Specific Aims

Obsessive Compulsive Disorder (OCD) affects about one percent of the nation [1]. OCD is defined by two major symptoms; obsessions are uncontrollable thoughts and actions, and compulsions are thoughts or rituals used to alleviate the anxiety that accompanies obsessions. OCD is a polygenic disorder, but one distinctive gene associated with it is the serotonin transporter SLC6A4. Uptake of serotonin is disrupted due to a mutation in the 5-HTT domain of SLC6A4, which induces the promoter and causes the gene to be overexpressed. This leads to high rates of serotonin reuptake and dampening of serotonergic messages.

The heritability of OCD is roughly 0.5, meaning that along with being polygenic, half of the disorder can be contributed to the environment [2]. Some factors that are thought to contribute to anxious behaviors include a lack of social bonds, sense of danger in their environment, and even learned behaviors from adults to children [3]. For several decades, mice and 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.

High levels of SLC6A4 as well as environmental components are known to affect OCD expression. However, the evolutionary conservation of *SLC6A4* as well as the comparative expressions of both DNA and proteins in healthy and affected organisms has not been explored. **To address this lack of knowledge we will test the hypothesis that conservation is necessary within OCD-linked genes to avoid anxiety-related diseases such that when mutations do occur, they cause measurable changes in gene expression and protein presence.**

The **long-term goal** of this study is to determine complex interaction of genes including *SLC6A4* as they relate to development of OCD. We hope to move toward this goal by using several bioinformatics and proteomic methods.

**Aim One:** Determine the conservation of the 5-HTT domain in SLC6A4 across a wide variety of organisms. **Approach:** Using BLAST and Homologene, protein homologs will be found for SLC6A4. All protein sequences can be entered into InterPro to determine the location of 5-HTT. An alignment of the regions can be created with ClustalOmega and their conservation determined. Those regions with more variability are likely regions related to diseases such as OCD, while regions maintained throughout evolution relate to more critical functions.

**Aim Two:** Determine the ontological differences between healthy and OCD-presenting rats. **Approach:** Neuronal tissue samples from both healthy and OCD-type rats will be collected and their mRNA used to make a labeled single-stranded cDNA library. These labeled cDNA strands will anneal to single-stranded DNA on a microarray. Spots that light up red will indicate genes overexpressed in OCD. These genes can be entered into the Gene Ontology Consortium (GO) to determine the similar biological processes, molecular functions, and cellular components that are likely implicated in OCD as well. STRING can also be used to map SLC6A4 relationships. Any interactions with the overexpressed proteins suggest a point of study using 5-HTT mutated organisms.

**Aim Three:** Determine how environmental factors alter the neuronal proteome in rats. **Approach:** Neural protein samples will be collected from healthy and OCD-affected rats. Healthy rats will then be exposed to different OCD-linked stressors such as isolation, threat of danger, rats that already exhibit anxious tendencies like ritualistic chewing. Neural proteins will be collected at several time points during and after each exposure. A proteomic assay will be performed to determine the proteome in the healthy, stressed, and anxious rats. This will indicate important differences between healthy and OCD-related proteomes.

Through the research proposed above, interactions between genes such as *SLC6A4* can be mapped and a greater understanding of the pathways implicated in OCD determined. With more known about these relationships, new methods of treatment can 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 a viable response for a wide variety of psychiatric disorders and better 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] Iervolino, A., Rijsdijk, F., Cherkas, L., Fullana, M., & Mataix-Cols, D. (2011). A Multivariate Twin Study of Obsessive-Compulsive Symptom Dimensions. Archives of General Psychiatry, 68(6), 637-644. Retrieved February 5, 2015, from <http://archpsyc.jamanetwork.com.ezproxy.library.wisc.edu/article.aspx?articleid=912992>

[3] In-Depth Report: Obsessive-Compulsive Disorder. (2013, March 11). Retrieved March 16, 2015, from <http://www.nytimes.com/health/guides/disease/obsessive-compulsive-disorder/risk-factors.html>

[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>