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Identification of a pathogenic intronic KIF5A mutation in an ALS-FTD kindred

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Not every gene nominated as a cause of human disease stands the test of time. As additional data become available, the evidence supporting the pathogenicity of a particular variant within a gene can be enhanced or diminished.[1] The amyotrophic lateral sclerosis (ALS) field, as much as any other, has been hesitant to address these controversies, leading to uncertainty among the research community.

In 2013, we published a study reporting that mutations in the *MATR3* gene were a cause of familial ALS.[2] That study was based, in part, on a pedigree in which we described p.Phe115Cys as the pathogenic variant based on exome sequence data obtained from 4 affected individuals. An additional member of this kindred (known as USALS#3, member III:10) was recently diagnosed as having ALS. Clinical genetic testing of this individual showed that they did not carry the *MATR3* mutation. Although this individual may be a phenocopy, a more parsimonious explanation was that a different mutation was responsible.

To address this issue, we performed whole-genome sequencing of this amyotrophic lateral sclerosis-frontotemporal dementia (ALS-FTD) family on an Illumina NovaSeq6000 sequencer to identify their true causative mutation (figure 1 and table). The participating institutions' institutional review boards approved the study (clinicaltrials.gov/ct2/show/NCT02014246), and informed consent was obtained from all subjects or their surrogate decision makers, according to the Declaration of Helsinki.

Analysis of the sequence data identified 218 variants that were rare and shared across the 5 affected individuals. One variant was located within intron 26 of the *KIF5A* gene, 14 base pairs from the start of exon 27 (chr12:57582588G>T, build hg38). Exon 27 within *KIF5A* is a known mutational hotspot underlying familial ALS.[3] Exon trap experiments on cDNA obtained from our proband confirmed that this intronic mutation led to aberrant splicing of the *KIF5A* mRNA transcript. The altered transcript sequence was identical to that produced by other mutations in this intronic region because of skipping of exon 27 (figure 2).[3] This family represents the most extensive kindred ascribed to a *KIF5A* mutation to date, and the affected individuals display both the short survival typically associated with ALS and the prolonged survival previously observed among some patients carrying mutations in this gene (table).[3]

Discussion

Our previous publication erroneously nominated the p.Phe115Cys variant in *MATR3* as the cause of disease within the USALS#3 kindred based on exome sequencing of affected

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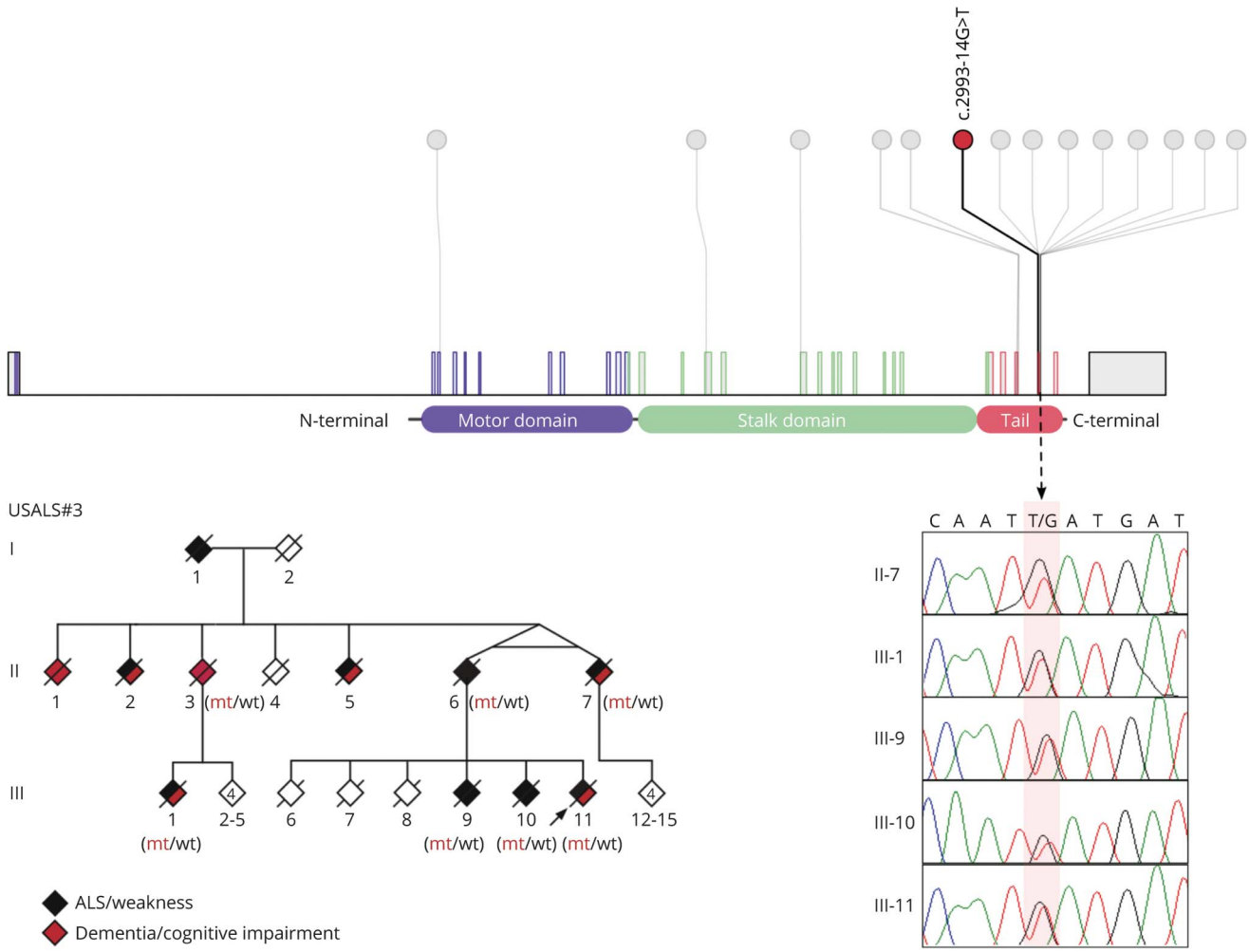
Figure 1 Intronic mutation in *KIF5A* identified in an ALS-FTD kindred



