**Spina Bifida,** characterized by a failure of the neural tube to fuse properly during embryonic development, is the most common birth defect [1] and often leads to lasting disability, including paralysis and bowel dysfunction.[2] The C677T SNP in the maternal ***MTHFR*** gene is associated with a modestly increased risk of having a baby with spina bifida when heterozygous and a significantly increased risk when homozygous.[3] The **MTHFR** enzyme converts 5,10 methylenetetrahydropholate into 5-methyltetrahydrofolate which is the primary circulatory form of folate and a necessary compound for methionine production.[4] Inadequate methionine levels leads to increased homocysteine, a compound that has demonstrated neurotoxic properties.[5] *Although folate metabolism is linked to the development of spina bifida, the mechanism through which dysfunctional folate metabolism disrupts neural tube closure is not characterized.*

The **long term goal** of this project is to identify therapeutic targets within the folate metabolic pathway that may reduce the incidence of spina bifida. My **primary objective** for this project is to characterize the mechanism through which folate metabolism and homocysteine remethylation facilitate closure of the neural tube during embryonic development. I **hypothesize** that elevated homocysteine levels in the developing embryo cause neural cell death and a failure of the neural tube to fuse. A zebrafish (*Danio rerio*) model will be used do to the ease with which spinal defects can be observed in zebrafish embryos as well as the rapid development and closure of the zebrafish neural tube, which occurs within 24 hours of fertilization.[6]

**Aim 1: Determine sequence conservation of *MTHFR* across species.**

**Approach:** A multiple sequence alignment assay will be performed in Clustal Omega to characterize the sequence conservation of the MTHFR domain. Zebrafish and human sequences will be compared and the human pathogenic C677T SNP will be created in a zebrafish line using CRISPR-Cas9.

**Hypothesis:** Due to the high conservation of the MTHFR domain across species, I expect the domain to display high sequence conservation between humans and zebrafish.

**Rationale:** The semi-dominant phenotype of the human C677T SNP enables investigation of the effect of varying levels of both functional folate metabolism and homocysteine levels on neurulation. The human mutation results in the substitution of an alanine residue for a valine residue.[7] Thus, delineation of the zebrafish MTHFR sequence will allow insertion of a SNP that will lead to the same substitution.

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