Effects of *Sptbn1* Variants on Corpus Callosum Morphology

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Background

- Spectrins are cytoskeletal proteins that help maintain membrane organization and integrity and aid in signaling events.
- βII-spectrin, encoded by the *SPTBN1* gene, is the most abundant β-spectrin in the nervous system.
- De novo variants in *SPTBN1* cause a novel neurodevelopmental syndrome (*SPTBN1* syndrome) with diverse and overlapping clinical phenotypes
- Brain imaging of patients show disrupted brain morphology, including a thinning of the corpus callosum (CC), a structure important for the inter-hemispheric neuronal signaling integration and circuitry.
- We generated two novel mouse models of the two most clinically severe variants (Sptbn1-KI^{T268S} and Sptbn1-KI^{L250R}) to characterize the pathogenesis of mutant βII-spectrin

SPTBN1 Variants and Functional Domains of βII-spectrin

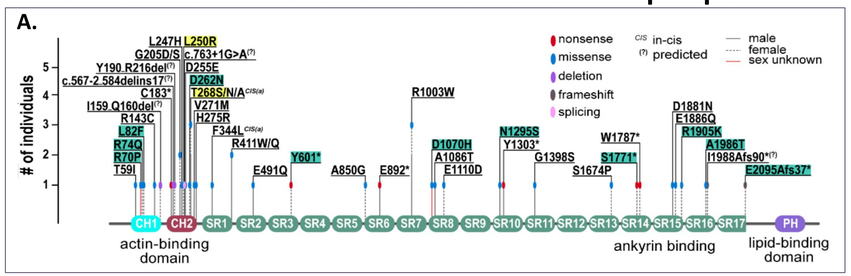


Figure 1: (A) Functional Domains of βII-spectrin. CH1 (calponin homology domain 1), teal; CH2, red. Locations of *SPTBN1* variants indicated. Both L250R and T268S are located in CH2.

SPTBN1 Syndrome Clinical Phenotypes

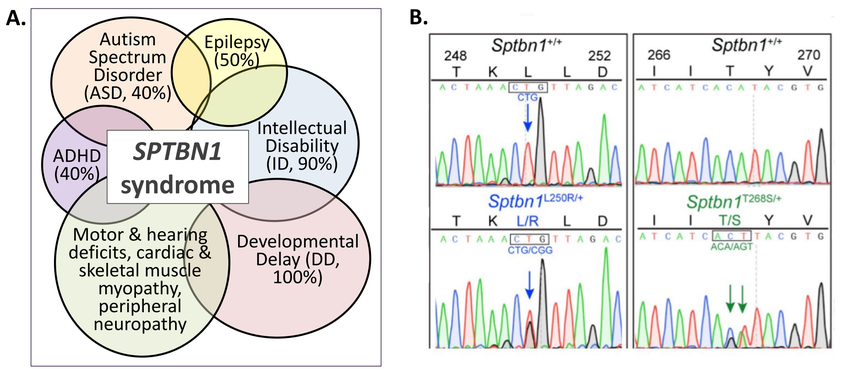
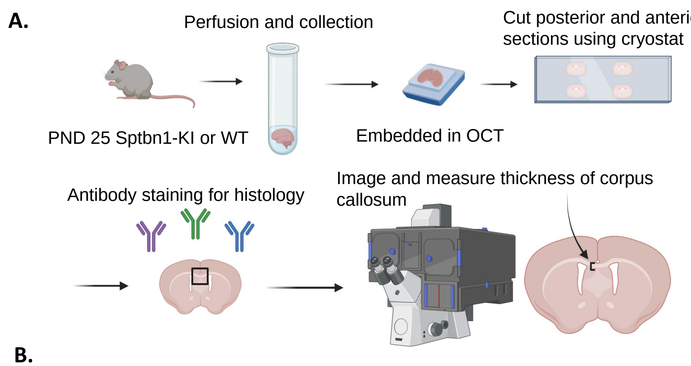


Figure 2: (A) *SPTBN1* Syndrome Clinical Phenotypes Diagram of overlapping clinical phenotypes associated with the different mutations causal of *SPTBN1* syndrome. **(B)** Sanger sequences of WT and heterozygous mice. Top row indicates normal sequences at each location. Bottom row indicates mutant sequences. In bottom left, leucine (L) is replaced by arginine (R) at position 250. In bottom right, threonine (T) is replaced by serine (S) at position 268. Colors of peaks indicate different nucleotides and height of peaks indicate amount of each nucleotide at that position. Blue and green arrows show where the mutations occur.

