correlation**

The clinical characteristics of the sporadic ALS cases carrying C9ORF72 expansion are reported in Supplemental Table. Twenty-five cases (36.2%) had FTD in addition to their motor dysfunction. In all cases, FTD presented with behavioral symptoms, while none of the cases had features consistent with semantic dementia or progressive non-fluent aphasia. Three cases had additional clinical symptoms of parkinsonism, with positive DAT scan, and three had psychotic symptoms (hallucinations and delusions).

Gender distribution and site of onset were similar in mutated and non-mutated cases (Table 1). Cases with C9ORF72 expansion had a slightly lower median age at onset than those without the expansion (59.0 [interquartile range 50.0–65.6], range 32.9–83.0, vs. 62.8 [interquartile range 54.0–69.7], range 20.5–89.6) (p=0.002) (Figure 1).

**Tracheostomy-free survival**

Survival of cases carrying the C9ORF72 hexanucleotide expansion was one year shorter than that of cases who did not carry this mutation (C9ORF72 expanded cases, median survival time 2.7 years, 95% c.i. 2.1 to 3.3; not expanded cases, median survival time 3.6; 95% c.i. 3.3 to 3.8; p=0.03) (Figure 2). The negative effect of C9ORF72 expansion on survival persisted in the Cox multivariate model (C9ORF72 status, not expanded vs. expanded, HR 1.79; 95% c.i. 1.26–2.98; p=0.008).

4. Discussion

C9ORF72 hexanucleotide repeat expansions have been detected in 3.7% of mainland Italian sporadic ALS of this series, which is representative of the Italian population, as it includes ALS cases originating from all Italian regions. Therefore, C9ORF72 hexanucleotide repeat expansions is by far the most common mutation in apparently sporadic ALS in the Italian population (Chiò et al., 2008; Lai et al., 2010; Del Bo et al., 2009; Corrado et al., 2010).

The frequency of Italian SALS carrying C9ORF72 hexanucleotide repeat expansion is lower than that reported in Finland (Renton et al., 2011), and in other north-European populations, such as Britons, Irish and Germans, as well in Caucasian Americans (Renton et al., 2010; Dejesuz-Hernandez et al 2011; Gijselinkck et al., 2012; Majounie et al., submitted).
In sporadic ALS of Sardinian ancestry, C9ORF72 repeat expansions were more frequent than in mainland Italy, accounting for about 7% of cases, but they are less frequent than the A382T missense mutations of the TARDBP gene, which accounted for about 23% of sporadic ALS in the same population (Chiò et al., 2011; Orrù et al., 2011). The high frequency of the C9ORF72 mutation in Sardinia is likely to be related to the ‘magnification’ of pathological genes in isolated populations, due to the so-called genetic drift phenomenon combined to the founder effect.

Sporadic ALS cases carrying the C9ORF72 hexanucleotide repeat expansion were phenotypically different from those not carrying the expansion. First, they were significantly younger, confirming previous observation in familial ALS patients carrying the same expansion (Chiò et al., in press; Majounie et al., submitted). Second, they had a more aggressive clinical course, with a median survival ~1 year shorter than cases without the expansion. According to the Cox multivariable analysis, the negative effect of C9ORF72 mutations on ALS survival is independent of age and site of onset. However, we did not include in the analysis the presence of FTD, a known negative prognostic factor in ALS (Elamin et al., 2011), which is known to have a higher frequency in ALS cases with C9ORF72 hexanucleotide repeat expansion than in those without this expansion (Renton et al., 2011; Dejesus-Hernandez et al., 2011). Finally, one third of cases with the expansion manifested cognitive impairment, consistent with previous reports of a high rate of FTD in this patient group.

The identification of the C9ORF72 expansion in apparently sporadic ALS may have different explanations, including poor diagnosis in the past, lack of knowledge of family history, mutation carriers in previous generations dying of other diseases prior to developing motor neuron degeneration, reduced penetrance of the gene, and even varying phenotypic manifestations among mutation carriers within the same family (Traynor and Singleton, 2009). An incomplete penetrance of C9ORF72 has been reported in a large multinational study (Majounie et al., submitted). However, the likely familial nature of these apparently sporadic cases is supported by several findings. First, the gender distribution of the cases carrying the C9ORF72 repeat expansion in our sporadic series is not different from what expected for an autosomal dominant mutation. Second, the frequent identification of relatives with FTD or dementia in the patients’ pedigrees support the notion that the C9ORF72 hexanucleotide expansion may manifest with different motor or cognitive phenotypes in the same pedigree. This observation implies that the definition of familial ALS should be enlarged to include patients with a positive family history for FTD, modifying the proposed criteria for definition of familial ALS (Byrne et al., 2011). Third, previous data indicate that ALS and FTD patients carrying the C9ORF72 mutation share a common haplotype and that all cases are in fact derived from a single founder (Mok et al., 2012).

