

SHORT COMMUNICATION

Identification of a *FUS* splicing mutation in a large family with amyotrophic lateral sclerosis

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Amyotrophic lateral sclerosis (ALS) is a severe neurodegenerative disease characterized by the degeneration of upper and lower motor neurons. Genetic studies have led, thus far, to the identification of 12 loci and 9 genes for familial ALS (FALS). Although the distribution and impact of superoxide dismutase 1 mutations has been extensively examined for over a decade, the recently identified FALS-associated *FUS* gene has been less studied. Therefore, we set out to screen our collection of FALS cases for *FUS* mutations. All 15 exons of *FUS* were amplified and sequenced in 154 unrelated FALS cases and 475 ethnically matched healthy individuals. One substitution located in the acceptor splice site of intron 14 was identified in all affected members of a large family, causing the skipping of the last 13 amino acids of the protein and the translation of 7 novel amino acids, resulting from the new translation of a part of the 3' untranslated region. Our study identified a new splicing mutation in the highly conserved C-terminal of the *FUS* protein. Thus far most *FUS* mutations are missenses, and our findings, combined with those of others, confirm the importance of the C-terminal portion of the protein, adding additional support for *FUS* mutations having a critical role in ALS.

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease affecting the upper and lower motor neurons. Specifically, neurons of the motor cortex, brain stem and spinal cord are progressively involved, causing gradual spasticity and muscle weakness starting in the limbs in 75% of cases and in the bulbar region in 25% of cases. In all, 90% of patients are believed to be sporadic (SALS), without any family history of the disease, while the other 10% of cases are familial (FALS), primarily segregating in an autosomal dominant manner.¹ SALS and FALS patients are clinically indistinguishable, except for the mean age of onset, which is 56 years for SALS, compared with an average of 46 years for FALS.² The overall prevalence is 4–6/100 000 and incidence is 1–2/100 000,³ which make ALS the most common of motor neuron diseases.

Mutations in the copper superoxide dismutase 1 gene were first shown to be ALS causative over 15 years ago and they account for 15–20% of all FALS cases, representing a proportion of 1–2% of all ALS cases.⁴ In the last 2 years, the identification of ALS causative mutations in the TAR-DNA-binding protein (*TARDBP* encoding TDP-43) gene in both SALS and FALS cases⁵ and in *FUS/TLS* encoding *FUS*,^{6,7} thus far mostly but not exclusively in FALS, opened a new era for the investigation of mechanisms underlying the disease. Whereas superoxide dismutase 1 mutations have been reported

throughout the full length of the protein, *TARDBP* and *FUS* mutations are mostly clustered to specific regions. For *TARDBP*, most of the mutations identified are in the glycine-rich region encoded by exon 6, and for *FUS/TLS*, which also contains a glycine-rich region, mutations are mostly in the extreme C-terminal part of the protein.⁸ The aim of this study was to evaluate the proportion of *FUS* mutations in a portion of our FALS cohort and to see if any mutations identified would be clustered to the same region.

The 15 coding exons of *FUS* were amplified and sequenced in 154 unrelated FALS cases, and only one variant was identified. It is an unreported substitution of an adenine to a cytosine in the acceptor splice site of intron 14 (c.1542-2A>C). The variant was present in six samples of a large ALS family (Figure 1) recruited in France: II:5, III:8, IV:1, IV:3, IV:4, and IV:6. Two of those individuals had an ALS phenotype (II:5, III:8), while the four unaffected mutation carriers are in the fourth generation and are younger than the average age of onset of the first ALS symptoms. By carrying the mutation, they confirm that their three affected fathers likely had the substitution. In addition, three mothers married to affected fathers (II:4, III:1 and III:7) were tested and were negative for the mutation. We prepared cDNA using total RNA from the immortalized lymphoblast cell lines of the two affected individuals with the adenine-to-cytosine

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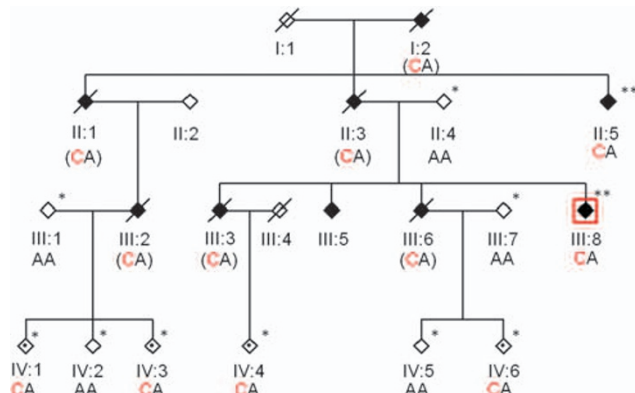


Figure 1 Pedigree of the family with the c.1542-2A>C variant. Black symbols represent affected individuals, while open symbols represent unaffected family members. Symbols with dots represent unaffected individuals carrying the substitution. Slashed symbols indicate deceased individuals. A red square around the individual III:8 symbol indicates the sample sequenced in the primary screening, and asterisks indicate family members for whom exon 15 of the *FUS* gene was sequenced in the secondary screening. Symbols with two asterisks represent individuals for whom cDNA was amplified. Genotypes at position c.1542-2 are indicated in black for the wild-type allele and in red for the mutant allele. Genotypes in parentheses are inferred.