Methods

Outline of Experimental Methodology and Analysis



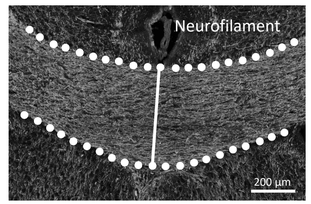
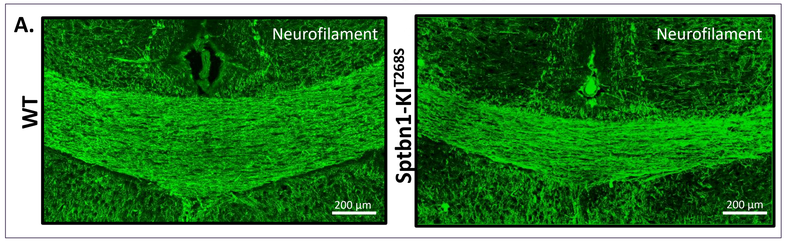


Figure 3: Outline of Experimental Methodology and Analysis. (A) Used to evaluate corpus callosum connectivity in WT and KI mice carrying *SPTBN1* variants associated with a neurodevelopmental syndrome. **(B)** Measurements starting at midline in 50 µm increments extending outwards for a total of 500 µm on either side.

Results

Evaluation of CC Morphology in *Sptbn1*$^{T268S/+}$ KI Mice



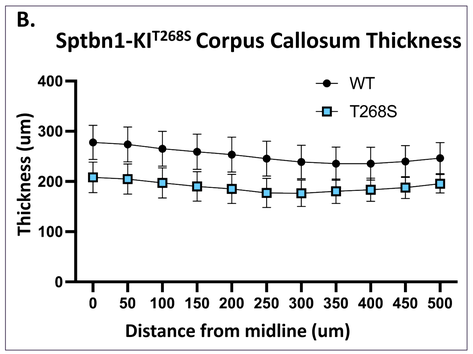
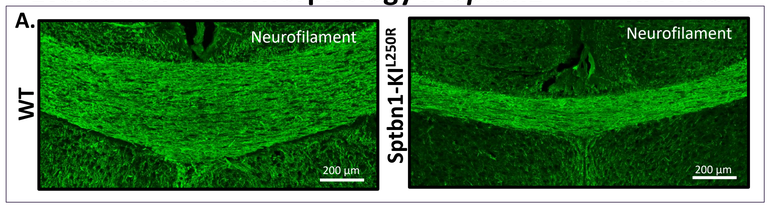


Figure 4: Representative Images and Quantification of Sptbn1-KI^{T268S} CC. (A) Images of coronal sections from PND25 mice stained for neurofilament. Scale bar, 200 µm. Left indicates WT mouse and right indicates T268S knock-in mouse. **(B)** Thickness of corpus callosum in 50 µm increments from midline with either side averaged using WT(n=4) and 4 T268S(n=4) mice to produce plot. Data in **B** represent SEM ± mean Evaluated using an unpaired t-test.

Results cont.

Evaluation of CC Morphology in *Sptbn1*$^{T268S/+}$ KI Mice



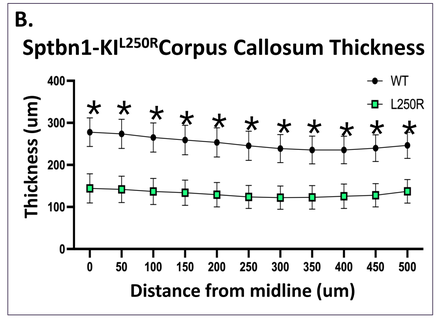


Figure 5: Representative Images and Quantification of Sptbn1-KI^{L250R} CC. (A) Images of coronal sections from PND25 mice stained for neurofilament. Scale bar, 200 µm. Left indicates WT mouse and right indicates L250R KI mouse. **(B)** Thickness of corpus callosum in 50 µm increments from midline with either side averaged using WT (n=4) and L250R (n=4) mice to produce plot. Data in **B** represent SEM ± mean. Evaluated using an unpaired t-test * <0.05.

Conclusion

- Significant thinning of the CC in PND25 L250R KI mice.
- Thinning of the CC in T268S KI that did not reach statistically significant difference (pending reevaluation with increased sample size).
- Alterations in CC morphology indicate that mutant βII-spectrin disrupts inter-hemispheric long-range axon projections and cortical circuitry, which may contribute to alterations in development, learning, sociability, and motor functions.
- CC thinning could be due to axon shortening, breakage, or misguidance during development.

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References

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