# Cellular characterization of *de novo* variants of *SPTBN2*

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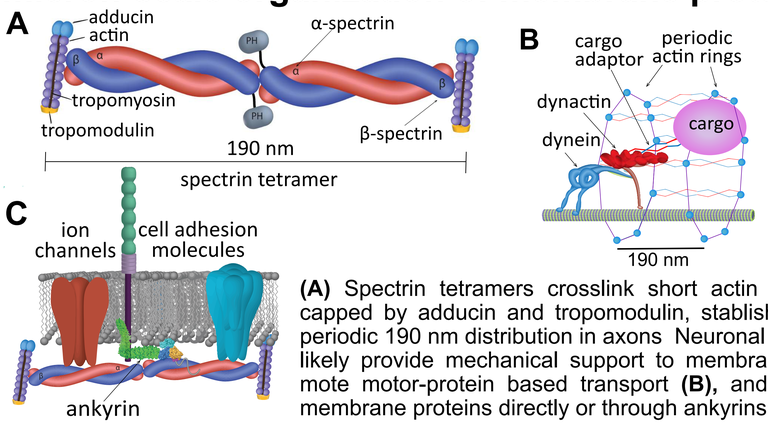
## Abstract

β-III 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. β-III spectrin, in addition to the other spectrins, is involved in various cellular processes, including axonal growth and maintenance, intracellular transport, and signaling transactivation. Unsurprisingly, mutations in β-III spectrin underlie neurodevelopmental, neurogenerative, and psychiatric disorders. Specifically, mutations in SPTBN2, the gene for β-III spectrin, have been found to be associated with infantile-onset spinocerebellar ataxias, global developmental delays and cognitive impairment. However, the pathogenic mechanisms are not fully understood.

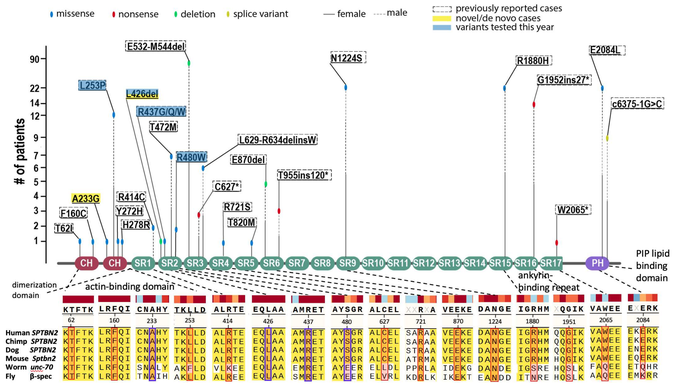
In order to better understand how β-III spectrin mutations manifest cellularly, we characterized various mutations in β-III spectrin including L426del, L253P, R437Q/G/W, and R480W through transfection of mutagenized GFP-β-III spectrin in HEK293T cells and evaluation of protein expression levels and binding interactions through Western blots and immunoprecipitation assays. Our preliminary findings indicate that two of the mutations show decreased protein expression levels relative to WT. Further studies are necessary to substantiate this data. All three mutations also seemed to demonstrate no significant disruption to β-III spectrin-αII-spectrin binding. However, further experimental data is needed to confirm or deny this observation.

## Spectrins provide membrane mechanostability and micron-scale organization of membrane proteins




**(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)**.

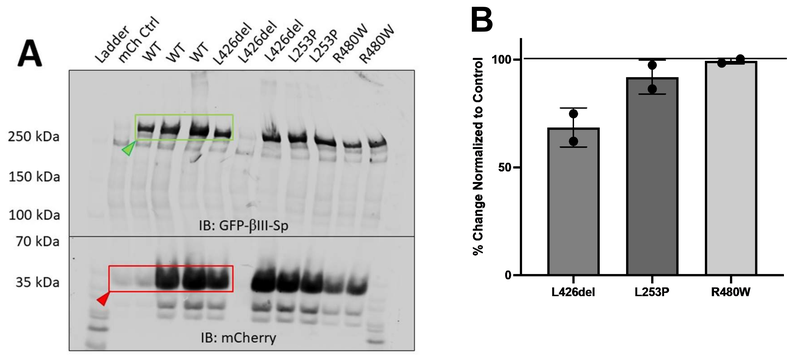
## Schematic of *SPTBN2* mutations




L426del is a *de novo* in-frame single amino acid deletion (p.Leu426del) in βIII-spectrin, identified in an 11-year old girl who presents with cerebellar ataxia, cerebellar hypoplasia, and learning disabilities (Figure A, "Duke" mutation). I will evaluate the effects of the Duke mutation and the SPTBN2 variant p.R480W, previously identified in patient with similar symptoms. The localization of these mutations within spectrin repeat 2 (SR2, Figure A) of βIII-spectrin, which is required for its dimerization with αII-spectrin, led us to hypothesize that these variants impair the formation of the spectrin tetramer. For comparison, I will also evaluate the p.L253P variant in the actin-binding CH domain identified in another SCA5 patient (Figure A).

