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 in females 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.

**Approach:** Use high throughput bisulfite-sequencing to compare the methylation pattern in male, non-pregnant female, and pregnant female zebrafish with normal and abnormal levels of NADPH. I will then identify if these sites are conserved using ClustalOmega, knockout the most conserved amino acids using CRISPR, and determine if there are distinct methylation patterns for pregnant zebrafish, compared to male, non-pregnant female zebrafish resulting in different levels of NADPH.

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

**Hypothesis:** There will be a small molecule that modifies G6PD methylation in pregnant females so that NADPH levels remain stable 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 high throughput bisulfite-sequencing to compare the G6PD methylation in all G6PD deficient pregnant zebrafish. I will then screen the Medicines for Malaria Venture Pathogen Box (400 small molecules) for a methylation altering drug [5]. After 24 hours of chemical exposure 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. I will then use bisulfite sequencing once again to compare the methylation patterns before and after treatment.

**Aim 3: Determine differences in protein interaction complexes required for NADP metabolism between methylated G6PD deficient pregnant zebrafish and unmethylated G6PD deficient pregnant zebrafish. Hypothesis:** Specific methylation patterns will enhance the interactions between G6PD enzyme and other proteins that play a role in the NADP metabolism process. I predict the unmethylated G6PD enzymes will have reduced protein interactions, leading to less NADPH production.

**Rationale:** Aside from G6PD, 8 other proteins are involved 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 groups I will quantify protein interactions using affinity pulldown followed by mass spectrometry. G6PD fragment- containing complexes will be copurified with the affinity-tagged G6PD-fragment proteins and analyzed by Mass Spectrometry.

References:

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[2] Baird K. (2015). “Origins and Implications of Neglect of G6PD deficiency and primaquine toxicity in Plasmodium vivax malaria”. *Pathogens and Global Health* 109(3): 93-106.

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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.

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