**SPECIFIC AIMS** – Selene Clay

Evolution of *EPAS1* Under Hypoxic Conditions in High Altitude Populations

Human populations and other mammalian species have been able to effectively adapt to high altitude environments, despite increased UV exposure, lower temperatures, limited food, and reduced oxygen availability which can lead to hypoxia. One human population that has successfully adapted to living in a high-altitude population is the indigenous Tibetan population, who have thrived in a hypoxic environment at an altitude exceeding 4,000m for thousands of years1–3. Several genetic variants have been identified that may contribute to adaptation to high altitude in Tibetans. One of the most notable variants is of the Endothelial PAS Domain Protein 1 (EPAS1), which has been shown to be a target of selection in Tibetans4,5 and was likely introduced into the Tibetan gene pool from Denisovan-like individuals6.

*EPAS1* is a chief component of the hypoxia-inducible factor transcriptional system and encodes for the transcription factor HIF2α, which plays a role in responding to oxygen, is central in coordinating a response to hypoxia, and aids in the stimulation and production of red blood cells5,7. Variants of this gene are also associated with adaptation to hypoxia in other mammalian species, such as the yak8, plateau zokor 9, Tibetan cashmere goat10, and Tibetan wolf11. However, despite the important role variants in this gene have towards multiple species’ fitness under hypoxia, little is known about the relationship between the sequence variation in *EPAS1* and physiological adaptation. I propose to better understand this relationship by identifying potential regions important for hypoxia adaptation in the Tibetan *EPAS1* variant and to compare how this variant, as well as ones selected for through a deep mutational scan, allow for increased fitness on cells under hypoxic conditions.

**Specific Aim 1: Comparison of *EPAS1* sequence in high-altitude populations and low-altitude relatives to characterize sites important for hypoxia adaptation**

I plan to analyze the sequence of *EPAS1* from Tibetan, Denisovan, and current low-altitude population genomes through alignments to identify the amino acids and regions putatively important for hypoxia adaptation. Additionally, I plan to look at orthologous sequences to determine which regions across the protein are invariant and which are more variable in order to characterize the variability of the regions that these functionally important variants fall in.

**Specific Aim 2: Characterization of Tibetan and introduced variants of *EPAS1* in cells under hypoxic conditions**

**Aim 2a:** To examine the effect of the Tibetan *EPAS1* variant on cells under hypoxic conditions, I will use CRISPR/Cas9 to introduce this variant into human endothelial lung cells. I will then compare the survival and proliferation of these cells with and without the variant under hypoxic conditions similar to that experienced at high-altitude to determine the effect of the variant on cell vitality.

**Aim 2b**: In order to test for selection for survival under hypoxia, I will then perform a deep mutational scan to introduce every possible amino acid change within the human EPAS1 protein in endothelial lung cells. These cells will be plated under hypoxic conditions to select for variants that allow for survival and proliferation under low-oxygen stress in order to compare the effect of the most optimal variants to the Tibetan variant on cell vitality.

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1) What is the theme of the proposed research? What are the major questions to be addressed?

This project is looking at the evoltion and diversification of the EPAS1 gene in various human population in order to understand the mutations responsible for the hypoxia resistance found in tibetan populations through a mixture of bioinformatic and wet lab experiments.

2) Do the methods and preliminary analyses used integrate concepts that lie at the interface of molecular evolution and biochemistry? Do they address the questions being asked?

The quesitons being asked and the methodology used seen to be more focused on the population genetics of the evolution of the particular protein. Even though there is no analysis of the structure of the protein, it is still focused on the evolution and elucidation of how these mutations affect its function.

3) What major improvements would you suggest?

I would highly suggest adding a section on the mechanism of how the protein enables it to tolerate lower oxygen enviorments. It would help the argument and seperate it a bit from the research done by Rasmus Nielsen at Berkeley who looked at the population genetics of the tibetans and give the project a unique twist.

Let me know if you need any information or help – feel free to email me or talk to me.