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A novel double mutation in FUS gene causing sporadic ALS

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Abstract

It has been shown that mutations in the Fused in Sarcoma gene (FUS) could explain up to 5% of cases with familial amyotrophic lateral sclerosis (ALS). Our mutation analysis of FUS in a Canadian ALS patient of Chinese origin revealed an unusual novel heterozygous double point mutation (R514S/E516V) confirming that exon 15 is a mutation hot-spot. The substitutions are in *cis* position to each other and affect highly conserved codons in the RGG-rich region of the FUS protein. The absence of clinical signs of ALS in the relatives of the affected carrier could indicate that this mutation is incompletely penetrant or *de novo*. The pathologic significance of the R514S/E516V mutation was confirmed by immunohistochemistry. FUS-positive cytoplamsic inclusions were noted in a moderate number in neurons and abundantly in glial cells in the motor cortex and the brainstem. Of interest, a significant number of neuronal and glial FUS-positive inclusions were found in the tegmentum of the brainstem. Importantly, some neurons with inclusions showed retention of the normal nuclear FUS immunostaining.

Keywords: Amyotrophic lateral sclerosis; FUS; Gene; Mutation

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease affecting motor neurons of the brain, brainstem and spinal cord. Several loci have been implicated in the familial form of ALS, which represents ~ 5% of all cases (Strong, 2010). It has been shown that mutations in the Fused in Sarcoma gene (FUS) could explain ~ 5% of familial ALS cases (Kwiatkowski et al., 2009; Vance et al., 2009). Most of the families demonstrate an autosomal dominant mode of inheritance; although the disease associated with FUS may also present as an incompletely penetrant, recessive, or sporadic disorder (Corrado et al., 2009). For example, a homozygous H517Q mutation was identified in an African ALS family (Kwiatkowski et al., 2009). To date, 24 different, mainly missense, FUS mutations have been identified, with a hotspot in exon 15 (alsod.iop.kcl.ac.uk/Als/misc/dataDownload.aspx). However, some of the reported FUS mutations have poor support for their pathogenic nature (i.e. absence of segregation studies or autopsy results).

FUS is a component of the complex responsible for regulating sensors of DNA damage and the normal function of the FUS protein is important for mRNA/microRNA metabolism and genomic integrity (Wang et al., 2008). ALS caused by FUS is associated with cytoplasmic retention and accumulation of mutant FUS protein, while wild-type FUS is exclusively localized to the nucleus (Kwiatkowski et al., 2009; Vance et al., 2009). However, neuropathological studies of affected patients are scarce. There are only two reports of patients with FUS mutations affecting the same codon (R521G, R521C, R521H) (Kwiatkowski et al., 2009; Vance et al., 2009). Both studies showed severe lower motor neuron loss in the spinal cord and to a lesser degree

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in the brainstem and motor cortex. Antibody to FUS labeled large cytoplasmic inclusions in the motor neurons, and ubiquitin showed diffuse positivity in nuclei, suggesting misfolding of a nuclear protein.

In this report, we describe a novel heterozygous double point mutation in the FUS gene detected in a sporadic Canadian ALS patient; and present detailed neuropathological and clinical findings in this case.

1. Methods

1.1. Genetic analyses

Patient #8176 was recruited at the ALS Clinic, Sunnybrook Health Sciences Centre (Toronto, Canada) and was diagnosed with ALS in accordance with the revised El Escorial criteria (Brooks et al., 2000). Informed consent was obtained from the participant in accordance with the ethical review board. Case #8176 was prioritized for mutation analysis of the FUS gene based on the neuropathology report (an absence of TDP-43 positive inclusions). DNA and RNA were isolated from frozen brain tissue using a QIAGEN kit. The entire open reading frame of FUS (including exon/intron boundaries) was analyzed in patient #8176 as described previously (Vance et al., 2009). The PCR product corresponding to exon 15, harboring the FUS mutation, was amplified with primers # 4543 (5′-ggttaggtaggaggggcaga) and # 4544 (5′-cttgggtgatcaggaattgg); and cloned directly into PCR 2.1-TOPO vector using the TOPO-TA cloning kit (K4500-01, Invitrogen, CA). Ten clones were sequenced. Mutations were searched by direct inspection of the fluorescent chromatographs using the SeqScape software (version 1.0) (Applied Biosystems, Foster City, CA). The cDNA was generated from total RNA (5 μg for 20-μL reaction) of cortex using the StrataScript kit (Stratagene, CA). Rt-PCR primers were designed for exon 11 (5′-tccggaaatcctatcaaggtc) and 3′-UTR (5′-caggaattggaaggttacaaaa). The PCR conditions were 94 °C for 5 minutes, followed by 35 cycles of 94 °C for 30 seconds, 58 °C for 30 seconds, 72 °C for 30 seconds, and 7 minutes at 72 °C.

