ELSEVIER

Neurobiology of Aging 33 (2012) 837.e1–837.e5

NEUROBIOLOGY OF AGING

www.elsevier.com/locate/neuaging

FUS mutations in sporadic amyotrophic lateral sclerosis: Clinical and genetic analysis

William Sproviero[a], Vincenzo La Bella[b], Rosalucia Mazzei[a], Paola Valentino[c], Carmelo Rodolico[d], Isabella Laura Simone[e], Giancarlo Logroscino[e], Carmine Ungaro[a], Angela Magariello[a], Alessandra Patitucci[a], Gioacchino Tedeschi[f], Rossella Spataro[b], Francesca Condino[a], Francesco Bono[c], Luigi Citrigno[a], Maria Rosaria Monsurrò[f], Maria Muglia[a], Antonio Gambardella[a,c], Aldo Quattrone[c], Francesca Luisa Conforti[a,*]

[a] *Institute of Neurological Sciences, National Research Council, Mangone, Cosenza, Italy*
[b] *ALS Clinical Research Center, Bio.Ne.C, University of Palermo, Italy*
[c] *Institute of Neurology, University Magna Graecia, Catanzaro, Italy*
[d] *Department of Neurosciences, Psychiatric and Anaesthesiological Sciences, University of Messina, Italy*
[e] *Department of Neurological and Psychiatric Sciences, University of Bari, Italy*
[f] *Second Division of Neurology, Second University of Naples, Naples, Italy*

Received 23 May 2011; received in revised form 3 October 2011; accepted 4 October 2011

Abstract

Fused in sarcoma (FUS) or translocation in liposarcoma (TLS), a DNA/RNA-binding protein, causes a dominant autosomal inherited form of amyotrophic lateral sclerosis (ALS), ALS 6. Its main role in neurodegeneration is highlighted by the presence of cytoplasmic accumulation of its mutant protein form in ALS patients. To further define the frequency and spectrum of *FUS* gene mutations, we have performed a molecular screening of a cohort of 327 Italian patients from Southern Italy with sporadic ALS (SALS). We identified 4 patients carrying 3 different missense mutations and several polymorphisms. Two different substitutions occurring in the same amino acidic position have been observed in 2 patients: R521G and R521C respectively; P525L mutation has been found in 2 additional cases. Most of the patients with *FUS* mutations showed early symptom onset and had short disease survival. We also detected 4 different polymorphic variants (3′-untranslated region [UTR] variant, c.*41G>A; c.523+3ins[GAGGTG]; c.335-15del[TTTT]; and rs13331793) in 9 patients from within our cohort. This study underlines the importance of population-based mutation screening of newly identified genes.

Keywords: ALS; *FUS* gene; Mutation; Sporadic

1. Introduction

Amyotrophic lateral sclerosis (ALS) is an adult-onset neurodegenerative disorder characterized by the loss of upper and lower motor neurons, resulting in progressive muscle weakness and atrophy, which causes death within 3 to 5 years from symptom onset.

In the last decade several genes implicated in the RNA processing pathways have been associated with ALS, including the fused in sarcoma/traslocated in liposarcoma gene (*FUS/TLS*) (Strong, 2010). FUS is a ubiquitously expressed 526 amino acid protein, encoded by 15 exons, that belongs to the FET/TET family (FUS, EWS and TAF15 proto-oncoproteins) of multifunctional DNA/RNA binding proteins. FUS contains an N-terminal Gln-Gly-Ser-Tyr-rich domain, a Gly-rich domain, an RNA recognition motif,

* Corresponding author at: Institute of Neurological Sciences, CNR, C. da Burga, Mangone 87050 (Cosenza), Italy. Tel.: +39 0984 9801216; fax: +39 0984 969306.
E-mail address: fl.conforti@isn.cnr.it (F.L. Conforti).

0197-4580/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
doi:10.1016/j.neurobiolaging.2011.10.005

multiple Arg-Gly-Gly repeats, a zinc-finger motif and a highly conserved extreme C-terminus that encodes a non-classic nuclear localization signal (NLS), recognized by transportin (Mackenzie et al., 2010).

Mutations in the *FUS* gene have been identified as the primary cause of ALS 6, an autosomal dominant form of familial ALS (FALS) linked to chromosome 16. The only exception is the Cape Verdean family for which the *FUS* mutation (p. H517Q) causes ALS only in a homozygous state (Lagier-Tourenne and Cleveland, 2009).

Subsequent reports of *FUS* mutations have also been described in sporadic patients (Talbot, 2009).

