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 LCT, which digests lactose2. There are many identified mutations, most of which 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.* LCT contains four repeated protein domains, which are all glycosyl hydrolase domains; this domain contributes the carbohydrate digestion function, and mutations in this region may have significant impacts in LCT activity. Studying the evolution of LCT and the conservation of the C-terminal domain can also elucidate its importance in the development of functional lactase among milk consuming organisms.

The **overall objective** is to understand how a single amino acid substitution mutation, R1587H, affects the structure, function, and interactions of LCT to determine how it may lead to CLD. The **central hypothesis**is that the R1587H mutation will alter the structure of the folded protein and affect subsequent interactions, resulting in loss of function. The mutation lies in a conserved activity region of lactase and changes of the structure or charge of this region could impact enzyme function or protein interactions. This study will use the model organism mouse (*M. musculus*) to study lactase because it is easy to manipulate its diet and observe the primary symptom--diarrhea. 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 importance of conservation of the C-terminus and R1587 for normal lactase function**

Approach: Compare the amino acid sequence of the C-terminal domain in homologs of LCT to trace the conservation of the domain and R1587 in milk consuming organisms compared to the others.

Rationale: Observing conserved regions can elucidate the importance of the conservation of the C-terminal domain and specific amino acids in functional LCT for organisms known to require lactose digestion due to diets. Important conserved amino acids may have significant impacts when mutated, which could explain the development of CLD.

Hypothesis: It is hypothesized that the arginine at position 1587 is conserved among the milk-consuming organisms, resulting in functional lactase to digest lactose in milk.

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

Approach: Make a mutant mouse line with R1587H and confirm it as a CLD model by observing diarrhea. Then perform RNA-seq on mutant R1587H and WT mice to determine differentially expressed genes. These genes will be sorted according to GO terms to 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 upregulated genes related to excretory functions and down regulated genes related to polysaccharide digestion and transport.

**Aim 3: Experimentally determine protein interactions of WT and R1587H mutant mice LCT**

Approach: Perform tandem affinity purification and mass spectrometry (TAP/MS) on LCT from WT and R1587H mutant mice. The resulting protein interactors will be sorted according to GO terms to determine biological processes.

Rationale: There is currently no published experimental data on the protein interactors of LCT in mice or humans, which both have similar predicted interaction networks. This study would elucidate which protein-protein interactions are being interrupted in mice by the R1587H mutation under native conditions, and which biological processes they contribute to. This will elucidate how the single amino acid substitution is affecting the interactions and thus function of LCT.

Hypothesis: It is hypothesized that the interaction network for WT LCT will involve many proteins involved in carbohydrate metabolism. The R1587H mutant LCT will likely be missing key interactions with carbohydrate metabolism proteins, explaining its loss of function causing CLD.

This work will contribute to the understanding of how missense mutations can contribute to development of metabolic diseases. This study will reveal the conservation of individual amino acids in the C-terminal domain of LCT in the context of dietary need for functional lactase. It will identify target genes that are differentially expressed in CLD caused by a missense mutation, and it will identify protein-protein interactions that are also altered. The discoveries regarding protein and gene loss of function may lead to potential treatments for CLD.

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