Specific Aims – Background and Gap, Aim 1 and 2

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 activates the classical complement pathway, which works by amplifying the strength of immune responses. 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 **primary goal** is to discover how C4A regulates synaptic pruning in schizophrenia. 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** is to understand the mechanism 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 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 dendritic proteins in 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. Knockouts will indicate which domains are crucial for pruning throughout zebrafish development.

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

**Aim 2: Identify small molecules that bind C4A domains participating in dendritic spine pruning.**

**Approach:** A high-throughput-assay is done using a diversity-oriented library. Small molecules that interact with C4A domains found in Aim 1 will be collected into a focused library and these molecules will be screened in zebrafish throughout development to determine which decrease pruning.

**Rationale:** Small molecules that interact with C4A and decrease pruning levels throughout development will further indicate which C4A domains are required in synaptic pruning and will yield possible future drug targets.

**Hypothesis:** Several small molecules having similar features to molecules present on dendritic spines will be identified through the screen that can bind to specific C4A protein domains.

**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.

ANOTHER POTENTIAL IDEA FOR AIM 2

**Aim 2: Determine C4A post translational modifications (PTMs) that activate/increase levels of synaptic pruning in schizophrenia.**

**Approach:** mass spectrometry

**Rationale:**

**Hypothesis:**

1. 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.