**Maple Syrup Urine Disease (MSUD)** results from the body’s inability to break down three amino acids: valine, leucine, and isoleucine. Initial symptoms lead to the sweet smell of maple syrup in urine, sweat, and ear wax. 1,2 MSUD is more common in populations with history of homozygosity, such as the Amish3. One gene associated with Maple Syrup Urine Disease is DBT. DBT encodes for the E2 subunit of the branched chain alpha-ketoacid dehydrogenase, which localizes in the mitochondria.1,14 The E2 subunit has been characterized as a core protein necessary for catalyzing the second step of the amino acid breakdown or acyltransferase step.5 Without treatment for prolonged time, the build up of the three amino acids can cause poor feeding, brain damage, developmental delays, seizure-like spasms, and can even be fatal.1,2 Other disorders known to affect mitochondrial function, similar to MSUD, have lead to GERD, which will affect feeding and esophageal function.9,10 Although poor feeding is a known symptom, *it is unclear how mutations in DBT lead to the poor feeding phenotype.*

My **objective** is to determine the role of DBT in esophageal development and in turn feeding behavior in infants. I **hypothesize** that DBT is important for proper esophageal function and mutations in DBT will result in dysmorphic and non-functional esophageal tissue that leads to the inability to eat. The mouse, *Mus musculus*, will be used in these experiments because their feeding behavior and urine can be easily assayed. 11 The **long term goal** of this study is to determine how pathogenic variants in DBT lead to poor feeding behavior.

**Aim 1: Identify conserved amino acids that are important for esophageal function and feeding behavior.**

**Approach:** NCBI Homologene and Ensemble will provide known homologs of the DBT gene. ClustalOmega and JalView will be used to align protein sequences. Any amino acid that is conserved among organisms with complex digestive systems but not simple digestive systems will be identified as potential amino acids to mutate for further study. These amino acids will be mutated via CRISPR/Cas9 in mice to have the amino acid from simple digestive system organisms. Mice will be assayed based on the milk present in their stomach (representative of feeding behavior), urine amino acid levels, and esophageal tissue that resembles that of GERD. Confirmatory sequencing will be done to verify which amino acids were mutated.

**Hypothesis:** Conserved amino acids among complex digestive system organisms, but not simple, are important for feeding.

**Rationale:** Phylogenetic trees demonstrate that there is a divergence between species with complex and simple digestive systems. 15 If it is conserved among all complex digestive system organisms, but not simple, it is plausible that it is part of mutations that arose during the divergence of those species. The conservation of that change among all complex digestive system organisms likely indicates its importance in some complex digestive function.

**Aim 2: Determine transcript levels that impact feeding and how DBT mutations impact transcript expression levels.**

**Approach:** Tissue will be extracted from the esophagus from wild type, DBT mutants, and DBT mutants with poor feeding. Those cells will be subjected to RNA-sequencing. A heat map will be generated and Gene Ontology will be used to determine which mitochondrial genes are differentially expressed between wild type and DBT mutants. Genes that show significant change in expression will then be knocked out in mice and assayed for feeding behavior and esophageal tissue phenotype.

**Hypothesis:** Mitochondrial genes will be differentially expressed in DBT mutants with poor feeding.

**Rationale:** DBT localizes in the mitochondria 14, therefore mutation that affect feeding will likely resemble esophageal dysmorphia caused by other mitochondrial disorders. Disorders that affect mitochondrial function have been characterized to present GERD symptoms in babies, which can destroy esophageal tissue.9,10,12 DBT protein in its mature form has been characterized to interact with a range of mitochondrial protein targets. 13 Therefore, since DBT interacts with mitochondrial proteins and mitochondrial disorders can lead to GERD, DBT’s effect on mitochondrial gene expression changes could impact feeding behavior.

**Aim 3: Identify proteins necessary for mitochondrial function and feeding.**

**Approach:** Tissue will be extracted from the esophagus from wild type, DBT mutants, and DBT mutants with poor feeding. Cells of the esophagus will subjected to Tandem Affinity Purification, SDS Page, Trypsin Digestion, and then Mass Spectrometry to determine changes in protein-protein interactions and identify those necessary for feeding. Protein interaction networks for all three test groups will be made and sorted using GO. Protein interaction that show significant loss or gain will then be knocked out in mice and assayed for feeding behavior and esophageal tissue phenotype.

**Hypothesis:** Mitochondrial transport proteins found in the esophagus will lose their interaction with DBT when DBT is mutated. This effect on mitochondrial transport will lead to poor feeding and symptoms and tissue phenotypes that resemble GERD.

**Rationale:** DBT interacts with the mitochondrial transport protein GREPL2 which is highly expressed in the esophagus.13,12 GREPL2 is involved in mitochondrial transport. Therefore, loss of this interaction and changes in other mitochondrial transport proteins will lead to poor feeding and esophageal function.

**Future Directions**: Many amino acids conserved in complex digestive species were found to be in a region that is not part of a characterized domain, specifically the region from amino acid K211-I216. This region is in between the E3 binding domain and the catalytic domain the human protein. Investigating this further could characterize a new domain of the DBT protein, providing insight into pathogenic variants.

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