**Specific Aims**

 Ehlers-Danlos Syndrome (EDS) is a heterogeneous group of connective tissue disorders with symptoms ranging from frequent joint dislocation to death. Characteristic symptoms include flexible joints, elastic skin, and fragile tissues (1). Vascular EDS, estimated to affect 1 in 90,000 people, is the most severe form of EDS with a median survival age of 51. Most deaths are due to unexpected arterial, intestinal, or uterine ruptures (2). Vascular EDS is caused by a mutation in the gene *COL3A1* that is responsible for the production of type III collagen (3). Type III collagen is an integral component of hollow organ walls. Interestingly, for individuals affected with Vascular EDS, 18% of males die from vascular rupture before the age of 20, whereas only 7% of females die of the same cause by the same age. This contributes to the overall disparity in median survival age between males and females of 46 years and 54 years respectively (2). *Few studies have been performed to identify factors that lead to lethal phenotypes in males more often than females with Vascular EDS during adolescence.*

 My **goal** is to identify factors that influence the male-biased lethality of those affected with Vascular EDS during adolescence. Gender differences in type III collagen production have been shown in mouse models: collagen levels are similar between males and females until puberty, when males exhibit a drastic increase in collagen production (4). In addition, androgen receptors have been shown to mediate the production of collagen in adolescent males but not females. **I hypothesize that hormone receptor activity during adolescence leads to differential protein expression and altered protein interactions in male and female hollow organ walls, contributing to the male-biased lethality of Vascular EDS.** I will investigate my hypothesis with the following aims:

**Aim 1:** Identify transcriptional targets of androgen and estrogen receptors.

**Hypothesis:** Androgen and estrogen, which act as transcription factors when bound to their respective ligands, will have different transcriptional targets, including COL3A1 and other proteins with similar gene ontology such as extracellular matrix components, resulting in different protein expression between males and females.

**Approach:** Use chromatin immunoprecipitation followed by next generation sequencing (ChIP-Seq) to identify the transcriptional targets of androgen and estrogen receptors in the hollow organ walls of adolescent mice (6 weeks).

**Rationale**: Gain an understanding of the regulatory mechanisms that cause gender differences in COL3A1 expression along with other proteins that make up hollow organ walls.

**Aim 2:** Identify proteins in the hollow organ walls of male and female mice and their expression levels at different points during development.

**Hypothesis:** Proteins that are shown to be hormonally regulated, including COL3A1 and other proteins with similar gene ontology such as extracellular matrix components, will be similar in male and female mice until puberty when differential expression will be established and maintained through adulthood.

**Approach:** Perform tandem mass spectrometry on male and female mice hollow organ samples isolated at 2, 6, and 16 weeks of age, corresponding to before, during, and after adolescence.

**Rationale:** Identify proteins that are uniquely expressed in male hollow organ walls during adolescence and thus may contribute to male-biased lethality.

**Aim 3:** Identify protein-protein interactions (PPI) in male and female hollow organ walls during adolescence.

**Hypothesis:** During adolescence, there will be unique PPI in the hollow organ walls of males, including interactions between different collagen proteins, resulting in the male-biased lethality of Vascular EDS.

**Approach:** Use the proteins isolated in Aim 2 including COL3A1 and other proteins that make up hollow organ walls in mice as baits in a TAP-Tag assay to identify protein-protein interactions (PPI).

**Rationale:** Identify PPI that are unique in adolescent males and may lead to male-biased lethality.

 It is important to determine how gender hormone differences regulate protein expression in hollow organ walls, what proteins are expressed in hollow organ walls during different time points in development as a result of gender hormone differences, and how these proteins interact with each other differentially in each gender. This information will provide more details on the mechanism of male-biased lethality of Vascular EDS and ultimately a starting point for drug targets and potential treatments.

References:

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