Functional characterization of *de novo* variants of *SPTBN2*

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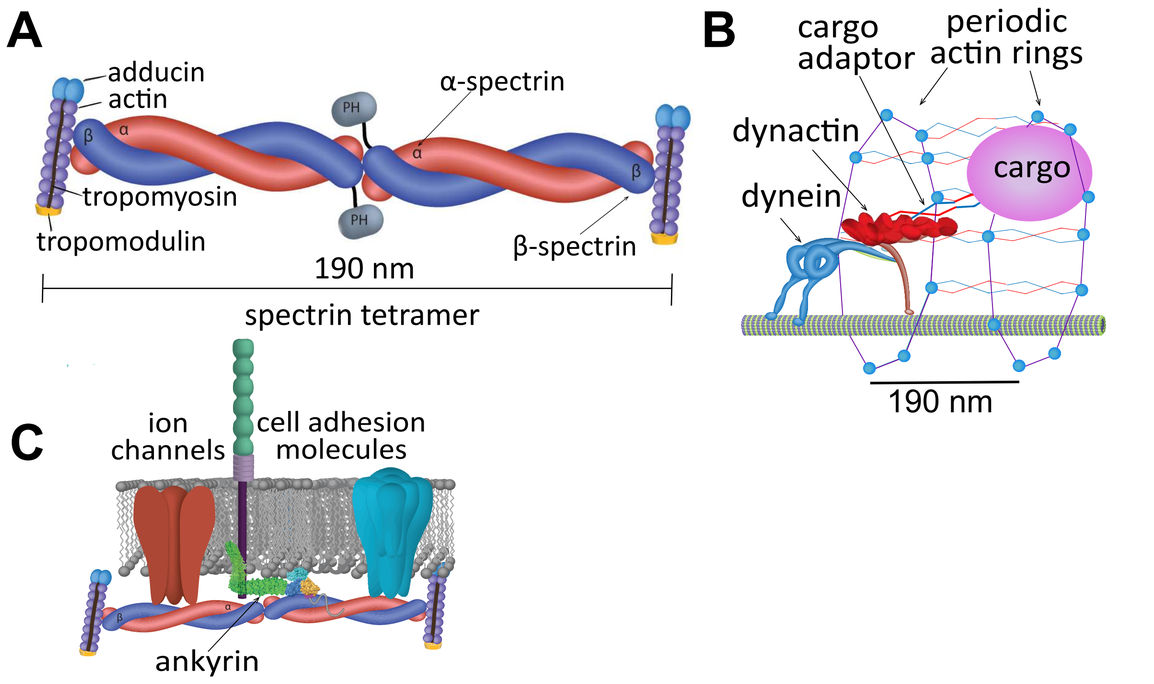
Abstract

βIII spectrin is a member of the spectrin meshwork in neurons formed by heterodimeric units of αII-spectrin and each of four β-spectrins (I-IV), which then form tetramers that crosslink F-actin to form periodic spectrin-actin arrays along axons and dendrites. β-spectrins also bind their molecular partners ankyrins to, together, stabilize ion channels, cell adhesion molecules, and membrane receptors. βIII-spectrin, in addition to the other spectrins, is involved in various cellular processes, including intracellular transport, dendritic development and signaling transactivation.[1,3,4] Unsurprisingly, pathogenic variants in β-spectrins underlie neurodevelopmental and neurogenerative disorders. Specifically, variants in *SPTBN2*, the gene encoding β III-spectrin, have been found in individuals affected with cerebellar ataxias, global developmental delays and cognitive impairments. However, the pathogenic mechanisms of *SPTBN2* variants are not fully understood.