The upper panel depicts a lollipop plot of *KIF5A* depicting the location of the intronic mutation identified in the USALS#3 kindred (red) and known mutations (gray). The chromatograms show mutant alleles from the indicated individuals (mutation is highlighted in pale pink). The pedigree shows the ALS-FTD kindred carrying the *KIF5A* intronic mutation (updated from ref 5). Individuals II:6, II:7, III:1, III:9, III:10, III:11 were diagnosed with ALS by neurologists. In addition, III:7 had executive dysfunction consistent with behavioral FTD based on formal neuropsychological testing, whereas III:1 and III:11 were observed to have mild cognitive impairment. The phenomenology of other individuals was reconstructed based on history from family members. Mt = mutant alleles; wt = wild-type alleles. Genotypes of presumed obligate carriers are in brackets. Arrow denotes proband. ALS = amyotrophic lateral sclerosis.

individuals.[2] Here, we correct the record to show that an intronic mutation within the known mutational hotspot of *KIF5A* is the actual cause of disease within this ALS-FTD family. The availability of DNA from an additional affected member within this pedigree was vital to identifying the causative mutation correctly. However, advancements within the genomics field and our understanding of ALS genetics were similarly crucial to resolving this family. In particular, our preexisting knowledge concerning *KIF5A* allowed us to single out that variant from the list of shared variants.[3]

Seven members of the kindred (figure 1 and table) developed executive dysfunction during their ALS illness, demonstrating a link between mutations in *KIF5A* and FTD. Mutations in *KIF5A* have now been linked to a wide variety of neurodegenerative conditions, including hereditary spastic paraparesis,[4] Charcot-Marie-Tooth disease,[4] ALS,[3] and, more recently, the *KIF5A* protein has been implicated as having a role in Alzheimer disease.[5] These discoveries show the importance of the kinesin protein complex and axonal transport within neurons. Aside from being a striking example of pleiotropy within a single gene, it also suggests that, cumulatively, mutations within *KIF5A* may be a significant cause of neurologic disease.

