Batten disease is an incurable juvenile neurodegenerative disorder, where deterioration of vision, cognitive function, and motor skills are not 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 lysosome; a membrane-enclosed vesicle containing enzymes that breakdown cellular waste [2]. CLN3 proteins are believed to be involved in the transport of cellular waste into the lysosome. [1,2]. *Although the CLN3 protein function is not yet known,* a buildup of substances such as cationic amino acids and gangliosides (glycosphingolipids with one or more sialic acids found on the surface of neuronal cells) in the cytoplasm of the neuron is suggestive of its function [2]*.* The buildup of cellular waste in neurons leads to cell death (or more specifically retinal degeneration), meaning it is essential to understand the role of the CLN3 protein, to prevent this disease from occurring [3].

**My primary goal** is to determine how the CLN3 protein is involved in the buildup of cellular waste and retinal degeneration in patients with Batten disease. **My hypothesis** is that the CLN3 protein is involved in the activation of RNA binding proteins (RBPs), which are essential for the translation of functioning neuronal lysosome transmembrane proteins [4]. My model organism will be the *Drosophila melanogaster,* due to the clear visibility of the wildtype and mutant phenotype (both in the eye and the neuronal lysosome), and because the anatomy of the *Drosophila’s* eye is highly studied and well understood [5]*.* **My long-term goal** is to understand the specific interaction between the CLN3 protein and RNA binding proteins; specifically relative to eye pathophysiology.

**Aim 1: Characterize the function of CLN3 protein motifs, in relation to RNA binding proteins.**

**Approach:** MEME will be used to identify conserved CLN3 sequence motifs in drosophila and humans. CRISPR/Cas9 will then be used to create three different mutants with knockout mutations in the conserved motifs: C-terminus knock out, N-terminus knock out, and transmembrane knock out. The phenotypes of the drosophila will be assayed for retinal degeneration and cellular waste buildup.

**Rationale:** Screening flies with different mutated regions of the CLN3 protein will determine motif that interacts with RNA binding proteins. The C-terminus and N-terminus are the most accessible regions of the CLN3 protein to RBPs, given they are not confined within the plasma membrane of the lysosome, nor are contained within the lumen of the lysosome.

**Hypothesis:** The C-terminus or the N-terminus motifs will be responsible for interactions with the RNA binding proteins, involved in the translation of lysosomal transmembrane proteins.

**Aim 2: Determine the effect of CLN3 mutations on lysosome transmembrane transcription levels.**

**Approach:** Cells from the retina of wild type and CLN3 mutant drosophila will be collected. The cells will undergo RNA sequencing, and a heat map will be generated. Gene Ontology will then be used to determine which genes involved in translation are differentially expressed between the wild type and CLN3 mutants. Genes that have distinct changes in level of expression will be knocked out via CRISPR/Cas 9 in drosophila retinal neurons, and assayed for retinal degeneration and cellular waste buildup.

**Rationale:** From this information, one would be able to deduce which genes’ transcription levels are influenced by CLN3. Genes involved in translation of lysosomal transmembrane proteins are of particular interest, because CLN3 mutants would likely have significant changes in transcription level of RNA binding proteins; thus be unable to translate lysosome transmembrane proteins that are essential for transport of cellular waste into the lysosome.

**Hypothesis:** Transcription levels of RNA binding proteins will be down-regulated in CLN3 mutants.

**Aim 3: Identify protein interactions of CLN3 involved in cellular waste metabolism in retina neurons.**

**Approach:** Neuronal retina cells will be extracted from wild type and CLN3 mutant flies. Cells will be subjected to Tandem Affinity Purification (TAP), then Mass Spectrometry to determine the difference in protein-protein interactions; specifically relative to RNA binding proteins. Gene Ontology will be used on the RBPs to analyze their protein interaction networks. Proteins that have significant changes in function between the wild type and the CLN3 mutants will be knocked out, and assayed in Drosophila for retinal degeneration.

**Rationale:** CLN3 interacts with RBPs, which are responsible for translation, and synthesis of RNA. Some lysosome transmembrane proteins are translated in the endoplasmic reticulum where CLN3 is synthesized. Therefore, should CLN3 involve the productivity of RBPs responsible for translating lysosomal transmembrane proteins, a mutation of CLN3 would prevent the proper formation of these proteins.

**Hypothesis:** Lysosome transmembrane proteins responsible for transport of cellular waste will not be translated in CLN3 mutants, thus will cause a buildup of cellular waste in the neuron.

**Future Directions:** ATPase involved in pumping cationic amino acids into the neuronal lysosome was found to be dysfunctional when CLN3 was mutated [5]. A dysfunctional ATPase leads to the increase in the pH of the lysosomal lumen, thus destroys the proton gradient necessary to move cellular waste into the lysosome. Investigating the relationship between CLN3 and the translation of specifically ATPase could resolve why there is significant cellular waste buildup in patients with retinal degeneration.

**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. 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.
5. 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.