Hereditary Spastic Paraplegia (HPS) is a rare group of neurological disorders associated with progressive weakness and extreme spasticity of the lower extremities. An autosomal dominant form of HSP results from mutations in the KIF5A gene [2,3,5], which leads to axonal degeneration in the lower motor neurons[1,2,3,5]. KIF5A is a kinesin motor involved in moving organelles and vesicles in axons [3,4,5]. *Although it is believed that the degeneration is linked to a faulty membrane transportation system, how the protein fails to localize to the extremities resulting in lower motor neuron degradation is still unknown.*

My **primary goal** is to determine why KIF5A localizes to the lower extremities. I will use *Danio Rerio* as a model organism because they serve as good model for observing behavior such as spasticity and are easy to genetically modify to have defective limbs. My **hypothesis** is that there are proteins and other post translational modifications that influence the localization of KIF5A that when altered result in spasticity, one of the symptoms of HSP. My **long-term goal** is to understand how KIF5A mutations leads to specific neurodegenerative effects of the lower extremities.

**Aim 1: Find conserved amino acids of KIF5A that are associated with phosphorylation sites and see whether they have a role in KIF5A localization.**

**Approach:** I will begin by using BLAST to find homologs for KIF5A and then ClustalOmega to perform sequence alignment. Additionally I will use NetPhos to view all the predicted phosphorylation sites and reference these to the conserve regions identified by ClustalOmega. I will then use CRISPR to create several mutants in zebrafish: S207A, S509A, S205A and S989A. I will then perform fluorescent tagging assay on all the mutants in addition to a positive control zebrafish, where there are no mutations, and a negative control, a zebrafish where KIF5A is completely knocked out. Using the results, I will be able to see where KIF5A and its altered counterparts localize within zebrafish. Additionally I will perform a phenotypic screen for spasticity with all zebrafish strains using high-throughput imaging. **Hypothesis:** Mutations in phosphorylation sites in the Kinesin motor domain (7aa-335aa) will impact KIF5A localization to various degrees, dependent on the mutation. **Rational:** The kinesin motor domain is the region in which KIF5A binds to microtubules (MT) for transportation, mutating this region could impact how far KIF5A travel along the MT or whether it binds at all.

**Aim 2: Perform a chemical screen in order to rescue mutated zebrafish and restore proper localization of KIF5A.**

**Approach:** I will perform a chemical screen using the same experimental and control zebrafish from Aim 1 with the addition of a zebrafish with a known mutation that results in HSP (610C>T). I will utilize a diverse chemical library to perform the screen so I can I identify new small molecule protein interactions. I will then introduce the small molecules to each strain of zebrafish and observe for any changes that may occur and if any of the mutated strains of zebrafish with spasticity have recovered. I will then fluorescently tag KIF5A once again and observe its localization with each mutant and recovered mutant. **Hypothesis:** I will see a recovered phenotype in S207A, S205A, and the zebrafish with the known HSP mutation. Fluorescence assay will show that zebrafish with the recovered phenotype will have proper localization of KIF5A again. **Rational:** If the zebrafish do not have spasticity in their extremities then one of the small molecules in the library have help with proper localization.

**Aim 3: Identify novel proteins that interact with KIF5A by localizing the protein to the legs.**

**Approach:**  For this aim I will introduce two mutations into exon 1 of Hox13 to create a zebrafish with defective limbs, as this has been shown to occur in previous studies. I will also have a control zebrafish with no mutations and fully functional extremities. First I will perform a fluorescence screen with KIF5A for both strains of zebrafish for localization. I will then perform a TAP Tag analysis on both the mutant and the control to see what proteins interact with the bait, KIF5A. If a protein is identified I will knockout the gene that codes for the protein of interest using CRISPR Cas 9 and insert a GFP tag creating my new mutant zebrafish. The control will have no knockout but a tag will be inserted. Localization of the protein will then be observed within the zebrafish. I will then create a protein interaction network to illustrate this proteins relationship to KIF5A. **Hypothesis:** I will be able to identify at least one protein that interacts with KIF5A in the control but not in the mutant zebrafish. **Rational:** If a protein is associated with zebrafish that has fins, but not with zebrafish without fins, than the protein must have some property that helps localize KIF5A to the extremities

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