Despite our recent findings, we maintain that mutations in *MATR3* are a cause of familial ALS. The p.Ser85Cys variant in *MATR3* remains the cause of neurologic disease within the other pedigree (USALS#4), segregating with disease among 11 affected members across multiple generations.[2] Although there is clear muscle involvement within this family, there is clinical evidence of upper and lower motor neuron involvement.[2] MATR3 protein is present within neuronal cytoplasmic inclusions of more than half of sporadic ALS patients,[6] and pathogenic MATR3 mutants

Table Clinical features of affected individuals in the USALS#3 kindred

	Clinical features			
Individual	**Diagnosis**	**Site of onset**	**Age at onset**	**Survival**
I:1	ALS	Lower limb	Died at age 47	Prolonged course
II:1	Dementia	Cognition	NA	NA
II:2	ALS	NA	NA	NA
II:3	Dementia	Cognition	Died at age 84	NA
II:5	ALS-FTD	Cognition	NA	NA
II:6	ALS-FTD	Upper limb	70	5 y
II:7	ALS-FTD	Upper limb	57	26 y
III:1	ALS-FTD	Lower limb	63	5 y
III:9	ALS	Upper limb	52	8 y
III:10	ALS	Limb	63	1.5 y
III:11	ALS-FTD	Bulbar	50	6 y

Abbreviation: ALS = amyotrophic lateral sclerosis.

display neurotoxicity that is mitigated by cytoplasmic redistribution.[7] Motor neuron loss and gliosis have been observed within the spinal cords of transgenic mice overexpressing mutant p.Ser85Cys MATR3.[8] Finally, there are reports of other *MATR3* mutations in patients diagnosed with ALS.[9]

In conclusion, we identified an intronic mutation in *KIF5A* that segregated with disease in a large, multigenerational pedigree. Our efforts highlight the rapid advancements that are taking place in our understanding of the genetic architecture of ALS and link mutations in *KIF5A* to cognitive impairment/frontotemporal dementia.

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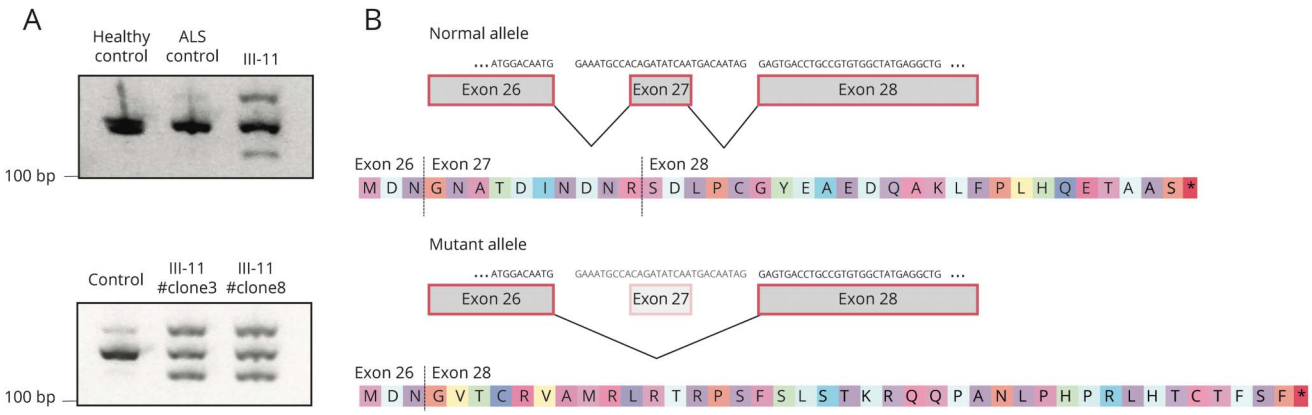
Figure 2 The intronic mutation alters the splicing of *KIF5A*



(A) RNA derived from blood (upper panel) from a healthy individual, an ALS patient not carrying the mutation, and individual III-11 carrying the *KIF5A* intronic mutation. RT-PCR was performed using RNA and previously described primers to amplify a wild-type (155 bp) splice form extending from exon 26 to exon 28.[3] An extra band was observed at 127 base pairs indicating aberrant splicing in individual III-11 that was not present in the healthy and disease control subjects. RNA obtained from an IPS cell line (lower panel) derived from fibroblasts of individual III-11 and a control IPS cell line (A18945) showed the same pattern. (B) Sanger sequence analysis of the 127bp transcript/band observed in the patient confirmed the skipping of exon 27 of KIF5A yielding an out of frame and extended disrupted C-terminal peptide sequence.[3] ALS = amyotrophic lateral sclerosis.

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Disclosure

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Bryan J. Traynor, MD, PhD	National Institute on Aging, Bethesda, MD	Design and conceptualized study, analyzed the data, and drafted the manuscript for intellectual content

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