1.2. Immunohistochemistry

The autopsy was performed 1 hour after death. The post mortem brain specimen was immediately submerged in neutral formalin and sectioned after 2 weeks. Tissue blocks were embedded in paraffin. Immunohistochemistry was performed on 5-μm thick paraffin sections using the Ventana BenchMark XT automated system. The primary antibody employed recognized FUS (Sigma-Aldrich anti-FUS; 1 : 100 with initial overnight incubation at room temperature, following microwave antigen retrieval). The reaction was developed with aminoethylcabizole (AEC). In addition, immunostains using commercial antibodies for tau (Dako, A0024), ubiquitin (Vector Laboratories, ZPU576), p62 (BD Transduction Laboratories), neurofilament-H (Sternberger Monoclonals, SMI31) and TDP-43 (ProteinTech Group, Inc.) were performed.

2. Results

2.1. Clinical findings

The proband # 8176 of Chinese origin (Hong Kong) was diagnosed with sporadic ALS at age 45 (Fig. 1A). The proband had predominantly lower motor neuron signs with only brisk reflexes in the lower limbs. Mental status remained normal with no evidence of Frontotemporal dementia (FTD). The ALS symptoms began at age 44 with progressive arm (followed by leg) weakness. Bulbar symptoms ensued and the patient died of respiratory failure as a complication of ALS at age 47. Samples from relatives were not available; however family members revealed that there is no history of neurological illnesses in the family. The father of the proband died in his 60s from a stroke, and the mother died in her 70s from colon cancer.

2.2. Genetic findings

In the proband we detected a novel heterozygous double point mutation in exon 15 of FUS: Arg514Ser and Glu516Val (R514S/E516V) at cDNA position c. [1542G > T; 1,547 A > T] and genomic Position g. [16268G > T; 16,273 A > T] (relative to nt1; NG_012889.1) (Fig. 1B). Both substitutions affect highly conserved codons in the RGG-rich region of the protein (Fig. 1C). To determine whether the two mutant nucleotides were on the same or different allele, exon 15 was amplified from the patient's genomic DNA and cloned into PCR 2.1-TOPO vector. The sequencing of 10 clones demonstrated that the substitutions are in *cis* position (Fig. 1B). Since the mutation is located at the exon-intron boundary, we assessed whether it influences alternative splicing of FUS. However, the Rt-PCR generated only a full-length product with an equivalent expression of the wild-type and mutant allele based on the sequencing diagram (data not shown).

2.3. Neuropathology

The diagnosis of ALS in patient #8176 was confirmed by autopsy. The brain weight was not recorded, however, except for some palor of the substantia nigra, there were no gross pathological changes and cortical atrophy. Spinal cord was not available, though a portion of the upper cervical cord at the medullary junction including the medial Motor nucleus (C1), and the accessory nucleus of XI as well as one block from the upper cervical cord were examined. Microscopic evaluation of the brain showed mainly lower motor neuron loss; and, to a lesser degree, cortical neuronal loss. Examination of the upper cervical and brainstem was very informative as the motor nuclei of numerous cranial nerves (7th and 12th mainly) showed extensive motor neuron loss and gliosis. There was no evidence of Bunina bodies but few spheroids were seen in areas showing neuronal loss.