Congenital hypothyroidism (CH) is an autosomal recessive hormone disorder resulting in a partial to nonfunctional thyroid gland. [1] One in every three thousand newborns are diagnosed with CH. [2] Without early newborn screenings and hormone replacement therapy untreated babies develop permanent intellectual disabilities and disrupted metabolisms. [3] Paired Box 8 (PAX8) is a transcription factor that regulates the thyroid stimulating hormone receptor (TSHR), which is involved in the development of the thyroid gland and thyroid associated metabolic processes. CH consists of multiple thyroid gland phenotypes ranging from mislocation, reduce size, to malformations. [4] The various phenotypes associated with CH are conserved among mammals, yet **no genotypes within the PAX8 gene have been identified in either human or mice model for each phenotype associated with CH.**

**Hypothesis: Each thyroid gland phenotype associated with CH has a distinct PAX8 genotype.**

**Primary Goal**: Investigate thyroid gland genotypes and identify specific nonsynonymous mutations associated with each phenotype (mislocation, reduce size, malformation) within the coding regions of PAX8.

**Long-term Objective**: Create PAX8 genotype-specific assay that corresponds to each thyroid gland phenotype to the improved hereditary predictions during the prenatal and newborn screening process.

**Aim 1**: Identify conserved genotypes for each thyroid gland phenotype that results in CH.

**Hypothesis**: Mice representing all the thyroid gland phenotypes carry identical mutation corresponding each phenotype (mislocation, reduced size, malformation).

**Approach:** Use Next Generation Sequencing to identify nonsynonymous mutations in the PAX8 coding regions in mice with various thyroid gland phenotypes associated with CH. Sequences will be aligned using CLUSTAL Omega, this will provide consensus genotypes for each thyroid gland phenotype.

**Rationale**: Genotype data for each thyroid gland phenotypes can lead to more accurate diagnosis and personalized treatment options for newborns with CH.

**Aim 2:** Introduce mutant PAX8 genotypes in wildtype (WT) mice to reproduce each thyroid gland phenotype to assess heritability of CH genotypes.

**Hypothesis:** Parental mice with integrated mislocation PAX8 genotype will develop a specific phenotype such as a mislocated thyroid gland and pass on both genotype and phenotype to offspring. Same will be true for all PAX8 mutant genotypes will develop their respective phenotypes in subsequent generations in a 3:1 ratio because of CH autosomal recessive nature.

**Approach:** Mutagenize mice using CRISPR- CAS9 Screen to integrate identified PAX8 genotypes to induce specific thyroid gland phenotypes. To determine heritability patterns of CH PAX8 mutant parent mice will be crossed to WT mice. Genotype and phenotype of offspring will be recorded and hopefully match that of their parents.

**Rationale**: CRISPR-CAS9 screen will confirm specific PAX8 genotypes cause a respective phenotype in replicate when testing heritability in mice models.

**Aim 3:** Determine protein interactions of PAX8 to TSHR for each thyroid gland phenotype.

**Hypothesis:** PAX8 and TSHR proteins should localize in the thyroid gland at different quantities and

abundance compared to WT mice model.

**Approach:** Use Mass Spectroscopy (MS) to determine the biological interactions of PAX8 and TSHR

proteins from mice with various thyroid gland phenotypes.

**Rationale** MS displays high confidence identifications of protein-protein interactions for each

thyroid gland phenotype.

**Conclusion:** CH is unique a hormone disorder with multiple phenotypes that result in the same

diseased thyroid gland with a one treatment option being hormone therapy starting infancy. A greater

genomic knowledge of CH can enhance diagnostic practices and personalized treatment

options for newborns can result of this study.

**Reference:**

[1] Ramos, H. E., et al. "Extreme phenotypic variability of thyroid dysgenesis in six new cases of congenital hypothyroidism due to PAX8 gene loss-of-function mutations." *European Journal of Endocrinology* 171.4 (2014): 499-507.

[2] Magliano, M. Pasca Di, R. Di Lauro, and M. Zannini. "Pax8 has a key role in thyroid cell differentiation." *Proceedings of the National Academy of Sciences* 97.24 (2000): 13144-3149.

[3] Park, S. M. "Genetics of congenital hypothyroidism." *Journal of Medical Genetics* 42.5 (2005): 379-89.

[4] Tell Gianluca, et al. "Structural defects of a Pax8 mutant that give rise to

congenital hypothyroidism." *Biochemical Journal* 341.1 (1999): 89.