

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 gene on chromosome 16. The C-terminal end of the FUS gene is involved in protein and RNA binding. It also 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 response¹. This impairment leads to FUS aggregate formation which is part of the ALS phenotype.

AIM: Mutation in the FUS nuclear localization sequence (NLS) induces impairment of DNA repair and cytoplasmic FUS mislocalization leads to FUS aggregate formation.

Methodology

Patient selection

R521C and R521H mutations are the most prevalent mutations within the NLS region of FUS. In addition of normal patients, fALS patients carrying diverse NLS mutations 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 human 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 mature enough.

CRISPR/Cas9 genome editing

To increase the significance of the research, mutations which have been linked to ALS will be performed using CRISPR/Cas9 vector and guide RNAs (gRNA) to create double strand break at the target site and insert the mutated sequence in the C-terminal nuclear localization sequence (NLS) of the gene². These proteins will be GFP-tagged to facilitate identification and observation.

Study

Electrophysiology of motor neurons will be performed to check overall neural activity (voltage-gated Na⁺ and K⁺ channels, action potentials and intracellular Ca⁺⁺). After extended maturation of the cultures, we will look for an increase of FUS aggregates and will compare DNA double strand breaks in mutated lines to normal cells.

Motivations for the specifics of the methodology

1) The reasons to select patients of mutated FUS gene is to compare their cells with the cells with the same mutated gene using CRISPR and acts as an additional validation step. 2) Because it is a genetic editing and also based on the scope of the study, CRISPR/Cas9 seems the natural choice for this experiment and seems simpler and cheaper compared to OOC, or KI mice models.

¹ See figure 1

² See figure 2 and 3

References:

https://en.wikipedia.org/wiki/RNA-binding_protein_FUS

Kwiatkowski et Al. - Mutations in the FUS/TLS gene on chromosome 16 cause familial amyotrophic lateral sclerosis, DOI: [10.1126/science.1166066](https://doi.org/10.1126/science.1166066) - <https://pubmed.ncbi.nlm.nih.gov/19251627/>

Marcel Naumann et Al – Nature Communication, volume 9, Article number: 335 (2018)- Impaired DNA damage response signaling by FUS-NLS mutations leads to neurodegeneration and FUS aggregate formation – Nature 20 January 2018

Lucia Corrade – Journal of Medical Genetics 47 (3): 19-4. DOI: 10.1136- [Mutations of FUS gene in sporadic amyotrophic lateral sclerosis](https://doi.org/10.1136/jmedgenet-2014-102511) - DOI: <https://jmg.bmj.com/content/47/3/190>

Zi Chao Zhang and Yuh Min Chook - Proceedings of the National Academy of Sciences of the United States - [Structural and energetic basis of ALS-causing mutations in the atypical proline–tyrosine nuclear localization signal of the Fused in Sarcoma protein \(FUS\)](https://doi.org/10.1073/pnas.1212111109)

Yulei Shang, Eric J. Hung Elsevier - <https://doi.org/10.1016/j.brainres.2016.03.036> - [Mechanisms of FUS mutations in familial amyotrophic lateral sclerosis](https://doi.org/10.1016/j.brainres.2016.03.036)

Caroline et al. – doi: [10.1093/hmg/ddt117](https://doi.org/10.1093/hmg/ddt117) - [ALS mutant FUS disrupts nuclear localization and sequesters wild-type FUS within cytoplasmic stress granules.](https://doi.org/10.1093/hmg/ddt117)

