



## A de novo nonsense mutation of the *FUS* gene in an apparently familial amyotrophic lateral sclerosis case

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### ABSTRACT

Mutations in *C9ORF72*, *SOD1*, *TARDBP*, and *FUS* genes account for approximately two-third of familial cases and 5% of sporadic amyotrophic lateral sclerosis (ALS) cases. We present the first case of an ALS patient carrying a de novo nonsense mutation in exon 14 of the *FUS* gene (c.1483c>t; p.R495X) with an apparently familial ALS. This mutation causes a phenotype characterized by a young age at onset, a rapid course (<24 months), and a bulbar onset with early respiratory involvement with a predominant lower motor neuron disease. De novo mutations could account for a sizable number of apparently sporadic ALS patients carrying mutations of ALS-related genes.

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## 1. Introduction

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder of the adult life, characterized by a progressive loss of cortical, bulbar, and spinal motor neurons. Approximately 5–10% of patients have a family history of disease, whereas the remaining 85–90% of cases appear to occur sporadically in the community. To date, mutations of at least 15 genes have been described to be related to familial ALS (FALS), the most common in Caucasian populations being *C9ORF72* (DeJesus-Hernandez, 2011; Renton et al., 2011), *SOD1* (Rosen et al., 1993), *TARDBP* (Sreedharan et al., 2008), and *FUS* (Kwiatkowski et al., 2009; Vance et al., 2009), accounting for ~60% of familial cases and 5% of apparently sporadic patients (Chiò et al., 2012; Kenna et al., 2013; van Blitterswijk et al., 2012). The detection of genetic mutations in apparently sporadic ALS cases has been variously explained as reduced gene penetrance, misdiagnosis of ALS or early death in preceding generations, nonpaternity, or de novo mutations (Chiò et al., 2013).

Here, we present a case of an apparently FALS patient carrying a de novo missense mutation of the *FUS* gene.

## 2. Methods

While performing mutational screening of large series of ALS cases in Piemonte region, Italy, we detected a young onset apparently FALS patient carrying the p.R495X nonsense mutation (c.1483c>t) in exon 14 of *FUS* that causes the truncation of the final 32 amino acids of the protein from the C-terminus of *FUS*, abrogating a putative nuclear localization signal (Bosco et al., 2010). A first cousin of her maternal grandmother also had ALS and was negative for this mutation. Because both her parents were still alive and not affected by ALS, we searched for the mutation in the parents.

### 2.1. Genetic analysis

Genomic DNA was extracted using a Biorobot MDX DSP (Qiagen Inc.). Exons 1–15 of *FUS* were sequenced as previously described (Chiò et al., 2009; Lai et al., 2010; Vance et al., 2009). To exclude that a single-nucleotide polymorphism under the primers could lead to selective amplification of only normal allele, a second polymerase chain reaction (PCR) sequence was performed using a second set of primers with a different binding site. PCR products were sequenced using the Big-Dye Terminator version 3.1 sequencing kit (Applied Biosystem) and run on an ABI Prism

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3100Avant genetic analyzer. Exon 14 was also sequenced in 368 control Italian individuals (Chiò et al., 2009; Lai et al., 2010). Quantitative fluorescence PCR was performed to assess paternity and maternity of the proband, with a multiplex analysis of short tandem repeats located on 5 chromosomes (Devys Resolution kit; Devyser). The electropherograms in all 5 chromosomes confirmed the paternity and the maternity of the proband.

## 2.2. Standard protocol approvals and patient consents

The study was approved by the Ethical committee of our institution. The patient and her family members signed a written informed consent.

