Alexander Disease (AD) is a fatal neurodegenerative disorder characterized by destruction of the myelin sheath of neurons and accumulation of aggregates called Rosenthal Fibers found in astrocytes [5]. This disease is caused by mutations in the intermediate filament protein, Glial Fibrillary Acidic Protein (GFAP) [1]. Rosenthal fibers consist of mutated GFAP aggregates and molecular chaperones, though their presence alone does not explain the degradation of the myelin sheath and resulting neuronal dysfunction [4]. Two studies suggested that glutamate transport and regulation were altered in astrocytes [3], and that heightened extracellular glutamate levels lead to the degradation of the myelin sheath, causing neuronal dysfunction [2]*.* Althoughthese studies implicate GFAP in glutamate regulation and transport, *the cellular mechanisms of how GFAP performs these functions in astrocytes is unclear.*

My **primary** **goal** is to determine the role of GFAP in glutamate uptake and neuronal regulation in astrocytes. I will utilize a mouse model, as GFAP mutant mice share similar astrocyte biology to human juvenile and adult patients with Alexander Disease. I **hypothesize** thatGFAP plays a role in mediating glutamate uptake and neuronal regulation in astrocytes. My **overall goal** is to characterize the cellular mechanisms behind the role of GFAP in astrocytes with hopes to create therapeutics for those that suffer from Alexander Disease.

**Aim 1: Identify conserved amino acids in GFAP necessary for regulating glutamate levels and transport in astrocytes. Approach**: I will first perform a multiple sequence alignment analysis, and I will identify conserved amino acids among all GFAP homologs. I will then mutate conserved amino acids in the mouse sequence using CRISPR/Cas9, and screen for mutations which result in significantly different glutamate levels in astrocytes and signs of neuronal dysfunction for the mutant versus that of the wild type. **Rationale**: Specific amino acids are important for GFAP functions involving glutamate uptake and neuronal regulation in astrocytes. **Hypothesis**: I hypothesize that distinct amino acids will be important for GFAP function in glutamate uptake and neuronal regulation in astrocytes.

**Aim 2: Identify novel genes that are differentially expressed in GFAP mutant brain that are important in glutamate regulation and transportation in astrocytes.** **Approach:** I will perform RNA-Seq on wild type and mutant mice to identify differentially expressed genes involved in glutamate regulation and transportation in astrocytes. Next, I will use Gene Ontology to identify genes that are repressed in GFAP mutant brains that are important for neuronal function. To determine if the new genes play a role in glutamate uptake and neuronal regulation, I will use CRISPR/Cas9 to knockout newly identified genes, and will screen for mutations which result in significantly different glutamate levels and neuronal function than in the wild type mouse. **Rationale:** Finding genes which are differentially expressed GFAP mutant mice that cause changes in glutamate levels and neuronal function can reveal new factors important for glutamate uptake and neuronal regulation. **Hypothesis:** I hypothesize there will be differentially expressed genes involved in glutamate uptake and neuronal regulation in GFAP mutant mice.

**Aim 3: Identify novel proteins which interact with GFAP necessary for glutamate regulation and transportation in astrocytes. Approach:** I will use TAP-MS and Y2H screening to identify proteins which interact with both mutant and wild type mouse GFAP homologs. I will then use Gene Ontology to screen for proteins which may be involved in glutamate uptake and neuronal regulation. Then I will use CRISPR/Cas9 to knockout newly identified proteins, and I will screen for significantly different glutamate levels and altered neuronal function when compared to the wild type mouse. **Rationale:** By comparing differential expression and protein-protein interactions between wild type and GFAP mutant mice, I can determine which proteins interactions are involved in glutamate uptake and neuronal function in astrocytes. **Hypothesis:** I hypothesize that new GFAP protein-protein interactors involved in glutamate uptake and neuronal regulation will play a role in glutamate transportation and regulation astrocytes.

By elucidating the cellular mechanisms behind how GFAP functions in glutamate uptake and neuronal regulation in astrocytes, it may be possible to reveal how GFAP functions in other cellular processes within the brain. These studies can help us further understand how the phenotype seen in Alexander Disease occurs, allowing researchers to focus on specific genes in these pathways and develop targeted future treatments. Though the road to discovering a cure for Alexander Disease is a long and challenging one, trying to tackle how GFAP functions one pathway at a time can allow for a comprehensive understanding of its role in astrocytes and how it causes Alexander Disease.

Work Cited:

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