Waardenburg syndrome type 2A (WS2A) is a rare autosomal dominant syndrome that affects the auditory system and causes pigmentation abnormalities in the skin, hair, and eyes. WS2A is caused by the loss of the MITF gene, which is important in the transcriptional activation of the tyrosinase gene and melanocyte differentiation [1]. Mutant MITF phenotypes can include full or partial hearing loss, a patch of white hair in the front hairline, and brilliant blue coloring of eyes [1]. In some cases of WS2A, only one eye is phenotypically affected by the mutation. In these WS2A patients, they fail to activate tyrosinase, a melanocyte-specific enzyme [1], *however little is known about MITF’s role in melanocyte differentiation in the eyes*.

My **long term goal** is to understand the incomplete penetrance of MITF mutations in the role of melanocyte differentiation in the eyes. My **primary goal** is to better understand MITF’s role as a transcriptional activator of the tyrosinase gene. My **hypothesis** is that MITF’s binding to the tyrosinase promoter is affected differently by different mutations resulting in incomplete penetrance in an individual.

**Aim 1: Identify the domains necessary in MITF for DNA binding and transcriptional activation of the tyrosinase promoter.**

**Approach:** Using domain analysis, different protein constructs will be made by deleting one domain at a time, or leaving only one domain and injecting them in zebrafish embryos. Using RNA-seq, cells will be tested for the expression level of the tyrosinase protein in the eyes. **Hypothesis:** The HLH domain, a DNA binding domain, will be the only domain to affect the binding of MITF to the tryosinase promoter effectively reducing expression seen. **Rational:** Learning which domains are important for MITF’s function will provide insight into the incomplete penetrance in MITF mutant individuals.

**Aim 2: Determine the strength of MITF binding domain, HLH, to the tyrosinase promoter.**

**Approach:** Using CRISPR/Cas9 in zebrafish, we will create different mutations in the HLH domain. Using EMSA (electrophoretic mobility shift assay), we will analyze the binding affinity of MITF to the tyrosinase promoter in each mutants. **Hypothesis:** Some mutations will weaken binding affinity more than others, with the result of some full phenotypic mutants and some partial phenotypic mutants. **Rationale:** This information is important for understanding if binding affinity has a role in the severity of the penetrance of MITF mutations.

References

1. Shi Y, Li X, Ju D, Li Y, Zhang X, Zhang Y. A novel mutation of the MITF gene in a family with Waardenburg syndrome type 2: A case report. Experimental and Therapeutic Medicine. 2016;11(4):1516-1518. doi:10.3892/etm.2016.3042