**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. Without treatment for prolonged time, the build up of the three amino acids can cause brain damage, developmental delays, seizure-like spasms, and can even be fatal.1,2 1,2MSUD is more common in populations with substantial history of homozygosity, such as the Amish.3 One gene associated with MSUD is DBT. This codes for the E2 subunit of the branched chain alpha-ketoacid dehydrogenase.1 The E2 subunit has been characterized as the core protein necessary for bringing the complex together to complete the reaction and catalyzes the acyltransferase step of the reaction (2nd step).5 There have been numerous variants in the human DBT gene classified as pathogenic or likely pathogenic.6 *It is currently unknown how pathogenic DBT variants in regions not characterized to a protein domain affect the E2 protein’s catalytic function in vivo.*

My **objective** is to classify how variations in the DBT gene, specifically in regions outside of characterized protein domains affect the acyltransferase activity and/or binding ability in the E2 protein subunit. The **long term goal** of this study is to use the information on variant’s effects on the E2 protein to predict disease manifestation and progression in order to develop appropriate treatment plans based on what is not functioning correctly. I **hypothesize** that variant mutation in these regions will affect protein shape and prevent E2 from acting as the center for complex formation, thus preventing the reaction progressing further than the initial step. The mouse, *mus musculus*, and zebrafish, *danio rerio*, will be used in this study. Zebrafish when mutated at the DBT gene have shown neurologic symptoms concurrent human disease manifestation7 and can be used to see fluorescent probes on proteins and other markers. Mice have shown similar phenotypic presentation when mutant for the DBT allele8 and will be used to determine in vivo phenotypes.

**Aim 1: Define conserved amino acid sequences of known pathogenic variants in regions outside of characterized DBT protein domains in well characterized DBT homologs.**

**Approach:** NCBI Homologene will provide known homologs of the DBT gene. FASTA amino acid sequences will be obtained for relevant homologs. ClustalOmega will be used to align protein sequences. Known human pathogens will be looked at in regions that are not classified to a DBT protein domain. Those that are highly conserved will be mutated in both mice and zebrafish. Mice urine output will be obtained and analyzed for BCAA levels and zebrafish neurological manifestation will be assessed for MSUD phenotypes.

**Hypothesis**: Variations in uncharacterized protein domains that are highly conserved will lead to metabolic and neurological disease manifestation.

**Rationale:** Although not in a protein domain associated with function, there are a handful of pathogenic variants outside of those domains. If found to be highly conserved among species, those variants can be mutagenized in mice and zebrafish. If confirmed to be disease causing in the assays, those mutants can be used later to determine specific effects on the E2 protein.

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