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. 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. 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. 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). 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).

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...
individuals. 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.

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, Charcot-Marie-Tooth disease, and, more recently, the KIF5A protein has been implicated as having a role in Alzheimer disease. 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. Although there is clear muscle involvement within this family, there is clinical evidence of upper and lower motor neuron involvement. MATR3 protein is present within neuronal cytoplasmic inclusions of more than half of sporadic ALS patients, and pathogenic MATR3 mutants...
display neurotoxicity that is mitigated by cytoplasmic redistribution.\textsuperscript{7} Motor neuron loss and gliosis have been observed within the spinal cords of transgenic mice overexpressing mutant p.Ser85Cys MATR3.\textsuperscript{8} Finally, there are reports of other MATR3 mutations in patients diagnosed with ALS.\textsuperscript{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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### Table Clinical features of affected individuals in the USALS\#3 kindred

| 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.

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.\textsuperscript{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.\textsuperscript{3} ALS = amyotrophic lateral sclerosis.
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The institutional review boards of participating institutions approved the study (NIH, 03-AG-N329), and informed consent was obtained from all subjects or their surrogate decision-makers, according to the Declaration of Helsinki.

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