## Results

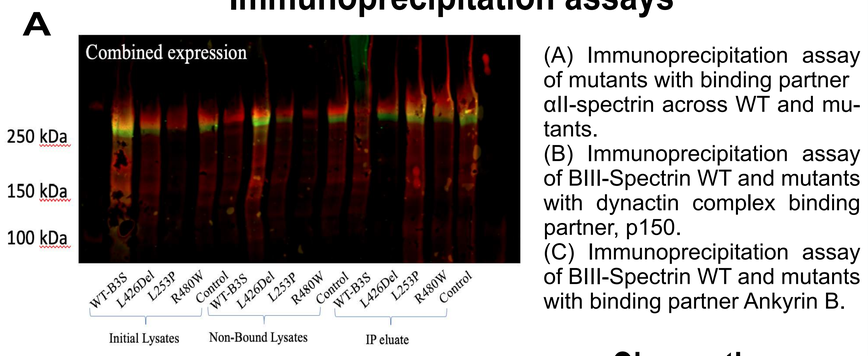
### GFP-βIII-spectrin expression of mutations




**(A)** Western Blot image of GFP-βIII-spectrin protein lysates cultured from HEK293T cells of WT, L426del, L253P, and R480W. **(B)** Graph depicting GFP-βIII-spectrin expression levels of mutants relative to WT by percent change.

The first replicate of WT-GFP-BIIIS showed anomalously high results compared to the other two WT replicates, indicating that its result may not be reproducible; it was left out of protein quantification. Similarly, the second replicate of L426del did not show significant protein expression, potenially indicating a problem with the efficacy of methodology.

## Immunoprecipitation assays




(A) Immunoprecipitation assay of mutants with binding partner αII-spectrin across WT and mutants.

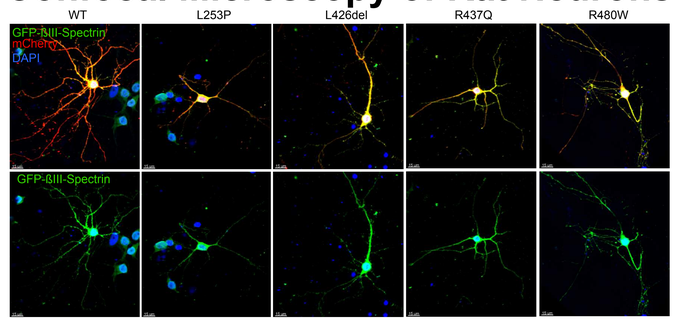
(B) Immunoprecipitation assay of BIII-Spectrin WT and mutants with dynactin complex binding partner, p150.

(C) Immunoprecipitation assay of BIII-Spectrin WT and mutants with binding partner Ankyrin B.

### Observations

The IP assay of GFP-βIII-spectrin and mCh-αII-spectrin indicates that overall, the mutants investigated do not seem to introduce any significant differences in binding interaction of β III-spectrin with its partner α II-spectrin as visualized by the red, green and combined yellow bands in both the WT and all the mutants. The IP assay of GFP-βIII-spectrin and dynactin complex p150 does show evidence of technical error, as the WT sample of IP eluate shows no band at the 250 kDa level which would mean that the binding of p150 and BIII-spectrin is not present, but studies do show that there is definite binding. Further replication is required.

## Confocal Microscopy of Rat Neurons

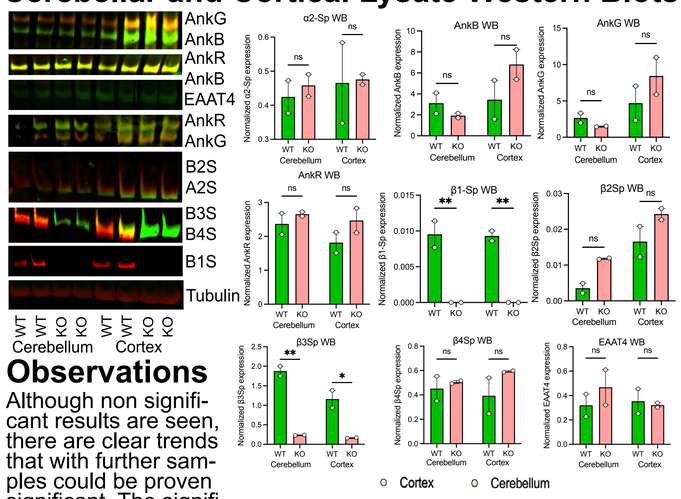



(A) These are confocal microscopy images of the WT BIII-Spectrin and 4 of the mutations we have studied. We have stained for GFP, RFP (similar to mCherry), MAP2- which is a dendritic specifc marker- and DAPI, which stains the DNA of the cell body.

### Observations

For these rat day in vitro 18 neurons, there is preliminary data suggesting that disease-linked variants are affecting dendritic development. Upon our preliminary analysis of these images, we can also see that the branching of the dendrites of the mutations is vastly different and diminished when compared to the WT control. Further images will be obtained and software will be utilized to quantify axonal length and dendritic branching.

## Cerebellar and Cortical Lysate Western Blots




### Observations

Although non significant results are seen, there are clear trends that with further samples could be proven significant. The significant result from Beta III-spectrin is indicative of a loss of expression within the KO mice. Additionally, loss of Beta III-Spectrin reduces Beta I-Spectrin levels in the cortex and cerebellum.

## Future Directions

1. Further replication of the immunoprecipitation assay will be conducted.
2. Further microscopy will be done for the remaining mutations (R437W/G) in rat neurons and HEK293T cells.
3. Previously fixed HEK cells will be imaged in the near future.
4. Behavioral studies with transgenic mice for all βIII- spectrin mutations created will be conducted.

## Acknowledgements

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