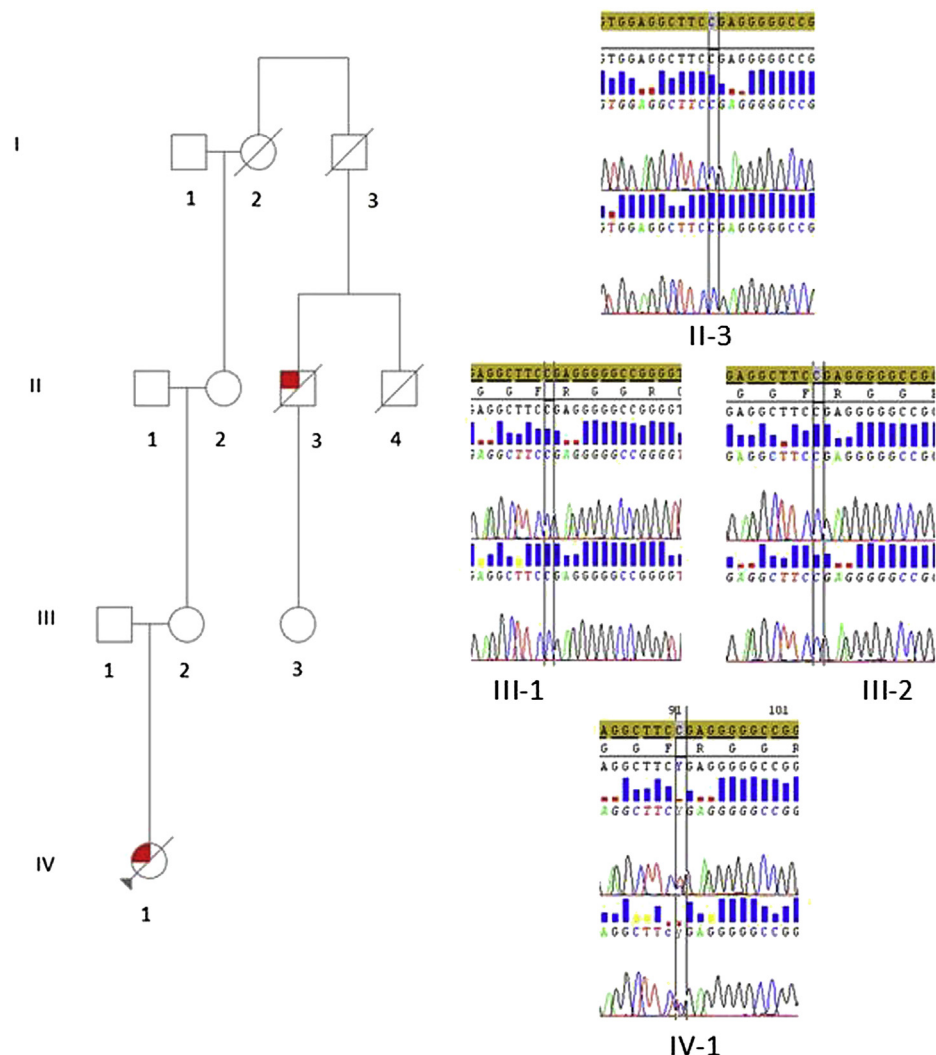
## 3. Case history

The patient's family pedigree is shown in Fig. 1. The patient (III-5) was a 30-year-old woman who developed mild dysphagia and dysarthria at the age of 28 years. One year later, she was referred to our ALS center because of a rapid worsening of bulbar symptoms and the onset of generalized asthenia. At neurologic examination, the tongue was atrophic with fasciculation. Diffuse

fasciculation were seen at upper and lower limbs, but muscle strength was normal. Deep tendon reflexes were hyperactive. Babinski and Hoffman signs were not present. She was cognitively normal. Neurophysiological examination demonstrated chronic and active denervation of tongue (genioglossus) and chronic denervation of proximal muscles of upper limbs, with normal repetitive nerve stimulation test. Cerebrospinal fluid examination was normal. Creatine kinase serum levels were raised. Head magnetic resonance imaging showed a cortical atrophy at the precentral gyri; brain spectroscopy revealed a reduction of the N-acetyl-aspartate to creatine ratio (NAA/Cr) ratio in the motor cortex, more pronounced at left. She was diagnosed as possible ALS. In the following months, she underwent percutaneous radiological gastrostomy because of worsening of dysphagia and weight loss and noninvasive ventilation because of a rapidly evolving respiratory failure. She refused tracheostomy and deceased from respiratory failure 24 months after the onset of symptoms.

