**Specific Aims**

Cri du Chat syndrome is a chromosomal deletion disease that occurs in 1 in 15,000 to 50,000 live-born infants.1 The homozygous deletion occurs in the short arm of the fifth chromosome, the loss of which also causes many well-known developmental diseases/syndromes, such as Autism, Parkinson’s disease, and mental retardation.1 Individuals with Cri du Chat syndrome exhibit symptoms that include small birth weights, microcephaly, poor muscle tone, cognitive impairment, and respiratory, circulatory, sensory, and skeletal problems.2 Mild cases of Cri du Chat syndrome are manageable with therapy, but more severe chromosomal deletions can result in critical medical conditions or organ defects, which are often fatal.

One of the genes responsible for Cri du Chat syndrome phenotypes is SEMA5A. Previous studies have shown that SEMA5A deletion can lead to abnormal brain development, but there have been few studies looking at SEMA5A involvement in other organ systems.3 A recent study showed that SEMA5A knockout mice (*Mus musculus*) die early because of SEMA5A involvement in vascular advancement in embryonic development.4 SEMA5A mutant mice exhibit abnormal vasculature localization, suggesting that SEMA5A is involved in vascular positioning. *Although there have been many studies on abnormal brain development associated with SEMA5A deletion, the functions of SEMA5A in vascular development are still unknown.*

**Hypothesis:** The SEMA5A gene is necessary for proper development in organisms with circulatory systems.

**Primary Goal:** Determine the proteomic changes that contribute to abnormal vascularization, which is potentially involved in fatal organ defects in individuals with Cri du Chat.

**Aim 1:** Determine predicted and conserved post-translational modifications in SEMA5A protein between different vascular organisms. **Hypothesis:** SEMA5A will have some functionally necessary phosphorylation sites that will be highly conserved. **Approach:** Perform NetPhos 2.0 analysis to predict phosphorylation sites in human SEMA5A protein. Align protein sequences in Clustal Omega to determine the evolutionarily conserved phosphorylation sites in SEMA5A. Use mass spectrometry to validate the predicted phosphorylated sites, *in vivo.* **Rationale:** Several signaling pathways depend on post-translational modifications for activity. Identification of important phosphorylation sites in SEMA5A will help determine the importance of SEMA5A in vascular patterning.

**Aim 2:** Determine unique protein interactions of SEMA5A that contribute to proper vascular development. **Hypothesis:** SEMA5A will interact with proteins involved in signaling pathways that are fundamental for vascular patterning. **Approach:** Perform co-immunoprecipitation on fruit flies with SEMA5A to isolate protein complexes. Mass spectrometry will be utilized to identify subsequent proteins and the protein interaction will then be compared to GO analysis. String Database will be used to visualize the unique protein interactions. The simple vasculature and sequenced genome of fruit flies make it an ideal organism for this experiment. **Rationale:** Several protein interactions functionally contribute to development. Identification of unique protein interactions will help determine the important SEMA5A protein interactions involved in vascular patterning.

**Aim 3:** Identify conserved phosphorylation sites in the Thrombospondin type 1 repeats domain region (a region potentially involved in vascularization) in complex vascular organisms, humans *(Homo sapiens),* and simple vascular organisms, fruit flies *(Drosophila melanogaster)*. **Hypothesis:** SEMA5A will have some highly conserved phosphorylation sites between *Homo sapiens* and *Drosophila melanogaster*. **Approach:** Perform NetPhos 2.0 analysis to predict phosphorylation sites in human SEMA5A protein. Align protein sequences in Clustal Omega to determine the conserved phosphorylation sites in human and fruit fly SEMA5A. Use CRISPR/cas9 to substitute different amino acidsin highly conserved regions. Determine the phosphorylation sites that function in vascularization. **Rationale:** Identification of conserved phosphorylation sites in SEMA5A will help determine the importance of SEMA5A in vascular patterning.

This project attempts to understand abnormal vasculature in Cri du Chat syndrome patients by studying SEMA5A at a genomic and proteomic level. This research could potentially discover patterns that lead to abnormal vascular development in Cri du Chat, Autism, Parkinson’s disease, and mental retardation patients. The goals of this study are to research the significance of SEMA5A in vascular patterning during embryonic development. Ultimately, this research could lead to new strategies for prenatal treatment of abnormal vascularization.

**References**

[1] Cerruti Mainardi, P. (2006). Cri du Chat syndrome. *Orphanet Journal of Rare Diseases*, *1*, 33. doi:10.1186/1750-1172-1-33.

[2] Manning, K. (1977). The larynx in the Cri du Chat Syndrome. The Journal of Laryn﻿gology & Otology, 91(10), 887-892.

[3] Genetic Science Learning Center (2014, June 22) Cri-du-Chat Syndrome. Learn.Genetics. Retrieved January 28, 2015, from [http://learn.genetics.utah.edu/content/disorders/chromosomal/cdc/](http://learn.genetics.utah.edu/content/disorders/chromosomal/cdc/" \o "" \t "_blank)

[4] Fiore, R., Rahim, B., Christoffels, V., Moorman, A., & Puschel, A. (2005). Inactivation of the Sema5a Gene Results in Embryonic Lethality and Defective Remodeling of the Cranial Vascular System. *Molecular and Cellular Biology,* *25*(6), 2310-2319, from <http://mcb.asm.org/content/25/6/2310.full>