Waardenburg syndrome type 2A (WS2A) is a rare autosomal dominant syndrome that affects the hearing and also leads to benign pigmentation abnormalities. Waardenburg syndrome is caused by the loss of the MITF gene, which is necessary for the transcriptional activation of the tyrosinase gene that induces melanocyte, pigmentation forming cell, differentiation [1]. Mutations in MITF can often cause a patch of white hair and brilliant blue coloring of eyes, and sometimes only one eye is affected [1], *but the molecular mechanisms for this incompletely penetrant phenotype in the eyes is unknown*.

My **long term goal** is to determine why mutations in MITF lead to incomplete penetrance of aberrant melanocyte differentiation in the eyes. My **primary goal** is to understand the role of MITF as a transcriptional activator of the tyrosinase gene. I will use mice as model organisms as they are easy to modify and show lack of pigmentantion in the eyes, in a short time period. My **hypothesis** is that MITF binds to the tyrosinase promoter differently during melanocyte differentiation during eye development in Waardenburg patients.

**Aim 1: Identify which MITF domains are necessary for the transcriptional activation of the tyrosinase promoter during eye development.**

**Approach:** Using domain analysis, different protein deletion constructs will be constructed by deleting one domain at a time, and injecting them in mice. Embryos with single eye pigmentation will be analyzed using fluorescence in situ hybridization on eye tissues to test for expression of tyrosinase in the eyes. **Hypothesis:** Loss of the HLH domain, a DNA binding domain, will lead to the differential pigmentation in the eyes because tyrosinase expression will decrease. **Rational:** Identifying MITF domains that are important for tyrosinase expression is important for understanding incomplete penetrance in MITF mutant individuals.

**Aim 2: Determine if melanocyte differentiation genes or eye development genes expression is affected by MITF.**

**Approach:** Mutations in the HLH domain will be created using CRISPR/Cas9 in mice. The resulting mice that contain two different color eyes, and WT mice, will be assessed using RNA-seq to test for global gene expression. The data between WT and mutants will be compared to discover any changes. **Hypothesis:** There will be a decrease of gene expression of melanocyte differentiation genes, and similar expression of eye development genes. **Rationale:** Understanding the changes of gene expression can help to understand the roles MITF takes within a cell and the pathways it is involved in.

**Aim 3: Discover other eye development proteins that MITF interacts with during development.**

**Approach:** Eye cells from wildtype (WT) and mutant mice will be collected and analyzed using co-immunoprecipitation, followed by mass spectrometry, to compare the protein interactions between the WT and MITF mutants. **Hypothesis:** There will be different protein interactions for MITF between the WT and mutant drosophila. A noticeable decrease in interactions with melanocyte differentiation proteins may be seen, as MITF will no longer be functioning in this role. **Rationale:** Discovering the differences of protein interactions can help explain what molecular pathways are being affected in a MITF mutant, and if MITF plays a bigger role in eye development.

These approaches are aimed towards understanding MITF’s role in transcriptional activation and melanocyte differentiation. The findings from these studies will help to understand the molecular processes effected in WS2A and similar disease.

**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
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