She had no mutation in *SMN1*, *SOD1*, *TARDBP*, and *C9ORF72*. She carried a c.1483c>t (p.R495X) truncating mutation of the *FUS* gene.

Her father (III-1), mother (III-2), and maternal grandmother (II-2) are 75, 70, and 91 years, respectively, and are healthy. Her maternal great grandmother (I-1) died at 87 years from heart failure.



**Fig. 1.** Family pedigree with chromatograms of part of exon 14 of *FUS* gene. Square indicates male; circle, female; slash, deceased; solid symbol, affected; and arrow, index patient. Chromatograms of part of exon 14 of *FUS* gene of the proband (IV-1), her parent (III-1 and III-2), and her grandmother's cousin (II-3) are shown.

**Table 1**Summary of *FUS* gene nonsense mutations identified in ALS patients

Mutation	Transmission	Gender	Age at onset (y)	Onset	Duration (mo)	Ancestry	Reference
p.G478LfsX23	Familial	1 M, 1 F	21–26	2 B	23–24	Caucasian	Waibel et al. (2013)
c.1483c>t (p.R495X)	Familial	2 M, 3 F	14–39	1 B, 1 S <sup>a</sup>	9–98	Caucasian	Yan et al. (2010)
c.1483c>t (p.R495X)	Familial	5 M, 3 F	16–59	4 B, 4 S	11–36	Caucasian	Bosco et al. (2010)
c.1483c>t (p.R495X)	Sporadic	F	29	B	NR	Korean	Kwon et al. (2012)
c.1483c>t (p.R495X)	Sporadic	F	19	B	24	Caucasian	van Blitterswijk et al. (2012)
c.1483c>t (p.R495X)	Sporadic	M	22	S	>5	Chinese	Zou et al. (2013)
c.1483c>t (p.R495X)	Familial	1 M, 2 F	31–36	3 B	12–18	Caucasian	Waibel et al. (2013)
c.1483c>t (p.R495X)	De novo	F	28	B	24	Caucasian	Present study
c.1555C>T (p.Q519X)	Familial	NR	NR	NR	NR	NR	Belzil et al. (2011)

Key: ALS, amyotrophic lateral sclerosis; B, bulbar; F, female; M, male; NR, not reported; S, spinal.

<sup>a</sup> The site of onset of 3 cases is not reported.

An 82-year-old first cousin of the proband's maternal grandmother (II-3) developed right foot drop, subsequently progressing to proximal muscles and to the left lower limb. He was referred to our ALS clinic 6 months after the onset of symptoms. Brisk reflex with clonus at lower limbs and bilateral Babinski sign were found. Wasting at the right hand was also observed, with diffuse fasciculations at all limbs. Creatine kinase serum levels were raised. Neurophysiological examination demonstrated chronic and active denervation at upper and lower limbs. He could not undergo magnetic resonance imaging because of claustrophobia. He was diagnosed as probable ALS. One year after the onset of symptoms, he developed respiratory failure and underwent noninvasive ventilation. He died 31 months after the onset of ALS. He was cognitively normal. He had no mutation in *FUS*, *SOD1*, *TARDBP*, and *C9ORF72* genes. His brother (II-4) died at 60 years because of renal failure. His daughter (III-3) is 65 years old and healthy. His father (I-2) died at 54 years from hepatic cirrhosis.

Neither the father, the mother, or the first cousin of the maternal grandmother carries the *FUS* mutation identified in the proband nor any other mutation of genes related to ALS.

