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Specific Aims Final Draft 14 April 2015

Emberger Syndrome (ES) is a rare autosomal dominant disorder characterized by the co-occurrence lymphedema with a predisposition to acute myeloid leukemia (AML). Lymphedema is the primary symptom in which fluid is retained and swelling of extremities occurs [1]. Other symptoms associated with ES include warts, deafness and physical anomalies such as neck webbing and slender fingers [2]. While there are multiple treatments to help improve the lives of patients with ES, including complete decongestive therapy (CDT), which decreases swelling and increases lymph drainage from the congested areas, a cure is unknown [3]. ES is caused by a series of eight mutations in the *GATA2* gene, which is a transcription factor necessary for the development and function of hematopoietic stem cells and the regulation of body fluid levels [4]. In ES patients, *GATA2* mutations lead to haploinsufficiency, which is when a patient only receives one functional copy of a gene. Although research has identified the mechanism of GATA2 haploinsufficiency, *how GATA2 regulates the function of genes involved in hematopoiesis remains unclear.*

The long-term goal of our research is to understand the pathology underlying Emberger Syndrome and *GATA2* deficiency. In order to do that, we must first understand the complete role of GATA2 in the development and function of hematopoietic stem cells. Here, we will test the hypothesis that mutations in *GATA2* regulate genes that affect the function of hematopoietic stem cells. To test our hypothesis, we will pursue the following aims:

Specific Aim 1: Identify proteins that interact with GATA2 that regulate hematopoietic function. **Approach:** Use STRING to determine GATA2 interactions and then determine the function of the interacting proteins through the gene ontology enrichment option. **Hypothesis:** GATA2 interacts with proteins involved in various processes related to phenotypes observed in Emberger syndrome, including hematopoiesis and lymphatic system development. **Rationale:** Emberger syndrome arises from mutations in *GATA2* that decrease the amount of protein product, likely limiting protein–protein interactions.

Specific Aim 2: Identify GATA2 interacting genes that are differentially expressed in mutant mice. **Approach:** Analyze wild type and mutant mouse bone marrow samples through the use of RNA sequencing. **Hypothesis:** Interacting genes involved in regulating hematopoiesis and development of the lymphatic system should decrease in expression while there should be minimal change in expression for interacting genes involved in the response to stimuli when *GATA2* protein is not functioning at a normal level. **Rationale:** Bone marrow is involved in hematopoiesis and produces the fluid that builds up in lymphedema, called lymphocytes. In ES patients, GATA2 interacting genes involved in the regulation of these two processes should decrease in expression.

Specific Aim 3: Determine if differentially expressed interacting genes cause ES phenotypes. **Approach:** Knock out genes found to decrease in expression in mutant mice by using CRISPR/Cas9 on wild type mice with fully functional GATA2. **Hypothesis:** Post knockout, mice will have abnormally high myeloblast counts and lymphedema. **Rationale:** Mutations in GATA2 restrict it from normal interactions, resulting in phenotypes observed in ES patients, such as AML and lymphedema.

1) National Lymphedema Network: http://www.lymphnet.org/le-faqs/what-is-lymphedema
2) Ostergaard, Pia et al. (2011). Mutations in *GATA2* cause primary lymphedema associated with a predisposition to acute myeloid leukemia (Emberger Syndrome). *Nature Genetics, 43(10): 929-930.*
3) Mansour, Sahar et al. (2010). Emberger Syndrome – Primary Lymphedema With Myelodysplasia: Report of Seven New Cases.*American Journal of Medical Genetics,* Part A: 2287-2296.
4) AmiGO 2: http://amigo.geneontology.org/rte

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Identifying and characterizing GATA2 interacting proteins that regulate hematopoiesis will provide insight into the pathological processes underlying Emberger Syndrome. Understanding these protein interactions and how they are disrupted in ES will help establish a better basis for research on potential treatments that could enhance the lives of patients.

1) National Lymphedema Network: http://www.lymphnet.org/le-faqs/what-is-lymphedema
2) Ostergaard, Pia et al. (2011). Mutations in *GATA2* cause primary lymphedema associated with a predisposition to acute myeloid leukemia (Emberger Syndrome). *Nature Genetics, 43(10): 929-930.*
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4) AmiGO 2: http://amigo.geneontology.org/rte