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C9ORF72 repeat expansion in a large Italian ALS cohort: evidence of a founder effect

Antonia Ratti[a,b,*], Lucia Corrado[c], Barbara Castellotti[d], Roberto Del Bo[b,e], Isabella Fogh[f], Cristina Cereda[g], Cinzia Tiloca[a], Carla D'Ascenzo[h], Alessandra Bagarotti[c], Viviana Pensato[d], Michela Ranieri[b,e], Stella Gagliardi[g], Daniela Calini[a], Letizia Mazzini[i], Franco Taroni[d], Stefania Corti[b,e], Mauro Ceroni[g,j], Gaia D. Oggioni[i], Kuang Lin[f], John F. Powell[f], Gianni Sorarù[h], Nicola Ticozzi[a], Giacomo P. Comi[b,e], Sandra D'Alfonso[c], Cinzia Gellera[d], Vincenzo Silani[a,b], and the SLAGEN Consortium

[a] *Department of Neurology and Laboratory of Neuroscience, IRCCS Istituto Auxologico Italiano, Milan, Italy*
[b] *Dipartimento di Fisiopatologia Medico-Chirurgica e dei Trapianti, 'Dino Ferrari' Center, Università degli Studi di Milano, Milan, Italy*
[c] *Department of Health Sciences, Interdisciplinary Research Center of Autoimmune Diseases, 'A. Avogadro' University, Novara, Italy*
[d] *Unit of Genetics of Neurodegenerative and Metabolic Diseases, Fondazione IRCCS Istituto Neurologico 'Carlo Besta', Milan, Italy*
[e] *IRCCS Foundation Ca'Granda Ospedale Maggiore Policlinico, Milan, Italy*
[f] *Department of Neuroscience, King's College London, Institute of Psychiatry, London, UK*
[g] *Laboratory of Experimental Neurobiology, IRCCS National Neurological Institute 'C. Mondino', Pavia, Italy*
[h] *Department of Neurosciences, University of Padua, Padua, Italy*
[i] *Department of Neurology, 'A. Avogadro' University and Maggiore della Carita' Hospital, Novara, Italy*
[j] *Department of Neurological Sciences, University of Pavia, Pavia, Italy*

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Abstract

A hexanucleotide repeat expansion (RE) in *C9ORF72* gene was recently reported as the main cause of amyotrophic lateral sclerosis (ALS) and cases with frontotemporal dementia. We screened *C9ORF72* in a large cohort of 259 familial ALS, 1275 sporadic ALS, and 862 control individuals of Italian descent. We found RE in 23.9% familial ALS, 5.1% sporadic ALS, and 0.2% controls. Two cases carried the RE together with mutations in other ALS-associated genes. The phenotype of RE carriers was characterized by bulbar-onset, shorter survival, and association with cognitive and behavioral impairment. Extrapyramidal and cerebellar signs were also observed in few patients. Genotype data revealed that 95% of RE carriers shared a restricted 10-single nucleotide polymorphism haplotype within the previously reported 20-single nucleotide polymorphism risk haplotype, detectable in only 27% of nonexpanded ALS cases and in 28% of controls, suggesting a common founder with cohorts of North European ancestry. Although *C9ORF72* RE segregates with disease, the identification of RE both in controls and in patients carrying additional pathogenic mutations suggests that penetrance and phenotypic expression of *C9ORF72* RE may depend on additional genetic risk factors.

Keywords: Amyotrophic lateral sclerosis; Frontotemporal dementia; *C9ORF72*; Repeat expansion; Mutation analysis; Haplotype analysis

1. Introduction

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder in which the relentless degeneration of motor neurons leads to a progressive muscular weakness and, ultimately, death. The etiology is sporadic (SALS) in the majority of cases, although familial forms (FALS) account

* Corresponding author at: Department of Neurology and Lab. Neuroscience, IRCCS Istituto Auxologico Italiano, Via Zucchi 18, 20095 Cusano Milanino, Milan, Italy. Tel.: +39 02 619113045; fax: +39 02 619113033.
E-mail address: antonia.ratti@unimi.it (A. Ratti).

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for about 10% and are mainly transmitted by an autosomal dominant inheritance (Hardiman et al., 2011). Until recently, the most common known genetic cause was mutations in *SOD1* gene which account for 20% of FALS cases. *TARDBP* and *FUS* genes account overall for about 10% of additional cases, while mutations in other genes, such as *VAPB, ANG, OPTN,* and *UBQLN2*, are responsible for a very small percentage of familial forms (Andersen and Al-Chalabi, 2011).

