Specific Aims

Obsessive Compulsive Disorder (OCD) affects about one percent of the nation [1]. OCD is defined by two major symptoms: obsessions, which are uncontrollable thoughts and actions, and compulsions, which are thoughts or rituals used to alleviate the anxiety that accompanies obsessions. These symptoms of OCD are often connected to levels of the neurotransmitter serotonin, a chemical involved in regulation of bodily functions such as sleep, appetite, and emotion [2]. One distinctive gene linked to OCD codes for the serotonin transporter SLC6A4. Normally this protein regulates reuptake of serotonin from the synapse, but previous studies have indicated that a missense mutation can cause SLC6A4 to be constitutively phosphorylated. This leads to its over-activation, bringing about higher rates of serotonin reuptake and the dampening of serotonergic messages [3]. The influence of phosphorylation on SLC6A4 activity gives some insight into mechanisms that may affect other proteins in relation to OCD and could help explain the polygenic nature of the disorder.

SLC6A4 over-activation is known to affect OCD onset. However, the effects of over-activation of SLC6A4 on protein kinase levels and other OCD-linked proteins have not been studied. **To address this lack of knowledge we will test the hypothesis that SLC6A4 over-activation leads to measurable changes in protein kinase levels, and in turn affects phosphorylation of other proteins.** For several decades, rats that exhibit symptoms such as ritualistic chewing behaviors and anxiety-like responses have been used to study OCD [4]. They will be used as a model organism for testing our specific aims.

The **primary goal** of these aims is to determine how over-expression of SLC6A4 may affect other proteins that are also involved in development of OCD. The **long-term goal** is to determine complex interactions between all proteins as they relate to the onset of OCD to fully characterize the disorder, leading to more effective treatments. We hope to move toward this goal by using several genomic, proteomic, and bioinformatic methods.

**Aim One:** Identify novel and conserved SLC6A4 interacting proteins in rats and humans. **Approach:** Using tandem affinity purification (TAP) tags, novel interactions between SLC6A4 and synaptic proteins from rats will be tested. Based on STRING, any new interactions discovered that were previously undocumented in rats will be entered into BLAST to find human homologs. **Hypothesis:** New interactions found through TAP tags will add several more protein kinases to the rat STRING network than the single kinase currently present. **Rationale:** Discovering new SLC6A4 interacting proteins provides candidates that may be involved in other behavioral changes noted in OCD.

**Aim Two:** Identify genes that are misregulated in SLC6A4 mutant rats. **Approach:** Neuronal tissue samples from healthy and SLC6A4 mutant rats will be collected and used to assess gene expression levels using RNA-seq. Genes that are differentially expressed in mutant rats will be entered into the Gene Ontology Consortium (GO) and grouped for similar biological processes. **Hypothesis:** Genes linked to signaling and modification GO terms will be over-expressed in SLC6A4 mutant rats. **Rationale:** Protein kinases have the potential to affect many other genes, and their over-expression would provide an explanation for characteristic behaviors seen in OCD that can not be explained by an SLC6A4 mutation directly.

**Aim Three:** Identify proteins exhibiting alternative phosphorylation due to interactions with misregulated kinases. **Approach:** For both SLC6A4 mutants and healthy rats, tandem pass spectrometry will be used to test neuronal tissue samples. The healthy and mutant samples will be compared to see if phosphorylation levels of any proteins besides SLC6A4 were affected. **Hypothesis:** Several proteins that interact with the kinases will exhibit over-phosphorylation in the mutant sample. **Rationale:** Proteins that show changes in phosphorylation are good candidates for other proteins involved in OCD.

This research will determine interactions between proteins involved in OCD and lead to a greater understanding of the molecular pathways implicated in the onset of OCD symptoms. With more known about these relationships, new and more effective treatment methods could be developed. Because there are so many other anxiety disorders that are highly similar to OCD, any information discovered about its manifestation may also be applicable to others. Future revelations may lead to drug treatments or behavioral modification approaches that are effective for a wide variety of psychiatric disorders, bettering the lives of millions.

[1] Facts & Statistics | Anxiety and Depression Association of America, ADAA. (2010, January 1). Retrieved February 23, 2015, from <http://www.adaa.org/about-adaa/press-room/facts-statistics>

[2] Mandal, A. (2014, February 13). Serotonin Function. Retrieved May 6, 2015, from <http://www.news-medical.net/health/Serotonin-Function.aspx>

[3] Ramamoorthy, S., Shippenberg, T., & Jayanthi, L. (2011). Regulation of monoamine transporters: Role of transporter phosphorylation. Pharmacology & Therapeutics, 129(2), 220-238. Retrieved April 15, 2015, from <http://www.sciencedirect.com/science/article/pii/S0163725810001932>

[4] Albelda, N., & Joel, D. (2012). Review: Current animal models of obsessive compulsive disorder: An update. Neuroscience, 211, 83-106. Retrieved February 23, 2015, from <http://www.sciencedirect.com/science/article/pii/S0306452211010281>