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Negative results

Pathogenicity of exonic indels in *fused in sarcoma* in amyotrophic lateral sclerosis

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Abstract

Insertion and deletion variants (indels) within poly glycine tracts of *fused in sarcoma* (FUS) were initially reported as causative of disease in amyotrophic lateral sclerosis (ALS). Subsequent studies identified similar indels in controls and suggested that these indels may confer susceptibility to ALS. We aimed to elucidate the role of previously published and novel exonic indels in *FUS* in an extensive cohort of 630 ALS patients and 1063 controls. We detected indels in *FUS* exons 5, 6, 12, and 14 with similar frequencies in patients (0.95%) and controls (0.75%). Exonic indels in poly glycine tracts were also observed with similar frequencies. The largest indel (p.Gly138_Tyr143del) was observed in 1 control. In 1 patient, a 3 base pair deletion in exon 14 (p.Gly475del) was identified, however in vitro studies did not reveal abnormal localization of p.Gly475del mutant FUS. These findings suggest that not all exonic indels in *FUS* cause disease.

Keywords: Amyotrophic lateral sclerosis; *Fused in sarcoma*; Mutation; Insertion; Deletion

1. Introduction

Fused in sarcoma (FUS) was recently identified as a novel amyotrophic lateral sclerosis (ALS) disease gene, with mutations clustering toward the C-terminus of the protein encoding the nuclear localization signal (Kwiatkowski et al., 2009; Vance et al., 2009). Insertion/deletion variants (indels) within glycine rich regions of FUS were also reported to be causative of ALS; however subsequent studies identified similar genetic changes in controls. This study aims to investigate the pathogenicity of published and novel indels throughout the entire *FUS* gene in ALS patients and controls.

2. Methods

We studied 148 familial and 482 sporadic ALS patients obtained from Coriell Repository and 1063 controls. To identify exonic *FUS* variants, polymerase chain reaction (PCR) amplification was performed for all 15 *FUS* exons using 1 fluorescently labeled primer, fragment length analysis was performed on an automated ABI3730 DNA analyzer and PCR products with abnormal allele lengths were sequenced. All ALS patients were further sequenced for *FUS* exon 15. The effect of exonic indels on FUS complementary DNA (cDNA) was determined by reverse transcription (RT)-PCR followed by sequencing analysis. For p.Gly475del, a V5-tagged mutant cDNA construct was created and immunocytochemistry of V5-FUS_{WT} (negative control), V5-$FUS_{Gly475del}$, and V5-$FUS_{\Delta 14}$ (positive control) was performed in N2A cells.

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Table 1
Exonic indels detected in ALS cases and controls

Exon	Indel	ALS		Controls	
		Number (n = 630)	Frequency (%)	Number (n = 1063)	Frequency (%)
5	p.Gly173_174del	3	0.48	4	0.38
5	p.Gly138_Tyr143del	0	0	1	0.09
6	p.Gly223ins	1	0.16	0	0
6	p.Gly223del	1	0.16	1	0.09
6	p.Gly223_Gly224del	0	0	1	0.09
12	p.Ser405del	0	0	1	0.09
14	p.Gly475del	1	0.16	0	0

Key: ALS, amyotrophic lateral sclerosis; indels; insertion and deletion variants.

3. Results

Fragment length analysis of all *FUS* exons detected 7 different exonic indels in a total of 6/630 ALS patients (0.95%) and 8/1063 controls (0.75%) (Table 1). Most exonic indels occurred within glycine rich regions encoded by exons 5 and 6. The most common indel was c.508_513delGAGGTG in exon 5, which we identified in 3 ALS patients (0.48%) and 4 controls (0.38%). cDNA analysis confirmed that this variant leads to the deletion of 2 glycine residues (p.Gly173_Gly174del) and excluded the degradation of the mutant transcript by nonsense-mediated decay. An 18 base pair deletion in exon 5 (p.Gly138_Tyr143del), outside of the glycine rich region, and a 3 base pair deletion in exon 12 (p.Ser405del) were detected exclusively in controls. Finally, we identified a 3 base pair deletion in exon 14 (p.Gly475del) in 1 ALS patient (ND07489) which was absent from controls. For this mutation, cDNA analysis also excluded degradation of the mutant transcript. Transient transfection of V5-tagged $FUS_{Gly475del}$ in N2A cells showed no difference in subcellular localization of FUS compared with FUS_{WT} transfected cells (Supplementary Fig. 1).

4. Discussion

We identified exonic indels in patients and controls with comparable frequencies, although a number of patient and control specific variations were observed (Table 1). Indels in polyglycine tracts were also observed with similar frequencies in patients (0.79%) and controls (0.56%). Although the indels observed in our control population could result from reduced disease penetrance, this is unlikely given similar indel frequencies in patients and controls. Despite previous reports of an enrichment of reduced polyglycine tract lengths in ALS patients and a converse increase in the polyglycine tract lengths in controls (Corrado et al., 2010), all 6 variants observed in controls in our study were deletions. The largest indel was an 18 base pair deletion in FUS exon 5 (c.412_429delGGACAGCAGCAAAGCTAT; p.Gly138_Tyr143del) identified in 1 control. Missense mutations and single amino acid deletions in this region of FUS were previously reported in ALS patients. In these patients, the disease was mostly sporadic or segregation of the mutation with disease could not be performed and autopsy confirming FUS pathology was not available. The identification of p.Gly138_Tyr143del in a control in this study therefore questions the pathogenicity of previously published *FUS* mutations outside the FUS C-terminal domain.

One patient was found to have a potentially pathogenic p.Gly475del mutation in exon 14, in a region of the gene where the ALS-causing missense mutations cluster. cDNA transcript analysis did not reveal alternative splicing events or degradation of the mutant transcript by nonsense-mediated decay. Cellular localization studies also revealed no obvious changes in the cellular localization of $FUS_{Gly475del}$. These findings suggest that p.Gly475del is not pathogenic due to FUS mislocalization, however we cannot exclude that this mutation may cause disease through an as yet unknown disease mechanism. Together this large study suggests that not all exonic indels in *FUS* cause disease. This finding has implications for genetic testing in ALS patients and should be taken into consideration when performing *FUS* mutation screening.

Disclosure statement

The authors disclose no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.neurobiolaging.2010.09.029.