Classic Galactosemia is characterized by the inability to metabolize the monosaccharide galactose, prompting a buildup of galactose in the bloodstream [1]. Galactosemia is caused by a variety of loss of function mutations in the *GALT* gene, which encodes a galactose-1-phosphate uridylyltransferase, which transfers a UDP between UDP-galalactose and UDP-glucose. [1]. Phenotypes of galactosemia are manifested in a wide variety of symptoms, including premature ovarian insufficiency in females [2]. Galactosemia is typically managed by excluding galactose from the diet, however, many symptoms of galactosemia can surface later in life. In fact, nearly 87% of female galactosemia sufferers displayed premature ovarian insufficiency, despite following a galactose restricted diet from birth [2]. Furthermore, known biochemical markers of galactosemia, namely elevated levels of erythrocyte galactose-1-phosphate and urinary galactitol, do not correlate with the long term outcome of premature ovarian insufficiency. *Further, it is unknown how perturbations in carbon metabolism caused by GALT deficiency cause* premature ovarian insufficiency *to arise despite elimination of galactose from the diet.*

My **primary goal** is to elucidate how *GALT* deficiency can lead to premature ovarian insufficiency and to illuminate the broader role of *GALT* outside its well-known role in galactose catabolism. My **hypothesis** is that previously overlooked metabolic roles of *GALT*, including glycosylation, play a central role in the pathology of classic galactosemia. I will use both the common mouse (*Mus musculus*) and *Saccharomyces cerevisiae* as model systems, due to their similar disease phenotypes to humans and ease of rapid analysis, respectively.

**Aim 1**: **Characterize and identify conserved amino acids of *GALT* that are critical for ovary development.**

**Approach**: I will screen the functionality of known Human *GALT* mutant alleles using a growth assay with *S. cerevisiae*. Next I will use sequence alignment methods to determine if loss of function mutations occur in evolutionary conserved sites. Finally, I will select of subset (mutated in conserved sites vs. non-conserved sites and functional vs. non-functional) of Human *GALT* mutants and use CRISPR/Cas9 to create transgenic mice lines with the various Human *GALT* disease alleles. I will then screen female mice for those that exhibit premature ovarian insufficiency**.**

**Rationale**: Not all females with galactosemia develop premature ovarian insufficiency, thus determining the mutations in *GALT* that lead to premature ovarian insufficiency will allow for better correlation of genotype to phenotype.

**Hypothesis**: I expect *GALT* alleles with mutations in evolutionary conserved sites will display the phenotype of premature ovarian insufficiency, while mutations in non-conserved sites will not.

**Aim 2**:**Characterize deferentially expressed genes across ovarian development in *GALT* deficient mice.**

**Approach**: I will perform RNA-seq on the ovaries of wild type and *GALT* deficient mice throughout ovarian development and into adulthood, mice will be fed a galactose free diet. RNA-seq data will be sorted using GO terminology and compared between both WT and *GALT* deficient mice and between the sampled time points.

**Rationale**: Genes that are deferentially regulated in the ovaries in the absence of *GALT* are possible targets for identifying novel processes that *GALT* may modulate. Further, determining the time-point of gene dysregulation will help elucidate the pathology of premature ovarian insufficiency.

**Hypothesis**: Since males with galactosemia do not exhibit infertility, I expect gene dysregulation to occur after sex determination happens in development. Further I expect genes involved in N- and O-glycosylation, ER stress, and various carbon metabolic pathways to be deferentially regulated.

**Aim 3: Characterize the ovarian glycoproteome of mice and the effects of *GALT* deficiency on the former.**

**Approach**:I will perform glycoproteomics by using mass spectrometry on ovarian tissue of adult WT and *GALT* deficient mice. Proteins that have altered glycosylation will be identified by comparing the glycoproteomes of WT vs. *GALT* deficient mice. Identified proteins will be sorted by their biological process and molecular functions.

**Rationale**: UDP-gal and UDP-glc are common carbohydrate donors for numerous galacto-/glycoproteins and galacto-/glycolipids, thus deficiency in *GALT* will result in aberrant glycosylation and metabolic dysregulation.

Therefore, identifying proteins that have altered glycosylation in ovarian cells of *GALT* deficient mice will provide insight to the pathology of premature ovarian insufficiency and other late life effects of galactosemia.

**Hypothesis**: I expect to see abnormal (hypo, hyper, or newly) glycosylation in the *GALT* deficient mice. This will lead to ER stress and changes of the glycosylation receptors as the cell deals with defective glycosylation.

**References**:

1. Isselbacher KJ, Anderson EP, Kurahashi K, *et al*. Congenital Galactosemia, a single enzymatic block in galactose metabolism. *Science* 1956;13:635–6.

2. Guerrero NV, Singh RH, Manatunga A, *et al*. Risk factors for premature ovarian failure in females with galactosemia. *J Pediatr* 2000;137:833–41

3. Berry GT. Classic Galactosemia and Clinical Variant Galactosemia. Retrived from: <https://www.ncbi.nlm.nih.gov/books/NBK1518/>

4. Berry GT, Moate PJ, Reynolds RA, *et al*. The rate of de novo galactose synthesis in patients with galactose-1-phosphate uridyltransferase deficiency. *Mol Genet Metab* 2004;81:22–30