Griscelli Syndrome Type II (GS) is a genetic disorder where patients have silvery skin and hair and immunodeficiency.[1,2] The disease has an autosomal recessive inheritance pattern. Type II is the most common form of GS and immunodeficiency is what sets it apart from the other types.[3] Partial albinism means that patients have silvery hair due to pigment being trapped in the hair follicles in big clumps. Similarly, in melanin producing cells the pigment gets trapped and is never exported. Type II is caused by mutations in the gene *RAB27A*. The protein encoded by this gene is a GTPase and is involved with the export of cellular products to the outside of the cell.[3] Failure to export pigment explains the observed abnormalities in hair and skin. GS patients have normal lymphocyte counts and yet are unable to fight off infections.[1,2] The link between *RAB27A* and immunodeficiency is that when the lymphocytes are unable to export components of the lysosome they are unable to fight off infections.[3] The current treatment is a bone marrow transplant to extend lifespan beyond a few years. Still unknown is why GS has very specific phenotypes when expression through the body is currently understood to have little tissue specificity.

My **objective** is to clarify the specific role of *RAB27A* in its transport pathway and why loss of its function results in such tissue specific symptoms despite the gene being expressed in most parts and cell types of the body. I **hypothesize** that the *RAB27A* mutants will show tissue specific differences either in the mRNA transcription profile or protein interactions when compared to wild type individuals. Mice will be used as a model organism for experiments because they have previous use in studies on RAB27A, the phenotype is easy to observe, and the mouse copy of *RAB27A* has already been mutated in lines for studies.[5] In mice the RAB27A mutant phenotype is referred to as ashen because the mice have silvery fur as the mouse equivalent to partial albinism associated with GS in humans. The **long-term goal** is to uncover mechanisms for successful exocytosis in the absence of functioning RAB27A protein.

Aim Number 1 – Identify essential amino acid differences in individuals with loss of *RAB27A* function.
Approach: A library of guide RNAs alongside the CRISPR/Cas9 system will be used to carry out a mutagenic screen on wild type (WT) mice. The library of guide RNAs will target different spots in the *RAB27A* gene in different mutant mice. The mutagenized mice will be visually assayed for the ashen phenotype, silvery fur, to identify mutants lacking functional RAB27A proteins. The ashen mice will then have their genomes sequenced to determine the nature of the mutation that resulted in the phenotype and which amino acids were affected.
Rationale: The very simple architecture currently understood for the *RAB27A* gene lacks multiple distinct domains to analyze. What can be done instead is to probe the currently understood single *RAB27A* domain in an attempt to understand which specific amino acids have reactivity important to function of the protein. In the mutagenic screen mutagenized individuals will have their gene altered at different locations due to difference in the guide RNA. Only mutations of certain amino acids will result in loss of protein function and those essential amino acids will be reveled when the identified mutants have their genes sequenced.
Hypothesis: Single nucleotide polymorphisms in key parts of the sequence of *RAB27A* will lead to loss of function mutations and the known reactivity of those amino acids will allow the generation of further hypothesis of which chemical reactions in the cell are part of wild type *RAB27A* function and if tissue specific chemical conditions might play a role in the tissue specific symptoms of GS.

Aim Number 2 – Identify differently transcribed genes that are implicated in exocytosis failure.
Approach: Using RNA-Seq transcription will be compared between wild type and ashen mice to seek out genes that differentially expressed in the mutant mice. Differentially expressed genes will then be sorted using gene ontology (GO) to identify those specifically relevant to exocytosis. CRISPR will then be used for targeted knock out of differentially expressed genes related to exocytosis by GO in WT mice. The resulting mutants will be visually assayed for silvery hair indicating the ashen phenotype to confirm that loss of these differently transcribed genes indeed results in partial albinism which indicates failed exocytosis.
Rationale: Differently expressed genes in ashen mutants vs wild type mice can be identified giving insights as to if functioning RAB27A is involved in the regulation of any other genes which might have tissue specific roles or expression.
Hypothesis: Genes differentially expressed in ashen mutants will show tissue specific roles or expression involving hair and the immune system which may play a role in the tissue specificity of GS symptoms.

Aim Number 3 – Identify differences in proteins interacting with RAB27A as part of the exocytosis pathway.
Approach: BioID tagging of interacting proteins will be used in both WT and ashen mutant mice to compare protein interactions with the RAB27A protein and look for differences. By coupling biotin ligase to RAB27A, functional protein for WT and nonfunctional protein for mutants, interacting proteins can be tagged. Differently interacting proteins will show up by differences in their biotinylation when compared to the wild type situation. Differently interacting proteins will be sorted using GO to identify if any are involved in exocytosis. I will then compare differently interacting proteins with the current STRING interaction network to see if any new protein interactions are discovered and to see if in the ashen mutants nonfunctional RAB27A is still forming its complex with MYO5A and MLPH.
Rationale: Studying interacting proteins in wild type mice allows us to clarify the currently understood network of RAB27A interactions. Differently interacting proteins can be discovered and expand the known network of RAB27A protein interactions to further understand the transport mechanism RAB27A participates in. If mutant RAB27A no longer forms its complex with MYO5A and MLPH then we will help clarify the exact nature of the failed exocytosis in mutants. If mutant RAB27A interacts with new proteins, then those newly identified proteins may help clarify why certain parts of the body are more affected by the change in RAB27A.
Hypothesis: Differentially interacting proteins in mutant RAB27A networks will show tissue specific roles or expression involving hair and the immune system which may play a role in the tissue specificity of GS symptoms.

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