Congenital Lactase Deficiency (CLD) is a severe, infant-onset form of lactose intolerance in which the body does not produce functional lactase enzyme to digest the sugar lactose, which is present in breastmilk and most dairy products, causing diarrhea1. This disease is caused by autosomal recessive mutations in the lactase enzyme gene LCT2. There are at least nine different identified mutations, of which most are nonsense mutations3,4,5,6. Truncated proteins cause obvious structural and functional problems, but it is still *unknown how single amino acid substitutions disrupt lactase function.* 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 for CLD.

The **long-term goal** of this study is to elucidate the mechanisms in which single amino acid changes cause lactase loss of function. The **overall objective** is to understand how the mutation R1587H (arginine to histidine substitution at 1587) affects the structure, trafficking, and substrate 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 pH and structure of LCT, which will cause decreased trafficking of the enzyme from the endoplasmic reticulum and decreased interaction of mature lactase with lactose. Studying the evolution of the gene can also help elucidate the function and importance. The mutation is located in the conserved region of mature LCT that encodes lactase activity3,7. The mutation could affect the overall charge balance and impact the structure of the folded protein. Misfolded proteins are in-turn less likely to properly bind to their substrate, so lactase would be rendered unable to bind to lactose. Misfolded proteins are also less likely to escape the endoplasmic reticulum (ER). This study may be able to further explain how single amino acid changes in the conserved mature LCT region cause the loss of function of lactase and development of CLD.

**Aim 1**: Understand the evolutionary history of LCT

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.

Approach: Use phylogenomics approach to create phylogenetic trees to trace the evolutionary history of the gene between organisms and its conserved domains.

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