Gaucher disease is a lysosomal storage disorder that results from an enzyme deficiency, leading to an accumulation of fatty substances in the liver and spleen, and the subsequent enlargement of these organs [1]. In the most severe cases of Gaucher disease, individuals experience neurological symptoms, including seizures and developmental delays, and death by the age of three [1]. Microglia are immune cells of the brain and an increase in their activation can induce neuroinflammation [2, 3]. **GBA1** is associated with Gaucher disease and regulates microglial cell proliferation, lipid storage, and lysosomal function [4, 5]. *Despite extensive research on Gaucher disease, the role of GBA1 in the regulation of lysosomal function in microglial cells in the brain remains unclear*.

My primary goal is to investigate the role of GBA1 in lysosomal function during microglial activation in the brain. I will use zebrafish *(Danio rerio)* because they are transparent vertebrates in which I can easily perform behavioral, microglial, and lysosomal assays [6]. Mutations at conserved serine sites may disrupt phosphorylation, and in turn, alter cell signaling and protein function [7]. Many individuals who experience the neurological symptoms of Gaucher disease have a mutation in their GH30_C domain [8, 9, 10, 11]. Therefore, I **hypothesize** that conserved Serines in the GH30_C domain of GBA1 are important for lysosomal function and microglial activation in brain tissue early during post-natal development. **My long-term goal** is to understand how GBA1 functions in microglial activation so that treatments can be formulated to prevent irreversible neurological damage in Gaucher disease patients.

Aim 1: Identify conserved amino acids of GH30_C in GBA1 that are essential for lysosomal function and activation of microglia early during post-natal development.
Rationale: Many Gaucher patients with neurological symptoms have a mutation in their GH30_C domain, indicating that this domain may be involved in microglial activation.
Approach: I will obtain FASTA protein sequence data of GBA1 orthologues from Ensembl and will use MEGA to conduct Multiple Sequence Alignment. I will use CRISPR/Cas9 to produce zebrafish that have mutations in a conserved serine site in the GH30_C region and in two regions outside of this domain. I will run a behavioral swimming assay on GBA1 wildtype, the GBA1 mutant, and on my Serine point mutants at the larval stage [12]. I will perform a microglia assay using a membrane-targeted fluorescent reporter, and Lysotracker, a dye used to track lysosomes [6, 13].
Hypothesis: Mutations at a conserved serine site within GH30_C will disrupt phosphorylation, leading to dysregulation of lysosomes in the microglia, and an increase in microglial activation early during post-natal development.

Aim 2: Perform a forward chemical screen using a target-focused library to identify small chemical compounds that rescue Serine mutant zebrafish.
Rationale: GBA1 interacts with four small molecules on PubChem, but it is unclear if any of these compounds affect GBA1 function in any way. One of these four molecules, $C_{17}H_{17}N_4O$, is a small molecular non-inhibitory chaperone. Small molecular non-inhibitory chaperones serve as a possible Gaucher disease treatment [14, 15].
Approach: I will treat wildtype, the GBA1 knockout mutant, and GBA1 Serine point mutants with chemical compounds from my target-focused library designed with compounds similar in structure to $C_{17}H_{17}N_4O$. I will perform a behavioral assay on all the zebrafish larvae to determine if abnormal swimming phenotypes are rescued by any small compounds in the chemical library. Then, I will conduct a microglia and lysosomal assay on the rescued zebrafish.
Hypothesis: Small molecules that rescue Serine mutants will likely modulate microglial and lysosomal function and serve as a possible cure for Gaucher disease associated neurological symptoms.

Aim 3: Use BioID to identify novel proteins that function with GBA1 in microglia in the brain.
Rationale: BioID is a tissue specific approach, which makes it ideal for identifying GBA1's interactions with other proteins in the brain.
Approach: I will tag GH30_C in wildtype, mutant GBA1, and GBA1 serine point mutants to mutated BirA in zebrafish brain tissue [16]. Then, I will run isolated brain lysates over a column to isolate proteins that are proximal to and/or interact with GBA1 [16]. To identify these proteins, I will run mass spectrometry [16]. Using the gene ontology terms, "microglial cell proliferation" and "lysosomes," I will sort the proteins. Then, I will employ CRISPR/Cas9 to knock out the proteins that are not found in the GBA1 serine mutant BioID assay and run behavioral, microglia, and lysosomal assays to validate my results.
Hypothesis: Proteins that directly interact with or are proximal to the GH30_C domain of GBA1 may function in the same neuroinflammatory pathway and regulate lysosomal function in the microglial cells of the brain.

These aims allow me to learn about the role of GBA1 in regulating lysosomal function, and in turn, microglial activation in the brain. I hope to uncover information that will alleviate the neurological symptoms associated with Gaucher disease.

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