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A *de novo* missense mutation of the *FUS* gene in a 'true' sporadic ALS case

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Abstract

Mutations in the Cu/Zn superoxide dismutase (*SOD1*), transactive response (TAR)-DNA binding protein (*TARDBP*) and fused in sarcoma (*FUS*) genes account for approximately one third of familial amyotrophic lateral sclerosis (ALS) cases. Mutations in these genes have been found in 1 to 2% of apparently sporadic cases. We present the first case of an ALS patient carrying a *de novo* missense mutation of the *FUS* gene (c.1561C>T, p.R521C). This report highlights the importance of screening ALS patients, both familial and sporadic, for *FUS* mutations and also suggests that *de novo* mutations is a relevant mechanism underlying sporadic neurodegenerative disease.

1. Introduction

Amyotrophic lateral sclerosis (ALS) is 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 90–95% of cases appear to occur sporadically in the community. To date, mutations in three genes have been described as common causes of familial ALS, namely *SOD1* (OMIM 147450), *TARDBP* (OMIM 605078), and *FUS* (OMIM 137070) (Rosen et al, 1993; Sreedharan et al, 2008; Kwiatkowski et al, 2009; Vance et al, 2009). Mutations of these genes account for about approximately one third of familial ALS cases. In contrast, the genetics of sporadic ALS is poorly understood. Genome-wide association studies of ALS have failed to yield a single replicating locus (Valdmanis et al, 2009), although possible susceptibility loci have been proposed (Van Es et al, 2009). Mutations in *SOD1*, *FUS*, and *TARDBP* have each been found in 1 to 2% of apparently sporadic cases, though it remains unclear if these cases are truly sporadic, or instead represent examples of familial ALS

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where the family history is not immediately obvious due to decreased disease penetrance or misdiagnosis in preceding generations (Sreedharan et al, 2008; Chiò et al, 2008; Corrado et al, 2009; Lai et al, 2010). There are two documented cases of genetically proven truly sporadic ALS patient, one resulting from a *de novo SOD1* mutation (Alexander et al 2002) and one heterozygous splice-site mutation in FUS intron 13 (IVS13-2A>G) (Dejesus-Hernandez et al, 2010).

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

2. Methods

While performing mutational screening of *FUS* in a large series of sporadic and familial ALS cases, we detected a young onset ALS patient carrying the p.R521C missense mutation (c.1561C>T) in exon 15 of *FUS* (Chiò et al, 2008; Lai et al, 2010. Since both his parents were still alive and not affected by ALS, we searched for the mutation in the parents and in the patient's brother.

2.1 Genetic analysis

To ensure against possible laboratory sample mix-up, fresh blood samples were drawn from the patient and his parents. Genomic DNA was extracted using a Biorobot MDX DSP (Qiagen Inc.). Exons 1 to 15 of FUS were sequenced as previously described (Vance et al, 2009; Lai et al, 2010, Chiò et al, 2009). PCR products were sequenced using the Big-Dye Terminator v3.1 sequencing kit (Applied Biosystem) and run on an ABIPrism 3100Avant genetic analyzer. Exon 15 was also sequenced in 368 control Italian individuals (Chiò et al, 2009; Lai et al, 2010). Quantitative fluorescence polymerase chain reaction (QF-PCR) was performed to assess paternity and maternity of the proband, with a multiplex analysis of short tandem repeats (STRs) located on five chromosomes (Devyser 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 his family members signed a written informed consent.

3. Case history

The patient's family pedigree is shown in Figure 1. The patient (III-5) was a 38 year-old man who developed muscle weakness and atrophy at the shoulder girdle at the age of 34 years, spreading rapidly to involve the flexor and extensor neck muscles. Deep tendon reflexes were normal, with the exception of ankle jerk reflexes, which were hyperactive with clonus. Babinski and Hoffman signs were not present. He was cognitively normal. Neurophysiological examination demonstrated chronic and active denervation of all four limbs, and of the axial muscles of neck and trunk. Head and spinal cord MRI and cerebrospinal fluid examination were normal. Creatine kinase serum levels were raised, and muscle biopsy of right quadriceps confirmed the presence of a neurogenic muscle denervation. He was diagnosed as probable laboratory-supported ALS. In the following months, he developed progressive weakness of his lower limbs and both hands. Two years after the onset of the first symptoms, he showed exertional dyspnea, and was treated with non-invasive ventilation. Subsequently, he opted for invasive ventilation via tracheotomy at age 37. Currently, he has flaccid tetraparesis, and communicates with an eye-tracking system. He has no mutation in *SMN1*, *SOD1*, *TARDBP* and *androgen receptor* (*AR*) genes.

He carries the c.1552A>G (p.R521C) missense mutation of the *FUS* gene (previously reported in Lai et al, 2010).

His father (II-3) is 75 years of age and is healthy. His mother (II-4) is 70 years old and was diagnosed with definite remitting-relapsing multiple sclerosis since age 45. Neither the father nor the mother carry the *FUS* mutation identified in the proband, nor any other mutation of genes related to ALS. The proband's sibs (III 1-4) are healthy and their ages range between 45 and 51.

4. Discussion

Here we report a proven case of *de novoFUS* mutation causing sporadic ALS. The parents and siblings did not carry the mutation, and highly informative polymorphic markers confirmed paternity and maternity. Furthermore, this particular mutation has previously been shown to segregate with disease in several ALS families, and has not been detected in a large number of neurologically normal controls (Kwiatkowski et al, 2009; Vance et al, 2009).