#### 4. Discussion

Here we report a proven case of de novo *FUS* mutation presenting as a FALS. The parents and ALS-affected first cousin of her maternal grandmother did not carry the mutation, and highly informative polymorphic markers confirmed paternity and maternity. This truncating mutation has been previously described both in familial (Bosco et al., 2010; Waibel et al., 2013; Yan et al., 2010) and apparently sporadic ALS patients (Kwon et al., 2012; van Blitterswijk et al., 2012; Zou et al., 2013). This particular mutation has not been detected in a large number of neurologically normal controls (Kwiatkowski et al., 2009; Vance et al., 2009; van Blitterswijk et al., 2012).

De novo mutations account for at least a part of patients affected by genetic diseases, as well known for familial adenomatous polyposis (Bisgaard et al., 1994) and Duchenne muscular dystrophy (Grimm et al., 2012). Recently, the detection of de novo mutations in patients with unaffected parents has led to the discovering of novel genes of autism (Neale et al., 2012; O'Roak et al., 2012; Sanders et al., 2012) and schizophrenia (Girard et al., 2011; Xu et al., 2011). In ALS, whole-exome sequencing in young-onset sporadic patients with unaffected relatives (so-called trios) represents a good resource for the discovery of new ALS genes, as recently demonstrated by a proof-of-concept study on 47 trios that identified several possible genetic mutations (Chesi et al., 2013).

The analysis of our pedigree raises some considerations. First, according to the proposed classification of FALS (Byrne et al., 2011), our patient was initially classified as possible FALS

because a first cousin of her grandmother also was diagnosed with ALS. The identification of the de novo mutation in the proband demonstrated that the 2 ALS cases in the same family were indeed phenocopies. The co-occurrence of 2 ALS cases in the same pedigree by chance is not negligible because the risk of developing ALS in the Italian population is 1 out of 300 males and 1 out of 450 females (Chiò et al., 2009).

Second, the proband carried a truncating mutation of *FUS*. Three different truncating *FUS* mutations have been described, 1 in exon 15 in a French case (c.1555C>T p.Q519X) (Belzil et al., 2011) and 2 in exon 14, that is, a large heterozygous deletion of 47 nucleotides (p.G478LfsX23) in a German patient (Waibel et al., 2013) and the single-nucleotide nonsense mutation found in our patients (c.1483c>t, p.R495X), which had been already described in FALS and sporadic ALS patients (Table 1). Truncating mutations of *FUS* are characterized by an age at onset between the second and fourth decades, a rapid course (usually <24 months) and a predominant bulbar phenotype. Interestingly, the p.R495X mutations have been detected both in Caucasian and in Asian patients.

Third, several de novo mutations involving the *FUS* gene have been described, including 2 missense mutations (c.1561C>T [p.R521C], 1 case, and c.1574C>T [p.P525L], 2 cases) (Chiò et al., 2011; Conte et al., 2012; Zou et al., 2013), a heterozygous 2-base pair deletion (c.1509\_1510delAG [p.G504Wfs\*12]) (Zhou et al., 2013), and a heterozygous splice-site mutation in *FUS* intron 13 (IVS13-2A>G) (Dejesus-Hernandez et al., 2010). Currently, only 1 de novo mutation in the *SOD1* gene has been reported (Alexander et al., 2002). The relatively high frequency of de novo mutations in the *FUS* gene could be related to the younger age at onset usually associated with mutations in this gene compared with other ALS-related genes (Chiò et al., 2012; Millecamps et al., 2012; Yan et al., 2010), so that it is more likely that both patient's parents are still available for DNA analysis. Another possibility is that *FUS* gene is particularly susceptible to mutations (i.e., "mutational hotspots"), presumably because of the characteristics of the surrounding sequence (Chiò et al., 2011).

We have identified a proven de novo mutation in the *FUS* gene in a patient with an apparently FALS. This finding, together with previous identifications of other de novo mutations in ALS-related genes, indicates that at least a part of apparently sporadic patients are in fact characterized by de novo mutations that cannot be demonstrated because of the unavailability of the DNA of patients' parents. The relative frequency of proven de novo mutations in the *FUS* is therefore likely to be because of the young age at onset of subjects carrying these mutations.

#### Disclosure statement

The authors have no actual or potential conflicts of interest. Financial disclosure: none declared.

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