Cognitive impairment occurs in up to 50% of cases and one in seven patients develop frank frontotemporal dementia (FTD) (Phukan et al., 2012). There is now compelling evidence that these two disorders are indeed linked by common pathogenic mechanisms. Families presenting with either ALS or FTD previously showed a linkage with chromosome 9p21 region and different genome-wide association (GWA) studies reported association with this region in both sporadic ALS and FTD cases (Mok et al., 2012). A major breakthrough was made when two independent groups identified in this region the presence of an expanded hexanucleotide repeat in the first intron of *C9ORF72* gene (DeJesus-Hernandez et al., 2011; Renton et al., 2011). This repeat (GGGGCC) is highly polymorphic in the normal population (2–23 units), but is expanded both in ALS and FTD patients (up to 1600 units). In the inbred Finnish population the mutational frequency of *C9ORF72* was reported to be as high as 46% in FALS and 21% in SALS (Renton et al., 2011). In other populations of European descent the mutational frequencies ranged from 23%–47% in FALS, 4%–5% in SALS to 12%–29% in FTD, and 6%–86% in ALS and FTD (ALS-FTD) patients (Byrne et al., 2012; DeJesus-Hernandez et al., 2011; Gijselinck et al., 2012; Simón-Sánchez et al., 2012; Snowden et al., 2012; Stewart et al., 2012).

Haplotype analysis showed that most of the *C9ORF72* expanded cases shared the same 20-single nucleotide polymorphism (SNP) haplotype within a 140 kb-long region surrounding the *C9ORF72* gene (Byrne et al., 2012) suggesting a common ancestor for all patients with expanded alleles.

In order to define the incidence of *C9ORF72* mutations in the Mediterranean area, we analyzed a very large cohort of Italian ALS patients composed of 259 FALS and 1275 SALS, including 76 patients presenting with ALS-FTD both in familiar and sporadic form.

2. Methods

2.1. Patients and controls

The 1534 ALS patients included in this study were recruited from 6 clinical Centers participating in the Italian SLAGEN Consortium. The cohort consisted of 259 unrelated FALS cases with a positive family history according to the recent criteria proposed for FALS classification (Byrne et al., 2011) and 1275 SALS patients, including 76 individuals (10 FALS and 66 SALS) with concomitant ALS and FTD. All patients fulfilled the El Escorial revised criteria for ALS and the Neary criteria for FTD diagnosis (Miller et al., 1999; Neary et al., 1998). The ALS cohort was characterized by a male to female sex ratio of 2:1 and a mean age of onset of 57.1 ± 12.7 years (FALS 53.2 ± 13.7; SALS 58.4 ± 12.1).

All FALS and SALS patients included in the study were screened for *SOD1*, *TARDBP*, and *FUS* gene mutations as previously reported (Corrado et al., 2006, 2009, 2010; Del Bo et al., 2009; Gellera, 2001; Ticozzi et al., 2009). Mutation analysis of *ANG, OPTN, VCP*, and *UBQLN2* genes was also performed in FALS cases (Corrado et al., 2007; Del Bo et al., 2011; Gellera et al., 2008; Tiloca et al., 2012, unpublished data). FALS and SALS subjects, included in this study with unknown genetic cause, were 204 and 1230, respectively. The control group consisted of 862 Italian healthy individuals (University and Hospital staff, blood donors) who did not have a personal or family history for neurodegenerative diseases.

2.2. Standard protocol approval and patient consent

We received approval for this study from the local Ethics committees on human experimentation of each participating Institution. Written informed consent was designed according to the Declaration of Helsinki and obtained from all patients and healthy subjects participating to the study.

2.3. Genetic analysis

Genomic DNA was isolated from peripheral blood according to standard protocols. The GGGGCC hexanucleotide repeat in *C9ORF72* was analyzed by a two-step protocol, including a first polymerase chain reaction amplification using the genotyping primers previously reported (DeJesus-Hernandez et al., 2011). The normal range fragment length analysis was performed both using GeneScan (Applied Biosystems, Foster City, CA, USA) and runs on 3% agarose gel, the latter to better discriminate hexanucleotide repeat alleles larger than 20 units but still in the normal range. Only those samples presenting with a single peak/amplification product were further analyzed in the second step by the repeat-primed polymerase chain reaction method (DeJesus-Hernandez et al., 2011) on a 3100 ABI Prism Genetic Analyzer (Applied Biosystems). The presence of *C9ORF72* repeat expansion (RE) was assigned when the sample displayed a typical electropherogram profile with decaying stutter amplification peaks with a 6 base pair periodicity that exceeded the upper detection limit of the assay, as previously described (DeJesus-Hernandez et al., 2011).

2.4. SNP genotyping

For haplotype analysis we utilized GWA data from our Italian SALS and control cohorts previously genotyped on Human660W-Quad BeadChip (Illumina, San Diego, CA,