Our finding, together with our previous publication of a *de novo* mutation in H80A *SOD1* (Alexander et al, 2002), and a recent publication of a heterozygous splice-site mutation in FUS intron 13 (IVS13-2A>G) (Dejesus-Hernandez et al, 2010) leading to C-terminal truncation of FUS, indicate that *de novo* mutations account for at least a portion of sporadic ALS cases. The rate of *de novo* mutations in the human genome is estimated to be ~2.5×10^{-8} mutations per nucleotide meaning that each individual carries ~175 mutations (Nachman et al, 2000). *De novo* mutations are a common cause of human disease. For example, 25% of familial adenomatous polyposis cases arise from *de novo* mutations in the *APC* gene (Bisgaard et al, 1994). The exact mechanism giving rise this type of mutations is not known, though certain spots in the genome are thought to be particularly susceptible to mutation (i.e. "mutational hotspots"), presumably because of the surrounding sequence. Indeed several lines of evidence now indicate that the c.1561C base pair represents such a hotspot for mutation: First, three different mutations of c.1561C base pair (i.e. C>A, C>G, C>T) have been described, each of which must have arisen by separate mutational events. Second, mutations of this particular codon account for more than 30% of all *FUS*-related ALS cases, making it by far the most common mutated codon in that gene (Kwiatkowski et al, 2009; Vance et al, 2009; Corrado et al, 2009; Lai et al, 2010; Belzil et al, 2009; Ticozzi et al, 2009; Groen et al, 2010; Drepper et al, 2009; Blair et al, 2009). Third, the c.1561C>T mutation must have arisen on at least two separate occasions (the current *de novo* mutation described in this paper and the occurrence of the same mutation in previously described familial ALS case).

In conclusion, we have identified a genetically proven case of a *FUS* mutation in a patient with true sporadic ALS, representing the first *de novo* mutation identified in this gene. Our data, in conjunction with other published studies, suggests that sequencing the FUS gene in both familial and sporadic ALS cases may be informative, particularly where the patient has a presentation characterized by a young age at onset, with rapid clinical progression and flail arm with dropped-head syndrome. This finding also indicates that *de novo* mutations is a relevant mechanism underlying sporadic neurodegenerative disease.

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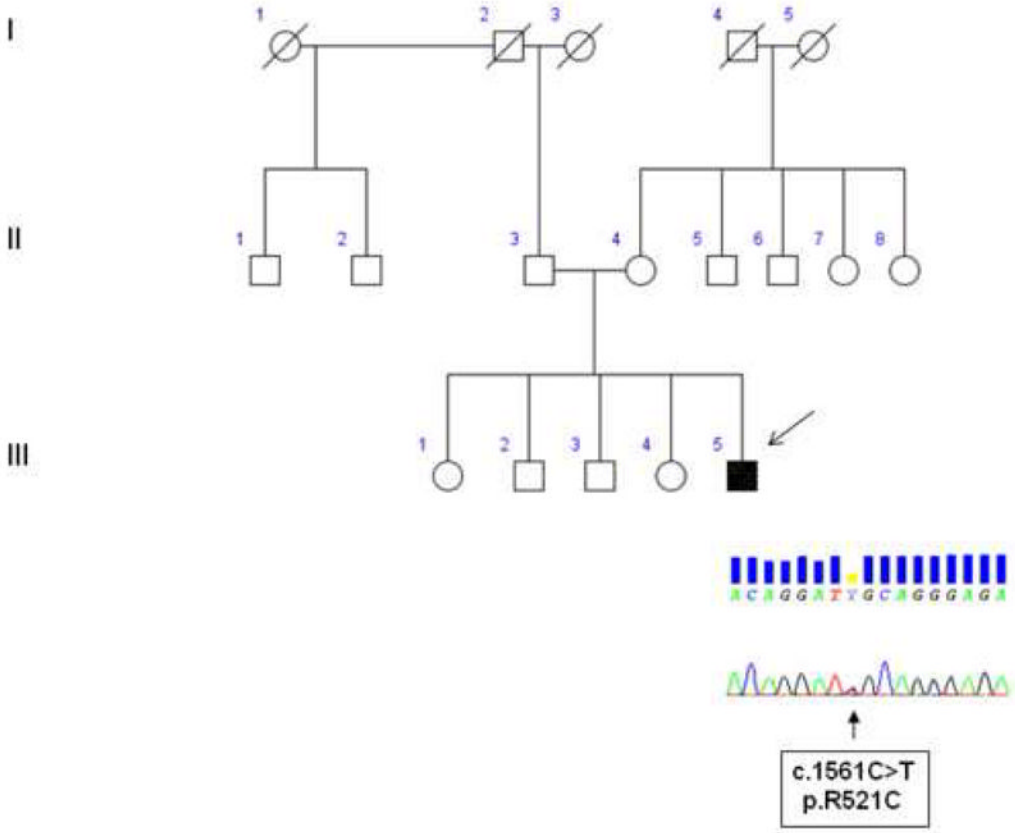

Figure 1.
Family pedigree with chromatograms of part of exon 15 of FUS gene. Square indicates male; circle, female; slash, deceased; solid symbol, affected; and arrow, index patient.