Amyotrophic lateral sclerosis (ALS) is motor neurodegenerative neuromuscular disease. ALS cases are classified as” sporadic” ALS (sALS) or “familial” ALS (fALS). With the fALS form, the disease is inherited. It has been reported that mutations of genes have been associated with fALS. And for this model, we will focus on the mutated FUS/TLS gene on chromosome 16. The FUS/TLS protein binds to RNA, single-stranded DNA and double-stranded DNA. It also interacts with gene-specific transcription factors and appears in double-strand break sites for repair of DNA damages. Mutations in the FUS NLS (Nuclear localization sequence) impairs the poly (ADP-ribose) polymerase (PARP)-dependent DNA damage response. This impairment leads to FUS aggregate formation which appear to be a common pathologic hallmark of ALS.

AIM: Mutation in the FUS nuclear localization sequence (NLS) induces impairment of poly (ASP-ribose) polymerase (PARP)-dependent DNA damage response and cytoplasmic FUS mislocalization leading to FUS aggregate formation. Along this process, we will develop motor neurons model of FUS-ALS with endogenously tagged proteins.

Methods

Patient selection

R521C and R521H point mutations are the most prevalent mutations within the NLS region of FUS. In addition of normal patients, patients carrying diverse NLS mutations (R521C, R521H) will be selected.

Line cells

Line cells will be established from biopsies (skin or hair cells) obtained after consent from the fALS patients and healthy individuals. The fibroblast lines will be plated in a media, and reprogrammed into induced pluripotent stem cells (hiPSCs) using “Yamanake-factors”. These vectors could be transfected into the cells with a transfection agent. These cell lines, then can be constantly regenerated in subcultures and will be monitored until colonies will develop enough.

CRISPR/Cas9 genome editing

Isogenic iPSC lines will be generated by CISPR/Cas9n from one of the clone of the R521C hiPSC line by generating a new mutation (P525L) carrying an additional c-terminal GFP tag.

Study

Electrophysiology of motor neurons will be performed to check presence of voltage-gated Na+ and K+ channels, action potentials and periodical increase of intracellular calcium. After extended maturation of the cultures, with cellular aging, we will look for an increase for FUS aggregates and will check for occurrence of DNA double strand breaks in isogenic lines compared to the P525L line.

The [N-terminal](https://en.wikipedia.org/wiki/N-terminus) end of FUS appears to be involved in transcriptional activation, while the [C-terminal](https://en.wikipedia.org/wiki/C-terminus) end is involved in protein and RNA binding.

<https://pubmed.ncbi.nlm.nih.gov/19251627/>

https://en.wikipedia.org/wiki/RNA-binding\_protein\_FUS

https://www.nature.com/articles/s41467-017-02299-1

https://www.researchgate.net/publication/38040513\_Mutations\_of\_FUS\_gene\_in\_sporadic\_amyotrophic\_lateral\_sclerosis