Amyotrophic lateral sclerosis (ALS) is a type of neurodegenerative disease characterized by motor neuron death, decreased muscle mass, and impaired movement1. Mutations in *FUS* concentrate in the non-conventional nuclear localization signal region (NLS), which result in a severe juvenile onset2,3. The FUS protein normally functions in DNA repair mechanisms and the regulation of translation4,5. More specifically, *FUS* alterations has been linked to defects in DNA repair components Ligase III and polymerase that can facilitate aggregate formation5,6. Unrepaired DNA single strand breaks in neuronal genomes has also been linked to neurodegeneration6. Although it is known that impaired DNA damage response mechanisms help facilitate FUS aggregation, it is *unknown* *how FUS-related DNA repair pathways interact and their specific downstream effect on neurodegeneration remains unclear*6,7,8*.*

The **objective** of this study is to determine which protein domains are necessary for DNA repair mechanisms that promote motor neuron function. I **hypothesize** that alterations in the NLS segment and critical protein domains will disrupt *FUS*-related DNA repair mechanisms and result in earlier neuron death compared to other regions. The **long-term goal** of this research is to determine the effect on neurodegeneration due to these alterations, while providing a comprehensive mechanism for the *FUS*-related DNA repair pathways. To achieve this goal, *D. rerio* will be used as a **model** due to conserved homology, transparency during development, and anatomical similarity of motor neurons7.

**Aim 1: Identify Conserved Amino Acids of FUS Critical for DNA Repair and Neurodegeneration**

**Approach:** I will perform a genetic screen using *D. rerio* to find regions associated with DNA repair in *FUS.* To begin, I will align protein sequences via ClustalOmega to identify conserved domains among organisms. I will then utilize CRISPR-Cas9 technology to induce specific mutations along those conserved regions. Next, I will screen for phenotypes showing behaviors associated with ALS in *D. rerio* and measure the amount of single/double stranded breaks (SSBs/DSBs) via a comet assay9.

**Hypothesis**: I hypothesize that *FUS* mutations in conserved domains will impact DNA repair and result in more SSBs/DSBs compared to non-conserved regions.

**Rationale**: Screening of *D. rerio* with these specific variants should result in a phenotype associated with neurodegeneration and increased SSBs/DSBs due to the proteins importance in DNA repair pathways. By determining these variants, additional protein interactions in the *FUS*-DNA repair pathways can be discovered.

References:

1. Shang, Y. & Huang E.J. (2016, September). Mechanisms of *FUS* mutations in familial amyotrophic lateral sclerosis. *Brain Research* 1647:65-78.
2. Zou, Z.Y., Liu, M.S., Li, X.G., Cui, L.Y. (2015, September). Mutations in *SOD1* and *FUS* caused juvenile-onset sporadic amyotrophic lateral sclerosis with aggressive progression. *Ann Translation Medicine* 3(15):221
3. Conte, A., Lattante, S., et al. (2012, January). P525L FUS mutation is consistently associated with a severe form of juvenile Amyotrophic Lateral Sclerosis. *Neurology Genetics* 2:63
4. Zhou, Y., Liu, S., et al. (2013, October). ALS-associated *FUS* mutations result in compromised *FUS* alternative splicing and autoregulation. *Nature Communications* 9:3683
5. Wang, H., Guo, W., et. Al. (2018, September). Mutant FUS causes DNA ligation defects to inhibit oxidative damage repair in Amyotrophic Lateral Sclerosis. *Nature Communications* 9:3683
6. Naumann, M., Pal, A., et al. (2018, January). Impaired DNA damage response signaling by *FUS*-NLS mutations leads to neurodegeneration and FUS aggregate formation. *Nature Communications*9:335
7. Penndorf, D., Witte, O., et al. (2018, February). DNA plasticity and damage in amyotrophic lateral sclerosis. *Neural Regeneration Research* 3(2): 173–180.
8. McGown, A., McDearmid, J.R., et al. (2012, October). Early interneuron dysfunction in ALS: Insights from a mutant *sod1* zebrafish. *Annals of Neurobiology* 73(2):246-258.
9. D’Costa, A.H., Shyama, S.K., et al. (2018, August). Induction of DNA damage in the peripheral blood of zebrafish (Danio rerio) by an agricultural organophosphate pesticide, monocrotophos. *International Aquatic Research* 10(3):243-251.