In conclusion, we found that C9ORF72 hexanucleotide repeat expansions represents a sizeable proportion of apparent sporadic ALS in the Italian and Sardinian population, representing by far the commonest mutation in Italy and the second more common in Sardinia, after the A382T missense mutation of the TARDBP gene. The high frequency of familial aggregates of ALS and dementia in the pedigrees of these apparently sporadic cases with the C9ORF72 mutation support the notion that most of these cases are in fact familial. An accurate study of the family history of ALS patients, as well the identification of co-morbid FTD, may therefore be useful to identify clinically the possible carriers of this mutation.
**Supplementary Material**

Refer to Web version on PubMed Central for supplementary material.

**Acknowledgments**

This work was supported in part by the Intramural Research Programs of the NIH, National Institute on Aging (Z01-AG000949-02), and NINDS. The work was also supported by the Packard Center for ALS Research at Hopkins (B.J.T.), the ALS Association (B.J.T., A.C.), Microsoft Research (B.J.T.), Federazione Italiana Giuoco Calcio (B.J.T., A.C.), European Community's Health Seventh Framework Programme (FP7/2007-2013) under grant agreement 259867 (A.C.), and ARISLA (B.J.T., A.C.). Dr. Traynor reports that a patent is pending based on the discovery of the hexanucleotide repeat expansion of C9ORF72. Funding organizations had no role in design and conduct of the study; collection, management, analysis, and interpretation of the data; and preparation, review, or approval of the manuscript.

**References**


*Neurobiol Aging. Author manuscript; available in PMC 2013 August 01.*


Appendix

Members of the ITALSGEN consortium

Stefania Cammarosano, MD, Giuseppe Fuda, Antonio Canosa, MD, Sara Gallo, MD (Department of Neurosciene, University of Turin, Italy), Laura Papetti, PharmD (Salvatore Maugeri Foundation, IRCCS, Scientific Institute of Milan, Italy), Giuseppe Lauria Pinter, MD (Department of Neurology, National Neurologic Institute Besta, Milan, Italy), Marco Luigetti, MD (Neurological Institute, Catholic University and I.C.O.M.M. Association for ALS Research, Rome, Italy), Serena Lattante, BS, Giuseppe Marangi, MD (Molecular Genetics Laboratory, Catholic University of Rome, Italy), Tiziana Colletti, MD (ALS Clinical Research Center, Bio.Ne.C., University of Palermo, Italy), Claudia Ricci, MD (Department of Neuroscience, Neurology Section, University of Siena, Italy), Paola Origone, PhD (Department of Neuroscience, Ophthalmology and Genetics, University of Genoa, Italy), Gianluca Floris, MD, Antonino Cannas, MD, Valeria Piras, MD, Emanuela Costantino, MD, Carla Pani, MD (Azienda Universitaria-Ospedaliera di Cagliari, and University of Cagliari, Italy), Leslie D. Parish, MD, Paola Cossu, BS (Department of Neuroscience, University of Sassari, Italy), Giuliana Solinas, PhD, Lucia Ulgheri PhD (Department of Biomedical Sciences, University of Sassari, Italy), Anna Ticca, MD (AO San Francesco, Nuoro, Italy), Francesco Izzo, MD, Anna Laiola, MD, Francesca Trojsi, MD (Department of Neurological Sciences, Second University of Naples, Naples, Italy), Simona Portaro, MD (Department of Neurosciences, Psychiatric and Anaesthesiological Sciences, University of Messina, Italy and IRCCS Centro Neurolesi “Bonino-Pulejo” Messina, Italy), William Sproviero, MD (Institute of Neurological Sciences, National Research Council, Mangone, Cosenza, Italy and University of Magna Gracia, Catanzaro, Italy)
Figure 1.
Cumulative probability of disease onset according to the age of patients. Comparison between sporadic ALS patients with, hexanucleotide repeat expansion of the C9ORF72 gene or unknown genetic mutation (p=0.003).
Figure 2.
Cumulative survival probability from time of disease onset. Comparison between sporadic ALS patients with hexanucleotide repeat expansion of the C9ORF72 gene and patients without known genetic mutation (p=0.023). Blue, unknown mutation; green, C9ORF72. Marks are censored patients.
Table 1

Comparison of clinical characteristics Italian and Sardinian sporadic ALS cases carrying and not carrying the C9orf72 hexanucleotide repeat expansion

<table>
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<tr>
<th></th>
<th>Italian sporadic ALS</th>
<th>Sardinian sporadic ALS</th>
<th>Overall</th>
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<tr>
<td>Gender (female)</td>
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<tr>
<td></td>
<td>30 (50.0%)</td>
<td>672 (43.0%)</td>
<td>0.28</td>
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<tr>
<td>Site of onset (bulbar)</td>
<td>21 (35.0%)</td>
<td>409 * (26.5%)</td>
<td>0.14</td>
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<tr>
<td>Median age at onset (years) (interquartile range)</td>
<td>58.6 (49.0–65.6)</td>
<td>62.9 * (53.6–70.0)</td>
<td>0.003</td>
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*Site of onset and age at onset were unknown in 17 not expanded cases.