Glucose-6-Phosphate-Dehydrogenase deficiency (G6PDd) is an X-linked enzyme disorder that causes severe hemolytic anemia when triggered by external factors, such as the antimalarial Primaquine [1]. Loss of G6PD enzyme function causes weakening of red blood cells, as the cells are no longer able to produce reducing agent NADPH, making the cells susceptible to toxic levels of reactive oxygen species. Unfortunately, Primaquine – the only drug available that can fully treat malaria– causes hemolytic anemia in G6PD deficient individuals [2], which is a major obstacle for malaria eradication. Primaquine is never prescribed to pregnant women due to the unknown risk of G6PD deficiency and increased severity of anemia-related health risks during pregnancy. Methylation of G6PD near the 3' end of the gene is associated with a high level of G6PD expression in males [3], however, epigenetic modifications are different between sexes [4], and **it is unknown** how methylation affects G6PD activity in pregnant females. Finding a methylation pattern that maintains normal levels of G6PD expression and protection of red blood cells could have substantial implications for future treatment of malaria during pregnancy.

My **primary goal** is to determine how methylation of specific sites on the G6PD gene will increase NADPH production in pregnant G6PD deficient females. I will test the **hypothesis** that G6PD expression and NADPH production can be controlled by regulating methylation of the G6PD gene, using zebrafish as a model organism because G6PD deficient zebrafish suffer from hemolytic anemia like humans, and the red blood cell phenotypes are easy to see in transparent zebrafish. My **long-term** goal is to evaluate the importance of epigenetic modifications as a treatment for G6PD deficiency and to improve treatment options for malaria during pregnancy.

Aim 1: **Identify conserved methylation sites of G6PD associated with normal levels of NADPH production in pregnant females**

Hypothesis: There will be a distinct and conserved pattern of G6PD methylated sites across homologous organisms of pregnant females. Differences in the methylation pattern will be associated with a difference in NADPH levels in pregnant females.

Rationale: High levels of methylation on G6PD sites near the 3' end is associated with a high level of G6PD expression in males. It is possible that a specific methylation pattern of the G6PD gene also plays a role in G6PD gene expression and NADPH production in females and pregnant females.

Approach: Use bisulfite-sequencing to compare the methylation pattern in male, non-pregnant female, and pregnant female zebrafish with normal and abnormal levels of NADPH. Next I will identify if the methylated sites are conserved in the three groups using ClustalOmega, knockout the most conserved amino acids using CRISPR, and determine if there are distinct methylation patterns for pregnant zebrafish that are correlated with different levels of NADPH.

Aim 2: Identify a small molecule that affects G6PD methylation and can be taken with Primaquine in pregnant females.

Hypothesis: There will be a small molecule that modifies G6PD methylation in pregnant females, resulting in stable NADPH levels when Primaquine is administered.

Rationale: As epigenetic modifications can be altered by environmental factors, there may be another small molecule that can modify G6PD methylation, resulting in a level of G6PD enzyme that can safely accept Primaquine treatment.

Approach: Utilize bisulfite-sequencing to compare the G6PD methylation in G6PD deficient pregnant zebrafish. Screen the Medicines for Malaria Venture Pathogen Box (400 small molecules) for a methylation altering drug [5]. After chemical screen of red blood cells, malaria parasite, and Primaquine to the molecules, I will assess whether or not the malarial parasite was destroyed and if there was hemolysis. Use bisulfite sequencing again to compare methylation patterns before and after treatment.

Aim 3: Determine differences in protein interaction complexes required for NADP metabolism between methylated and unmethylated G6PD deficient pregnant zebrafish.

Hypothesis: Unmethylated G6PD will have reduced protein interactions, leading to less NADPH production. I hypothesize that the mechanism by which methylation sites influence NADPH production is through the alteration of interactions between G6PD and other proteins involved in NADP metabolism.

Rationale: In normal individuals, G6PD works with eight other proteins in the NADP metabolism process. I want to elucidate if methylation affects the protein-protein interaction network responsible for NADPH production.

Approach: Use CRISPR to knockout methylation sites in the treatment group. For treatment and control (no knockout) groups, G6PD fragment-containing complexes will be copurified with the affinity-tagged G6PD-fragment proteins and analyzed using Mass Spectrometry. I will then compare the pregnant zebrafish to male and non-pregnant females.

References:

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[4] Hall, E. (2014). "Sex Differences in the Genome-Wide DNA Methylation Pattern and Impact on Gene Expression, microRNA levels, and Insulin Secretion in Human Pancreatic Islets." *Genome Biology* 15(12): 522.

[5] "The Pathogen Box." Medicines for Malaria Venture, Medicines for Malaria Venture, 2018, www.mmv.org/research-development/open-source-research/pathogen-box.