**Functional impact of CRH on placental transcriptional regulatory networks and birth timing**

**SPECIFIC AIMS**

Known as the “placental clock”, corticotropin hormone (CRH) is produced by the placenta in massive amounts thereby influencing the in utero environment. CRH is traditionally associated with stress related glucocorticoid signaling, but during pregnancy CRH influences placental growth and function, fetal adrenal activation, and maternal serum cortisol levels. Placental CRH modulates metabolic pathways related to fetal growth and development, such as glucose transport(1). Placental CRH is an driver of parturition, and maternal plasma CRH levels are positively associated with gestational length(2) and elevated CRH is predictive of preterm birth(3). In order to understand the molecular mechanisms altered during preterm birth, there is a ***critical need*** to understand the interplay between placental CRH production and gestational length. ***We hypothesize that*** CRHlevels in mid pregnancy alter the placental gene expression landscape, with downstream consequences on placental function and gestational length. Using existing software tools from the Price lab, we have generated a large scale transcriptional regulatory network (TRN) of the human placenta. In this proposed analysis, I will use this TRN to understand the consequences of CRH on the placental transcriptome and on pregnancy timing. This collaborative project will use samples generated as a part of the University of Washington ECHO PATHWAYs analysis; a multi-year NIH center grant focused on examining prenatal environmental exposures, placental transcriptomes, and childhood health outcomes. This study is not designed to interrogate preterm birth, but the rich CRH measurements collected in a cohort of women with mostly asymptomatic pregnancies presents a unique opportunity to investigate the role of CRH in gestational length. This will build upon the skills and techniques that I have acquired in my post doctoral fellowship, and will provide avenues for me to conduct independent research.

**Specific Aim 1(Mentored):** *Construction of Placental TRN using ECHO PATHWAYs data.* The goal of this aim is to construct a genome scale model of transcriptional regulatory networks specific to the human placenta, using transcriptional regulatory network analysis (TRENA), which defines relationships between transcriptional factors and the genes they regulate, produced in a tissue specific manner using DNAse hypersensitivity data from the placenta. We will use the transcriptomics data from the ECHO cohort (N=1200) to construct a new placental TRN (Aim1A). We will perform in vitro validation the utility of our TRN by knocking out transcription factors within JEG3 placental derived cells, and quantify corresponding changes to gene expression in their target genes (Aim 1B). This aim will produce a genome scale map of gene regulatory connections, which can be utilized by a large community of placental biology researchers.

**Specific Aim 2 (Mentored):** *Identification of shared and distinct genes associated with CRH in mid-late gestation and gestational length*. We hypothesize that both maternal plasma CRH and gestational length are associated with changes in gene expression, and that there may be overlaps in this differential gene expression between these phenotypes. Using transcriptomics data from the ECHO cohort (N=1200), we will use a series of linear regression models implemented within EdgeR to identify differentially expressed genes related to gestational length (Aim 2A) and plasma CRH in both mid and late gestation (Aim 2B). With these gene lists, we will identify the intersection of these genes, and use enrichment analysis to identify shared and distinct molecular pathways (Aim 2C). This aim will deliver a list of genes and pathways in which gene expression is altered in relation to both CRH expression and gestational length.

**Specific Aim 3 (Independent):** *Leveraging the placental TRN to identify shared regulatory drivers mediating expression of both CRH and gestational length***.** We hypothesize that there are shared and distinct transcriptional drivers which regulate the expression of the differentially expressed genes related to both CRH and gestational length. In aim 3A, using our placental TRN, we will identify transcription factor (TF) modules; which are networks of genes and transcription factors which are regulatory drivers of a list of genes. We will identify overlaps between the TF modules related to genes associated with maternal plasma CRH and to gestational length identified in Aim 2. In aim 3B, we will validate the influence of CRH on these transcription factor modules in vitro by treating JEG3 placental cells with exogenous CRH, and quantifying changes in genes in these TF modules. By finding overlapping modules between between these 2 pathologies, we will gain insight into how transcriptional regulation by CRH may influence gestational age.

**Expected Outcomes:** Through these aims, I will perform the first thorough characterization of relationships between plasma CRH and placental transcriptomics**.** This will provide crucial insights into underlying biological mechanisms altered in response to CRH, and compare them to mechanisms related to gestational age, which may provide actionable targets for therapeutic intervention in preterm birth. I will increase my data analysis skills, building upon the strengths of my mentors in a way that is independent of their work. I will build a pipeline which can be used to interrogate relationships between other data generated in the ECHO cohort which can be used for subsequent R01 submissions, allowing me to establish an independent line of research.