Batten disease is an uncurbable juvenile neurodegenerative disorder, where deterioration of vision, cognitive function, and motor skills aren’t apparent until the age of four, and progress throughout one’s shortened lifespan [1]. Effecting 4 in 100,00 children, the gene associated with juvenile Batten disease is the CLN3 gene, which when mutated, has a total loss of function [1]. The CLN3 gene encodes Cln3 proteins, which are transmembrane proteins that reside in the membrane of the neuronal contractile vacuole; a membrane-enclosed vesicle containing fluid [2]. These proteins are also believed to be involved in osmoregulation [1,2]. *Although the Cln3 protein function is not yet known,* a buildup of substances such as water and gangliosides (glycosphingolipids with one or more sialic acids found on the surface of neuronal cells) are suggestive of its function [2]*.* The buildup of gangliosides inhibits the passing of neurotransmitters through the synapse, meaning it is essential to understand the role of the Cln3 protein, to prevent this disease from occurring [3].

**My long-term goal** is to understand the effects of various mutations on the Cln3 protein function; specifically relative to eye pathophysiology. **My primary goal** is to better understand the role the Cln3 protein has with trafficking gangliosides into the neuronal vacuoles. **My hypothesis** is that the Cln3 protein transports gangliosides into the neuronal vacuole, which when “full” is expelled from a presynaptic cell via vesicles. My model organism will be the *Drosophila melanogaster,* due to the clear visibility of the wildtype and mutated phenotype, and because the anatomy of the *Drosophila’s* eye is highly studied and well understood [4]*.*

**Aim 1: Characterize conserved amino acids of Cln3 to identify a potential protein function.**

**Approach:** To begin, I will use BLAST to find the homologs of CLN3 gene. I will then use multiple sequence alignments via MUSCLE to optimize the amount of conserved regions within the homologs. Next, I will compare protein domains using the patterns of the motifs, via the PROSITE database. Then, I will analyze the data to determine which homologs share similar motifs with the Cln3 protein. Using CRIPSR-Cas9, I will then mutate different conserved amino acid regions of the Cln3 protein to understand how the mutations influence eye pathology. Finally, I will compare the phenotype of the mutated *Drosophila’s* eye to the control fly.

**Rationale:** Screening flies with different mutated regions of the Cln3 protein will determine motif that regulates trafficking of lipids to the neuronal vacuole.

**Hypothesis:** Conserved domains within homologs of Cln3 proteins will give insight to what lipids the protein is transporting across the neuronal vacuole membrane.

**References:**

1. Mathavarajah, S., Mclaren, M. D., & Huber, R. J. (2018). Cln3 function is linked to osmoregulation in a Dictyostelium model of Batten disease. *Biochimica Et Biophysica Acta (BBA) - Molecular Basis of Disease,1864*(11), 3559-3573. doi:10.1016/j.bbadis.2018.08.013
2. Somogyi, A., Petcherski, A., Beckert, B., Huebecker, M., Priestman, D., Banning, A., . . . Tikkanen, R. (2018). Altered Expression of Ganglioside Metabolizing Enzymes Results in GM3 Ganglioside Accumulation in Cerebellar Cells of a Mouse Model of Juvenile Neuronal Ceroid Lipofuscinosis. *International Journal of Molecular Sciences,19*(2), 625. doi:10.3390/ijms19020625
3. Boswell-Casteel, R. C., & Hays, F. A. (2016). Equilibrative nucleoside transporters-A review. *Nucleosides, nucleotides & nucleic acids*, *36*(1), 7-30.
4. Perland, E., Bagchi, S., Klaesson, A., & Fredriksson, R. (2017). Characteristics of 29 novel atypical solute carriers of major facilitator superfamily type: evolutionary conservation, predicted structure and neuronal co-expression. *Open biology*, *7*(9), 170142.

\*\*Do not grade citations below. They are for my next aims

Schultz, M. L., Tecedor, L., Lysenko, E., Ramachandran, S., Stein, C. S., & Davidson, B. L. (2018). Modulating membrane fluidity corrects Batten disease phenotypes in vitro and in vivo. *Neurobiology of disease*, *115*, 182-193.