substitution. These cDNAs were PCR amplified, and two products of 425 and 167 bp were observed on agarose gel (Figure 2a), demonstrating that the mutant allele was not degraded by nonsense-mediated mRNA decay. The heterozygous sequence trace was analyzed, as well as the separate sequence of the two different alleles after a gel extraction of the two products (Figure 2b). Sequencing showed that patients with the c.1542-2A>C change expressed a mutated allele missing the 40 bp coding sequence of exon 15 as well as the first 203 bp of the 3' untranslated region. More precisely, the sequence of the mutant mRNA brings together the last 2 bp of amino acid 514 located in exon 14 and the nucleotide at the c.*204 position in the 3' untranslated region, as an AG alternative acceptor site located at position c.*202_*203 is encountered (Figure 2c); translation subsequently ends with a stop codon at position c.*228. Consequently, the mutant protein undergoes a silent change at amino acid 514 (p.R514R), followed by seven new amino acids (SMSRSGR) at the end of the protein. The adenine of the wild-type acceptor site of intron 14 located at base pair 31110219 on chromosome 16 is highly conserved across species and is not listed as a known SNP. The Alternative Splice Site Predictor bioinformatics program attributed a score of 15.533 to the wild-type AG acceptor splice site, and a score of 4.249 for the acceptor splice site with the cytosine substitution. The alternative AG acceptor splice site located in the 3' untranslated region at position c.*202_*203 had a score of 4.620 and is suggested to

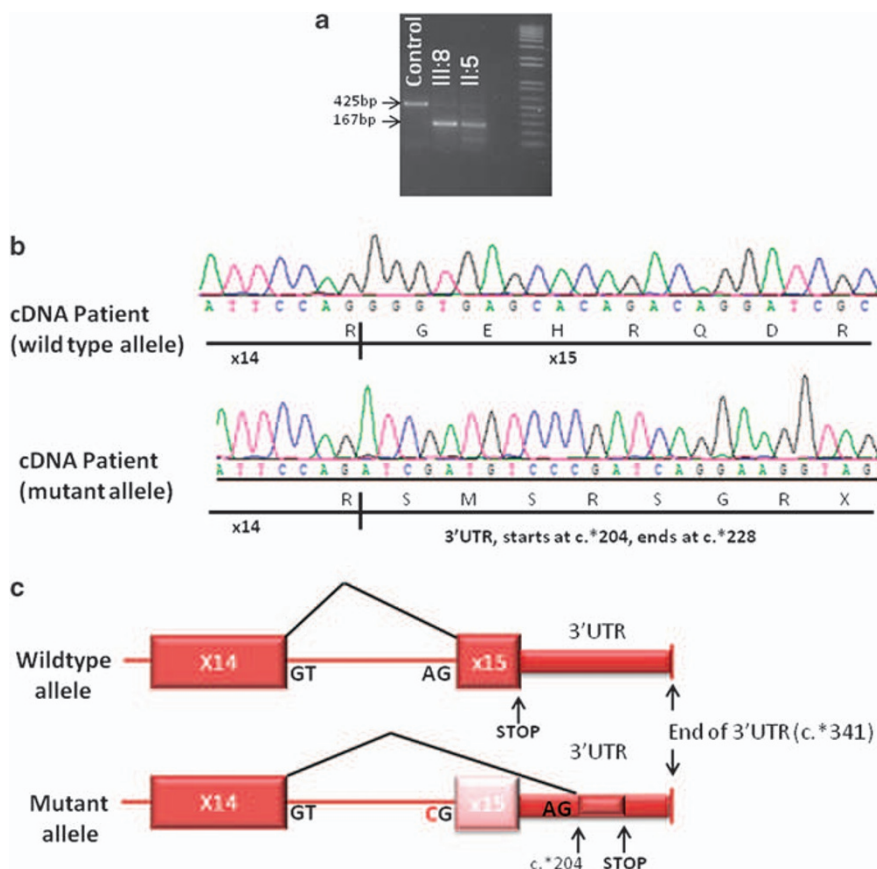


Figure 2 Splicing mutation in *FUS*. (a) Agarose gel electrophoresis of *FUS* cDNA amplified in one unrelated control, and two affected family members (II:5, III:8), showing the wild-type allele (425 bp) and the mutant allele (167 bp) for the fragment amplified using forward primer 5'-GAGGGGG ACCAGGTGGCTCTCAC in exon 14 and reverse primer 5'-TCATTTGGCCTTCTCCCGAACAC in the 3' untranslated region (UTR). (b) cDNA sequence chromatogram of the wild-type and mutant alleles in one patient, confirming the skipping of exon 15 and the first 203 bp of the 3' UTR. (c) Schematic (not to scale) representation of exons 14, 15 and the 3' UTR of the *FUS* gene for the wild-type and mutant alleles. In the mutant allele, a part of the 3' UTR is translated, which adds seven new amino acids to the protein (SMSRSGR).

be responsible for this new isoform. As this *FUS* mutation segregates in all affected individuals of the family as well as in the children of deceased affected members, and was not present in 475 control participants, we believe that it is likely responsible for the ALS phenotype observed in this family.

The substitution is located in the RGG domain enriched in arginine–glycine–glycine motifs, where most other *FUS* mutations were reported. Importantly, truncated proteins were previously observed with the two other major ALS causative genes.^{9–11} But, more interestingly, this is the second splicing mutation reported in *FUS*, as another one was recently shown to lead to the skipping of exon 14 after the substitution of the adenine located in the acceptor splice site of intron 13.¹² Unfortunately, no clinical information could be retrieved for the family presented in our study, so we were unable to compare the disease evolution caused by our splicing mutation in intron 14 with the splicing mutation in intron 13 reported by Dejesus-Hernandez *et al*.¹² The splicing mutation identified here removes the last 13 amino acids that partly encode the C-terminal portion of *FUS*, in which a number of mutations have been associated with ALS. Our finding reinforces the notion that this portion has a key role in the pathology of ALS, most likely by way of affecting the RNA-binding domain it encodes for. In the future, such splicing defect causative mutations may become treatable through drugs or gene therapies aimed at correcting splicing, as explored for a number of disorders.^{13–15}

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