Amyotrophic lateral sclerosis (ALS) is a type of neurodegenerative disease characterized by motor neuron death, decreased muscle mass, and impaired movement1. Mutations in the gene *FUS*, which normally functions in DNA repair and splicing, lead to severe juvenile onset of ALS2,3,4,5. Altered *FUS* facilitates aggregate formation, which directly lead to neurodegenerative disease5,6. Although it is known that impaired DNA damage response mechanisms help facilitate FUS aggregates, it is *unknown* *how defects in the DNA repair pathways lead to neuron defects*,7,8*.*

The **objective** of this study is to determine how FUS-related DNA repair leads to proper motor neuron function. I **hypothesize** that FUS domains play a role in DNA repair mechanisms, due to their conserved nature and role in DNA binding. The **long-term goal** of this research is to determine how FUS-related DNA repair leads to neurodegeneration. To achieve this goal, *Danio rerio* will be used as a **model** due to homology, anatomical similarity, and simplistic motor movement screens9.

**Aim 1: Identify conserved amino acids of FUS critical for DNA repair and neurodegeneration**

**Approach:** I will perform a protein alignment to generate CRISPR mutants in order to find conserved regions associated with DNA repair in *FUS.* To begin, I will align protein sequences via ClustalOmega to identify conserved amino acids in the RNA recognition motif, zinc-finger domain, and those that contain conserved pathogenic variants, like G187C2. I will then utilize CRISPR-Cas9 technology to induce specific mutations along those conserved regions. Sanger sequencing will then be used to confirm on target alterations. Next, I will screen for phenotypes showing defective motor movements*.* For those showing motor defects, I will measure the amount of single/double stranded breaks (SSBs/DSBs) via a comet assay when subjected to UV damage6.

**Hypothesis**: I hypothesize that *FUS* mutations in the zinc finger domain will impact DNA repair and result in more SSBs/DSBs, due to zinc finger domains emerging roles in genome stability10.

**Rationale**: Screening of *D. rerio* with these specific variants should result in a phenotype with increased SSBs/DSBs, which would define DNA repair binding regions important for genome stability and neuronal function.

**Aim 2: Identify small molecules that rescue FUS mutant phenotype**

**Approach:** I will perform a chemical screen by using a known CRISPR-Cas9 mutant, R521C, that shows increased SSBs/DSBs and motor movement impairments9. To begin, I will utilize a chemical library of small molecules that regulate DNA repair proteins involved with SSBs/DSBs, like those in homology directed repair and base excision repair 5,11. I will perform a motor movement screen and comet assay in order to identify small molecules capable of restoring proper motor neuron function and DNA repair.

**Hypothesis:** Small molecules associated with the downregulation of proteins that terminate repair complexes will rescue the FUS mutant phenotype, due to increased retention time at SSBs/DSBs sites.

**Rationale**: By identifying small molecules that restore DNA repair and motor neuron function, FDA approved cancer drugs that target DNA repair pathways could easily be transitioned to ALS patients12.

**Aim 3: Identify new FUS protein-protein interactions in DNA repair**

**Approach:** Utilizing lysed neuronal cells from *D. rerio*, I will subject samples to both wild type and mutant FUS baits (R521C). Tandem affinity purification and mass spectrometry will then be used to purify and obtain MS/MS data. Computer software would provide sequence coverage of these captured protein interactions. To confirm these interactions are legitimate, CRISPR-Cas9 will be used to make knockouts of potential FUS binding sites determined by BLAST. A comet assay and motor movement screen will identify DNA repair proteins as those with increased SSBs/DSBs and possible impacted neurodegeneration. Proteins will be sorted by GO terms, which would elucidate a more extensive protein interaction network of FUS in DNA repair.

**Hypothesis:** TAP-MS will provide new FUS interactions associated with DNA repair in *D. rerio* due to the presence of high levels of SSBs/DSBs commonly found in FUS mutants.

**Rationale:** Currently, databases like String show limited DNA repair protein interactions with FUS in *D. rerio*14. Eukaryotic DNA repair pathways are conserved, and this data could suggest similar mechanisms in humans13.

Through these approaches, the role that FUS plays in DNA repair and motor neuron function can be identified. There are no known treatments that improve neuron function for FUS-ALS patients. By understanding targets of FUS in the DNA repair pathways, current FDA approved cancer drugs could easily be administered to those with FUS-ALS. This would not only help restore genomic stability, but also allow restoration of neuron function in patients. Ultimately, this understanding would highlight how DNA repair defects lead to neurodegeneration.

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