Alkaptonuria is an autosomal recessive genetic disorder where patients develop joint pain early in life, followed later by hearing loss, calcification of the heart valves, and increased risk of kidney and gallbladder stones [1]. Alkaptonuria is caused by mutations in the HGD gene, which is responsible for the breakdown of homogentisic acid. Homogentisic acid is a by-product in the pathway that synthesizes phenylalanine and tyrosine, and is converted into a melanin-like protein that is deposited onto the collagen of various tissues. This inhibits the tissue’s functioning and stains it black [1]. The HGD gene is highly expressed in the prostate, and some cases of alkaptonuria result in prostate stones [1]. *It is unknown what role HGD plays in the metabolic functioning of the prostate gland.*

My **primary goal**is to determine the role of HGD in amino acid metabolism in the prostate. I will use the mouse (*Mus musculus*), as it does not suffer from the plaques associated with alkaptonuria but still exhibits high urinary levels of homogentisic acid. Mice also have analogous prostate anatomy to humans. I **hypothesize** that HGD-mutant on a high-tyrosine diet will exhibit the formation of prostate stones, and will also exhibit higher levels of HGD expression. My **long-term goal** is to better understand the role of HGD in prostate function.

Aim 1: Identify HGD mutations that result in severe prostate stone phenotypes.

**Approach:** I will identify and align HGD homolog protein sequences to identify several highly conserved regions. Then, using CRISPR/Cas9, I will create male mouse mutants that have one of the identified regions mutated and feed them a diet high in tyrosine [2]. A control group will also be fed a standard diet. At the end of their natural lifespan, they will be assessed for the presence and quantity relative to body mass of prostate stones. **Rationale:** Different HGD mutations in humans can result in phenotypes with different amounts of severity, and a mouse model would be useful for understanding how different mutations can relate to risk for prostate stones in humans. **Hypothesis:** I hypothesize that only the mice fed the high tyrosine diet will have prostate stones, and that the amount of prostate stones will vary across the different mutations.

Aim 2: Identify differences in prostate gene expression in HGD mutants

**Approach:** Using the same lines of mice created in the first aim, I will use RNA sequencing to quantify expression of genes by isolating prostate tissue from wild type and HGD mutant male mice at different age classes fed on normal and high tyrosine diet. The differences in gene expression will then be sorted by GO terms to determine which biological processes are affected in male mutants. Genes from this list will then be validated by generating mice lines with those genes knocked out via CRISPR. Mice from those lines will also be assessed for prostate stone development. **Rationale:** Identifying how gene expression responds to high homogentisic acid levels can help narrow down or identify possible causes of prostate stones in HGD-mutant individuals, as well as uncover previously unknown roles the gene may play. **Hypothesis:** I hypothesize that genes involved with metabolic breakdown pathways will have their expression increased.

Aim 3: Analyze the importance of protein phosphorylation locations in HGD function

**Approach:** I will use NetPhos to identify phosphorylation locations on the mouse and human HGD protein sequences [3]. Where the phosphorylation locations are identical for both species, I will generate lines of mice where the phosphorylation location is mutated, and their prostate stone phenotype on normal and high-tyrosine diets will be assessed, as per the first aim. **Rationale:** Phosphorylation sites are important for the function of many proteins, and a protein as small as HGD might be highly susceptible to a change in just one site. **Hypothesis:** I hypothesize that mutations at phosphorylation sites will cause an impediment to HGD function, but will only do so significantly when the site occurs in a highly conserved (as identified in the first aim) region of the sequence.

References:

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[3] Blom, Nikolaj, Steen Gammeltoft, and Søren Brunak. "Sequence and structure-based prediction of eukaryotic protein phosphorylation sites1." *Journal of molecular biology*294.5 (1999): 1351-1362.