Schizophrenia is a chronic, heritable brain disease characterized by cognitive, perceptual, and behavioral impairments1. Schizophrenic individuals have decreased grey matter in their cerebral cortexes, which contain fewer and sparser dendritic spines2. Dendritic spines normally function in synaptic storage and electrical transmission between neurons. In some schizophrenia patients, overexpression of the complement component 4A (C4A) gene activates the classical complement pathway, which is commonly known for amplifying the strength of immune responses. However, classical complement activation also plays a role in shedding neuronal connections during childhood and adolescence, known as synaptic pruning. Synaptic pruning increases in schizophrenic patients during the progression into adulthood, yet *how C4A specifically regulates dendritic spine pruning and immune response is unclear.*

My **primary goal** is to discover how C4A regulates synaptic pruning in schizophrenia patients. My **hypothesis** is that C4A plays a role in dendritic pruning by increasing complement activation at dendritic spines. *Danio rerio* is a great model organism to study pruning because neuronal and whole-brain changes can be easily visualized, development patterns are well established, and the C4A ortholog is well conserved. My **long-term goal** is to understand mechanisms of synaptic pruning in schizophrenia to determine effective treatments and preventative measures.

**Aim 1: Determine which C4A domains participate in the pruning of dendritic spines during the transition from embryo to adult.**

**Approach:** Domain analysis is conducted on C4A orthologs using SMART, PFAM and Clustal Omega to identify conserved regions between humans and zebrafish. CRISPR-Cas9 will be used to knock-out the most conserved domains in zebrafish embryos and adults. Then, synaptic pruning will be visualized by immunofluorescence imaging of dendrites in young and adult zebrafish brain neurons to find domains which reduce the amount of pruning in adults. Those domains will be GFP-tagged to determine their neuronal localization in zebrafish throughout development.

**Rationale:** C4A has at least nine domains, all of which may not participate in synaptic pruning. Knockouts will indicate which domains are crucial for pruning throughout zebrafish development.

**Hypothesis:** Specific domains or amino acid sequences in C4A are necessary for pruning of dendritic spines from the embryo-to-adult transition in zebrafish.

**Aim 2: Identify small molecules that rescue dendritic spine pruning defects in adult C4a mutant zebrafish.**

**Approach:** A high-throughput chemical genomic assay will be performed using a diversity-oriented library. Small molecules will be screened using young and adult wildtype and C4A mutant zebrafish from Aim 1 to identify molecules that function in the pruning process.

**Rationale:** Small molecules that interact with C4A and decrease pruning levels throughout development will further indicate which C4A domains are required for dendritic spine pruning.

**Hypothesis:** Several small molecules having similar features to molecules present on dendritic spines will be identified through the screen that can rescue defects in C4A mutants.

**Aim 3: Determine how other proteins interact with C4A to regulate dendritic spine pruning.**

**Approach:** C4A protein interactors found using STRING will be analyzed for shared GO terms using PANTHER. The interacting proteins involved in immune system processes will be knocked out with CRISPR-CAS9 in zebrafish embryos and adults. Behavioral observations and immunofluorescence imaging of dendrites will be used to screen zebrafish with pruning defects. Small molecules from Aim 2 will be screened on defective zebrafish to determine whether C4A interactors have similar binding and activity as C4A. **Rationale:** C4A interactors will reveal more about how the C4A protein causes dendritic spine pruning. **Hypothesis:** Knocking out C4A interactors that are involved in similar pathways and mechanisms as C4A will cause similar pruning defects in zebrafish.

The results of this study will give valuable insight into how C4A regulates dendritic spine pruning through immune activation, and will also provide potential drugs that could yield preventative and curative treatments for schizophrenia.

**References:**

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2. Garey, L., Ong, W., Patel, T., Kanani, M., Davis, A., Mortimer, A., … Hirsch, S. (1998). Reduced dendritic spine density on cerebral cortical pyramidal neurons in schizophrenia. *Journal of Neurology, Neurosurgery, and Psychiatry*, *65*(4), 446–453.