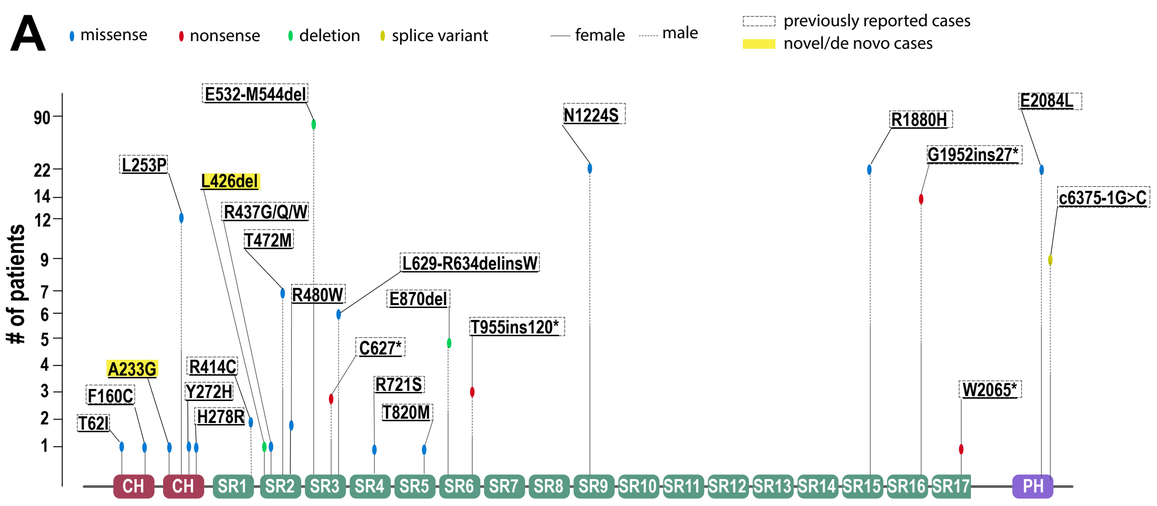
Here, we generated expression plasmids bearing a subset of disease-linked βIII-spectrin variants. We compared the effects of GFP-tagged willd-type (WT) βIII-spectrin and of mutant p.L426del, p.L253P, and p.R480W GFP-β III-spectrin on protein expression levels and binding interactions through western blots and immunoprecipitation assays. Our preliminary findings indicate that expression of p.L426del decreased protein expression levels relative to WT. In constrast, none of the variants analyzed caused significant disruption of βIII-spectrin binding to αII-spectrin. Future work will evaluate the effect of the variants on binding to other critical cytoskeletal partners, on the distribution of βIII-spectrin, and on neuronal morphology.

Spectrins provide membrane stability and micron-scale organizaton of membrane domains



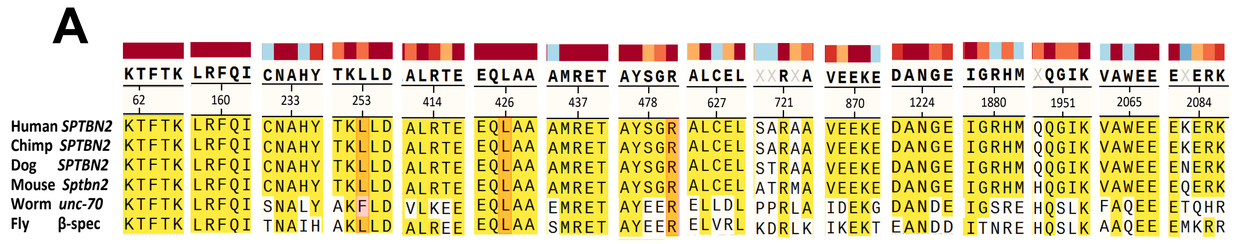
(A) Spectrin tetramers crosslink short actin filaments capped by adducin and tropomodulin, stablishing their periodic 190 nm distribution in axons Neuronal spectrins likely provide mechanical support to membranes, promote motor-protein based transport **(B),** and stabilize membrane proteins directly or through ankyrins **(C)**.

