Functional Characterization of de novo variants of SPTBN2

Gephyrin spectrin is a member of the spectrin meshwork in neurons formed by heterodimeric units of gephyrin- and each of four gephyrin-spectrins (I-IV), which then form tetramers that crosslink F-actin to form periodic spectrin-actin arrays along axons and dendrites. Gephyrin-spectrins also bind their molecular partners ankyrins to, together, stabilize ion channels, cell adhesion molecules, and membrane receptors. Gephyrin-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 gephyrin-spectrins underlie neurodevelopmental and neurogenerative disorders. Specifically, variants in SPTBN2, the gene encoding gephyrin-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 gephyrin-spectrin variants. We compared the effects of GFP-tagged wild-type (WT) gephyrin- and of mutant p.L426del, p.L253P, and p.R480W GFP-gephyrin 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 contrast, none of the variants analyzed caused significant disruption of gephyrin-spectrin binding to gephyrinII-spectrin. Future work will evaluate the effect of the variants on binding to other critical cytoskeletal partners, on the distribution of gephyrin-spectrin, and on neuronal morphology.