Severe Combined Immunodeficiency Disorder (SCID) is an immunological deficiency disorder disturbing T-, B- and NK-cell function and consequently compromising antibody responses[1]. Around 15 % SCID cases are due to a mutation in the Adenosine Deaminase (ADA1) enzyme. ADA1 deficiency *disrupts adenosine metabolism* & causes an *accumulation of adenosine* deoxyribonucleotides (dATPs) inhibiting ribonucleotide reductase activity essential for cell proliferation and DNA repair [2]. There is growing evidence that ADA-SCID is involved in various non-immunological defects. These include non-infective, *adenosine metabolic disorders*, such as neurocognitive, auditory, and pulmonary airway dysfunction[3][4]. It is *still unclear* how the *adenosine metabolism disruption* contributes to these pulmonary airway abnormalities.

**My primary goal:** is to determine the role of *ADA1 deficiency* in pulmonary dysfunction.A Danio rerio (zebrafish) model will be used to first study gills as they share the conserved respiratory function of lungs, have an ADA homolog (ada), produce many offspring frequently, and their transparent, externally developing embryos facilitate *in-vivo* modifications to observe potential differential gill development/inflammation. I will also use a Mus musculus (mouse) model because they share very similar lung physiology, have exhibited severe pulmonary inflammation in ADA-deficient lines[6], and I will be able to conduct a *transfer impedance* experiment to test for normal lung function[5]. I **hypothesize** that accumulated levels of adenosine increases cell signaling pathways responsible for hyper-constriction of the lungs and increased inflammation. **My long-term goal:** is to better understand the role ADA1 has on the lungs.

**Aim 1:** Determine conserved amino acid sequences in ADA1 necessary for normal pulmonary/gill function. **Approach:** I will use ENSEMBLE and ClustalWOmega to obtain and align homologous ADA1 protein sequences from different model organisms to identify conserved regions of ADA1 homologs that are only in organisms with lungs or gills. I will then use CRISPR-Cas9 to knock out the conserved zebrafish ada regions and search for individuals showing deficient gill development/inflammation. **Rationale:** Identifying ADA knockout zebrafish with gill dysfunction will confirm the conserved, non-immunological, role of adenosine in respiration, also being the main role of the pulmonary system. **Hypothesis:** The ADA1 knockout zebrafish will show deficient gill development due to the increased levels of adenosine.

**Aims 2:** Use RNA-Seq on ADA1 knockout zebrafish to identify differentially expressed transcripts **Approach:** I will sequence isolated zebrafish RNA from both a control ada+/ada+ group and from the zebrafish of aim 1 that showed gill deficiencies. I will next Gene Ontology to identify clusters of gill development/functional genes that are lost or downregulated in the ada knockout group. Next, I will use CRISPR-Cas9 to knockout the mice homologs of the differentially expressed transcripts from aims 1 and test for signs of pulmonary defects through a *transfer impedance test*. **Rationale:** Online literature indicates ADA1 is linked to lung alveolus development and negative regulation of inflammatory response, two pathways affected in pulmonary dysfunction[7]. Identifying expression changes in these transcripts will demonstrate the role of adenosine in lung dysfunction. **Hypothesis:** I expect to see pulmonary irregularities in the new knockout mice.

**Aims 3:** Identify novel protein interactors with ADA1 mutants. **Approach:** I will use In-Vivo SILAC labeling on wt+ vs ADA knockout mice from Aims 2 with a “heavy isotope” 13 Carbon Lysine diet fed to the ADA knockout group. Lysated protein isolates from both groups will be analyzed by *high-resolution liquid chromatography-tandem mass spectrometry* (nLC MS/MS) to identify the differences in protein ratios of the control and mutated groups. **Rationale:** Ubiquitination is known to have a regulatory role during periods of chronic inflammation in the lungs[9]. Assessing the protein ratios of ubiquitin in both wt+ vs ADA mutants could lead to a better understanding of the correlation between concentrations of adenosine and the positive anti-inflammatory responses ubiquitin brings to the lung cells. **Hypothesis:** I hypothesize that ubiquitin protein ratios will be significantly lower in the 13 Carbon Lysine metabolic diet of the ADA mutant mice group caused by indirect signaling of adenosine on a ubiquitin-like protein messenger.

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