Congenital Lactase Deficiency (CLD) is a severe, infant-onset form of lactose intolerance that leads to diarrhea1. This disease is caused by autosomal recessive mutations in the lactase enzyme gene LCT2. There are at least nine identified mutations, of which most are nonsense mutations3,4,5,6. Truncated LCT proteins cause obvious structural and functional problems, but it is *unknown how the single amino acid substitutions in the C-terminal glycosyl-hyrdolase domain, such as R1578H, disrupt lactase function.* Studying the evolution of LCT and its conserved regions can also help elucidate important functional protein domains. Mutations in the conserved lactase activity region could affect the overall charge and pH, impacting the structure of the folded protein and its subsequent trafficking and interactions. This study will demonstrate how a single amino acid substitution can lead to disease.

The **overall objective** is to understand how the mutation R1587H (arginine to histidine substitution at 1587) affects the structure, function, and interaction of LCT to understand how it may hinder the enzyme function. The **central hypothesis**is that the R1587H mutation will cause significant changes in the structure and interactions of lactase, resulting in loss of function. The mutation lies in conserved activity region of lactase and causes an amino acid change that could impact the overall charge and pH, changing the structure and hindering the interactions. This study will use the model organism mouse (*M. musculus*) to study lactase function. Mouse has a homologous gene LCT which maintains the same functions as human LCT, and it is easy to observe the symptoms, so it will serve as an excellent disease model for CLD. The **long-term goal** of this study is to elucidate the mechanisms in which single amino acid changes in the C-terminal glycosyl-hydrolase domain cause lactase loss of function.

**Aim 1**: **Understand the evolutionary history of LCT and conservation of the C-terminus and R1587**

Approach: Use phylogenomics to create phylogenetic trees based on the homologs of LCT to trace the evolutionary history of the gene between organisms and trace the conservation of R1587.

Rationale: Understanding the evolutionary history could elucidate the importance of LCT in development. Creating phylogenetic trees based on the gene and protein could identify important evolutionary patterns and conserved regions.

Hypothesis: It is hypothesized that the arginine at position 1587 is widely conserved within the milk-consuming organisms in the phylogenomic tree. Additionally, the protein will have evolved in a manner that matches current known information about diet as it is related to lactose consumption.

**Aim 2**: **Determine differentially expressed genes and their functions in R1587H mutant mice**

Approach: Perform RNA-seq on mutant R1587H and WT mice to determine differentially expressed genes. Then sort these genes according to GO terms and identify genes involved in carbohydrate metabolism and excretory functions.

Rationale: Determining differentially expressed genes in the R1587H mutant will elucidate which biological processes are being disrupted and identify target genes that may interact with LCT. This will help to determine how a single amino acid substitution in the C-terminal glycosyl-hydrolase domain causes CLD.

Hypothesis: It is expected that R1587H mutant mice will have differentially expressed genes related to excretory functions and polysaccharide digestion or transport.

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