Much evidence suggests that mutations in *FUS* are associated with earlier onset of ALS than the general mean age of approximately 60 years (Corrado et al., 2010; Kwiatkowski et al., 2009; Vance et al., 2009).

In most cell types, FUS is present in both the nucleus and cytoplasm, but in neurons the proportion of FUS is higher in the nucleus than in the cytoplasm and in glia FUS is exclusively nuclear (Neumann et al., 2009). Neuropathological analysis of brain and spinal cord of ALS patients carrying mutations showed cytoplasmic retention and the formation of FUS ubiquitin-positive neuronal aggregates (DeJesus-Hernandez et al., 2010).

In only 2 years, 35 different pathogenic *FUS* mutations have been identified; they account for about 4% of familial ALS cases, but less than 1% of sporadic ALS cases. Most of these mutations (21 missense mutations) are clustered in the NLS region (encoded by exons 14 and 15) and may exert their pathogenic effects by disrupting the subcellular distribution of the protein, assuming a toxic gain of function effect (Hewitt et al., 2010; Mackenzie et al., 2010).

In this study, in order to investigate the presence and frequency of *FUS* mutations in our cohort of south Italian ALS patients, we performed a mutational screening of the *FUS* gene in 327 *SOD1*-negative, *ANG*-negative, *VAPB*-negative, and *TARDBP*-negative sporadic ALS (SALS) cases. We also wished to report the clinical findings of those ALS patients carrying *FUS* mutations.

2. Methods

2.1. Patients

A total of 327 unrelated Caucasian patients from southern Italy (185 men and 142 women; mean age at onset 56.5 years, SD 12.2381) were included in this study. Each patient underwent a full neurological evaluation including electromyography, clinical laboratory testing, and imaging as appropriate to establish the clinical diagnosis of ALS according to the El Escorial criteria (Brooks et al., 2000). Patients were classified as sporadic when none of them had a known family history of ALS. Age at onset, gender distribution, site of symptom onset (bulbar and/or spinal-onset), predominance of upper/lower motor neuron signs, and disease duration were recorded (Table 1). Mini Mental State Examination (MMSE), Frontal Assessment Battery (FAB) and Beck Depression Inventory were administered in *FUS* mutation carriers to evaluate general and severe cognitive deterioration, frontal cognitive impairment, and severe depression, respectively.

Table 1
Clinical and genetic details of amyotrophic lateral sclerosis (ALS) patients

Sporadic ALS cases	$n = 327$
Mean age at onset, y	56.55
Male (%)	185 (56.6)
Female (%)	142 (43.4)
Site of symptom onset (%)[a]	
Bulbar-onset disease	55 (17.2)
Spinal-onset disease	261 (81.6)
Lower	125 (47.9)
Upper	122 (46.7)
Mean disease duration (mo)[b]	42.03
SOD1-, VAPB-, ANG-, TARDBP-positive	None

[a] Site of symptom onset data were not available for 7 cases.
[b] Disease duration data were not available for 40 cases.

All patients tested negative for mutations in *SOD1*, *ANG, VAPB*, and *TARDBP* genes.

Control DNA was obtained from 100 unrelated, ethnically matched unaffected subjects with a negative personal and familial history for neurodegenerative diseases. Informed written consent was obtained from each individual, and appropriate institutional review board approval was obtained concerning human subjects. Blood samples were collected and DNA was extracted using standard protocols.

2.2. Molecular analysis

Until now, the previously reported variants, predicting an amino acid change, were all located in exons 3, 5, 6, 12, 14, and 15, which are the only *FUS* regions affected by mutations in ALS patients; therefore, we specifically screened these exons in 327 SALS patients. Twelve primer pairs were designed from genomic DNA to amplify, by polymerase chain reaction (PCR), 6 coding exons and relative intron/exon boundaries of the *FUS* gene. The amplicons for exons 3, 5, 6, 12, and 14 were analyzed by denaturing high-performance liquid chromatography (DHPLC) (Wave Transgenomic, Mountain View, CA, USA), while exon 15 was amplified and directly sequenced in each individual using the BigDye terminator v3.1 sequencing chemistry (Applied Biosystems, Foster City, CA, USA) and run on an ABI PRISM 3130 Genetic Analyzer (Applied Biosystem). In each case, forward primer or reverse primer was used for sequencing and variations were confirmed by sequencing an independent polymerase chain reaction product. Nucleotide numbering of *FUS* variations reflects cDNA numbering with +1 corresponding to the A of the ATG translation initiation codon reported in the GenBank reference sequence NM_004960.2, according to the journal guidelines (www.hgvs.org/mutnomen/). The initiation codon is codon 1.