Specific Aims – Background and Gap and Aim 1

Schizophrenia is a chronic, heritable brain disease characterized by cognitive, perceptual, and behavioral impairments (1). Schizophrenic individuals have decreased grey matter in the frontal and temporal lobes of their cerebral cortexes, and these regions contain fewer and sparser dendritic spines (2). Dendritic spines normally function in synaptic storage and electrical transmission between neurons. In some schizophrenia patients, overexpression of the complement component 4A (C4A) gene affects classical complement activation which works by strengthening defenses against pathogens. Classical complement activation also plays a role in the shedding of neuronal connections during childhood and adolescence, known as synaptic pruning. Synaptic pruning often increases in schizophrenic patients during the progression into adulthood yet *how C4A specifically regulates dendritic spine pruning is unclear.*

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 visualized easily, development patterns are well established, and the C4A ortholog is well conserved. My **long-term goal** to discover how C4A regulates synaptic pruning.

**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 14 C4A orthologs using SMART, PFAM and Clustal Omega. CRISPR-Cas9 will be used to knock-out the most conserved areas of different domains in zebrafish embryos and adult trials, then synaptic pruning is visualized by fluorescence imaging of the cortex neurons. Knocked out domains which reduce the amount of pruning in adults will be of interest, and those domains will be tagged with GFP to determine their neuronal localization in zebrafish throughout development.

**Rationale:** C4A has at least 9 domains, all of which may not participate in synaptic pruning so knockouts will tell me which are crucial for pruning throughout zebrafish development.

**Hypothesis:** There is a specific domain or AA sequence in C4A that is necessary for pruning of dendritic spines.

**References:**

1. Sekar, A., Bialas, A. R., de Rivera, H., Davis, A., Hammond, T. R., Kamitaki, N., … McCarroll, S. A. (2016). Schizophrenia risk from complex variation of complement component 4. *Nature*, *530*(7589), 177–183. http://doi.org/10.1038/nature16549
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.
3. Zorzetto, M., Datturi, F., Divizia, L., Pistono, C., Campo, I., De Silvestri, A., … Ricevuti, G. (2016). Complement C4A and C4B gene copy number study in Alzheimer's disease patients. *Current Alzheimer Research*, Epub ahead of print.

Ideas:

C4A is overexpressed in the brains of schizophrenic patients and localizes to neurons and their synapses (does this only happen at dendritic spines?), so what is signaling the C4A to activate the complement pathway in these regions? What is the C4A targeting?

This can lead to new drug targets for treatment and/or prevention of schizophrenia