SPTBN2 variants associated with neurodevelopmental and movement disorders



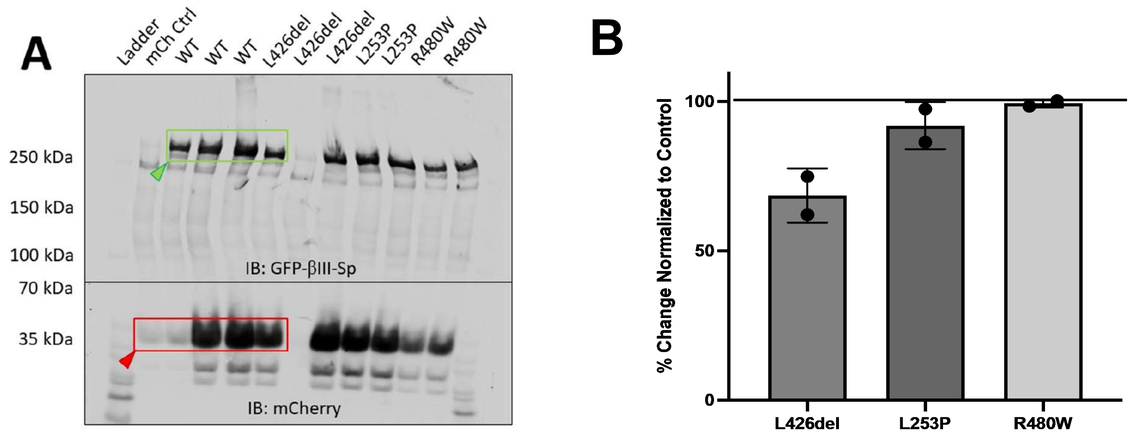
(A) Schematic representation of functional domains of βIII-spectrin. CH1=calponin homology domain 1 (teal), CH2=calponin homology domain 2 (red), SR=spectrin repeat (green), and PH=pleckstrin homology domain (purple). The locations of disease-linked *SPTBN2* variants are indicated. Variants evaluated in this study are shown in yellow.

SPTBN2 variants are evolutionarily conserved



(A) Amino acid alignment of βIII-spectrin mutations across six species. The position of the variants evaluated is indicated by orange boxes.

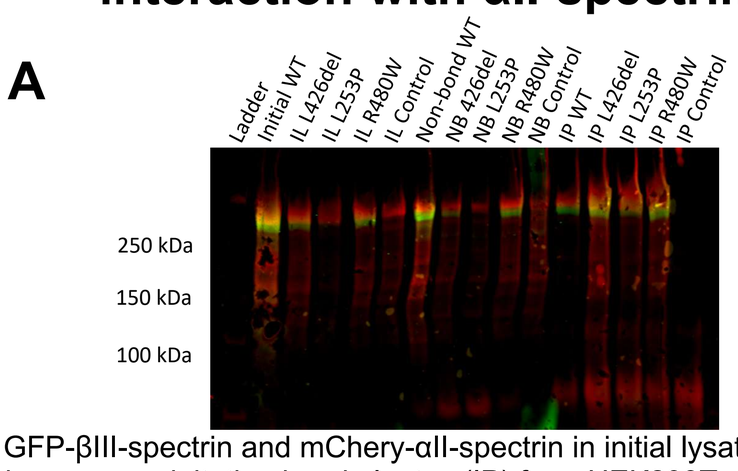
The L426del variant leads to reduced protein expression in HEK293T cells



(A) Western Blot image of GFP-βIII-spectrin and mCherry expression in protein lysates from HEK293T cells expressing WT, L426del, L253P, and R480W GFP-βIII-spectrin plasmids

(B) Graph depicting GFP-βIII-spectrin expression levels of mutants relative to WT by percent change

SPTBN2 variants do not affect interaction with αII-spectrin



(A) Levels of GFP-βIII-spectrin and mChery-αII-spectrin in initial lysates (IL), non-bound(NB) fractions and immunoprecipitation bead eluates (IP) from HEK293T cells expressing WT, L426del, L253P, and R480W GFP-βIII-spectrin together with mChery-αII-spectrin. Isotype rabbit IgG was used as control in the IP experiments

Conclusions

1. Disease-linked *SPTBN2* variants are evolutionarily conserved
2. Preliminary data suggest that the p.L426del variant reduced protein expression in HEK293T cells
3. Variants do not affect interaction with αII-spectrin

Future Directions

1. IP assays to evaluate binding to other known partners like Ankyrin B and dynactin
2. Assesment of localization of mutant proteins in HEK293T cells and in primary cortical and cerebellar neurons and of their effects on neuronal morphology and survival
3. Behavioral studies in knockout mice to explore phenotypic manifestations

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