**Specific Aims**

Anxiety disorders affect over 40 million adults in the United States, making them the most diagnosed mental illnesses in the country. One such disorder is known as Obsessive Compulsive Disorder (OCD), which affects about one percent of the nation [1]. OCD is defined by two major symptoms: obsessions, or uncontrollable thoughts and actions, and/or compulsions, thoughts or rituals used to alleviate the anxiety that accompanies the obsessions. Based on previous studies, the heritability of OCD is roughly 0.5, meaning approximately half of the disorder is affected by a genetic component [2]. In the last few decades, it has been suggested that the neurotransmitter serotonin is disrupted due to a mutation in the promoter region of the SLC6A4 gene. This transcribes a transmembrane protein in neurons that takes up serotonin from the synaptic gap. Thus, when more of these proteins are transcribed more serotonin will be removed from the synaptic gap and its signal will be dampened.

Currently, it is unknown the exact neuronal and genetic contributors to OCD. Research has shown that it can be passed through families, but since the heritability rate is 0.5 this also means the environment significantly contributes to its expression as well. This is also a polygenic condition, so the totality of all genes involved is actively in research. While there are some treatments, including pharmaceuticals like serotonin reuptake inhibitors (SRIs), that are known to be effective against OCD, the mechanism is still being determined. **In the interest of addressing this lack of knowledge, we will test the hypothesis that low serotonin levels strongly contribute to development of OCD and these low levels are, at least in part, connected to overexpression of SLC6A4 or its homologs.** Mice and rats have been studied for several decades on symptoms such as ritualistic chewing behaviors and anxiety-like responses and their relation to OCD [3]. They will be used as a model organism for testing this hypothesis, and are likely candidates for future research on the topic as well.

The **long-term goal** of this study is to determine the degree to which the SLC6A4 gene affects expression of OCD in humans, as well as its interactions with other genes that may also contribute to its development. We hope to move toward this goal by systematically analyzing SLC6A4 itself though several genomic, proteomic, and bioinformatics methods.

**Aim One:** What is the total effect of the SLC6A4 gene on organisms expressing OCD-like symptoms? **Approach:** Rats that have overexpression of SLC6A4 gene due to the promotor mutation addressed above will be created using homologous recombination for gene targeting. Observed differences can be noted between the mutants and wild-type rats to indicate the degree to which the mutation alters phenotypic expression.

**Aim Two:** Is there an optimal amount of serotonin loss at which anxiety disorders such as OCD are most likely to be observed in model organisms? **Approach:** Starting with wild-type rats, we will use a CRISPR/Cas9 approach to knock out the gene involved with serotonin production. The only serotonin that will be experienced by these rats will be injected in various concentrations, up to the concentration found in a wild-type condition. Any groups that show higher proportions of OCD symptoms will indicate the concentration at which serotonin most strongly supports its expression.

**Aim Three:** Through what mechanisms does the SLC6A4 gene interact with other genes to additively contribute to the OCD phenotype? **Approach:** Using SMART, we will enter the known sequence of the human SLC6A4 protein. Based on the *Interactions* tab on the results page, connections between this protein and various other proteins in the human body are shown and can be reviewed. This will not only suggest proteins that may be of future interest for the study of OCD, but could also become candidates for targeted treatment of the disorder.

Through the cumulative research proposed, the degree to which the SLC6A4 gene determines development of OCD will be examined. With more known about the proteomics of the serotonergic system, new methods of treatment can be developed. Because there are so many other anxiety disorders that are highly similar to OCD, any information that is discovered about its manifestation may also be applicable to the 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] Albelda, N., & Joel, D. (2012). Review: Current animal models of obsessive compulsie disorder: An update. *Neuroscience,* *211*, 83-106. Retrieved February 23, 2015, from <http://www.sciencedirect.com/science/article/pii/S0306452211010281>