<https://medlineplus.gov/genetics/gene/fus/>

<https://www.genecards.org/cgi-bin/carddisp.pl?gene=FUS>

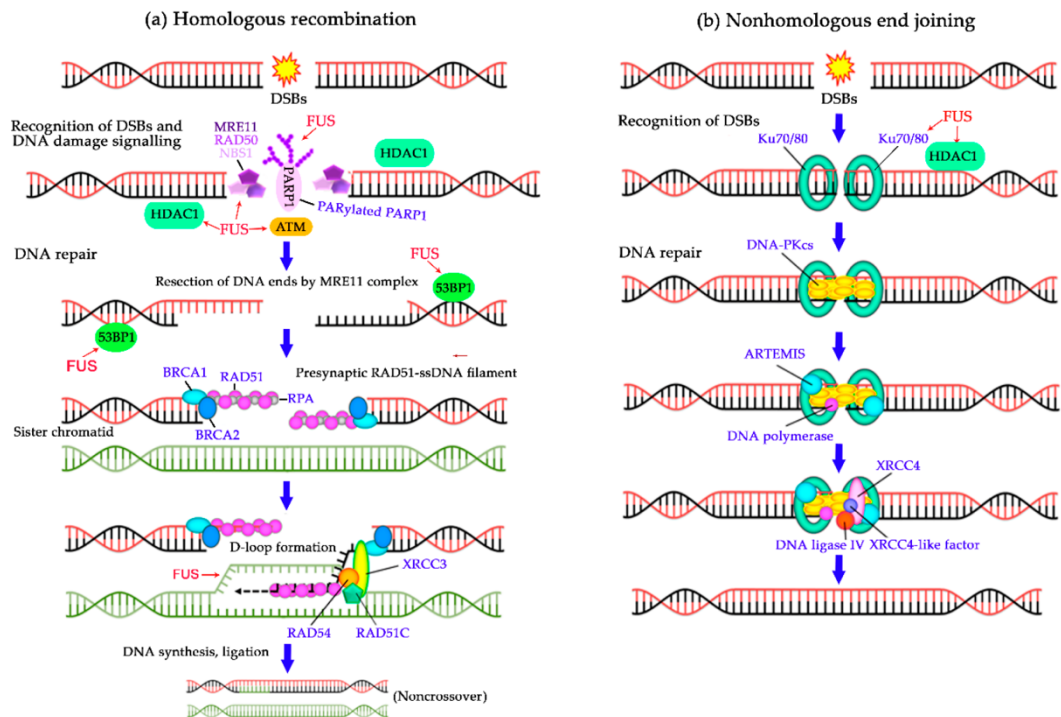


Fig 1. FUS DNA Repair mechanism

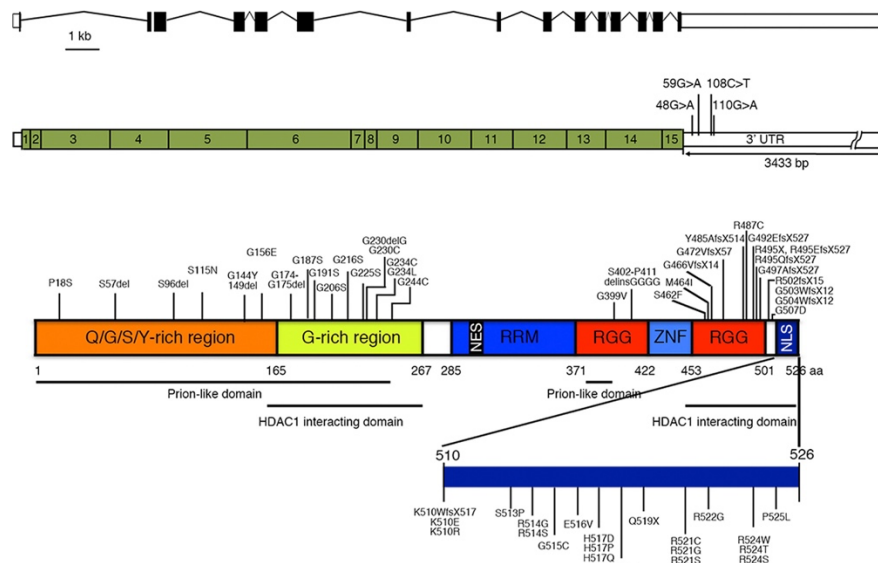


Fig 2. FUS NLS mutations

Mutation	Nucleotide change	Exon	Patients	Controls
p.Gly 171_174del	*c.515delGAGGTGGAGGTG	5	1/1009	0/621
p.Gly 173_174del	*c.508delGAGGTG	5	2/1009	3/621
p.G191S	c.571G → A	6	1/1009	0/793
p.R216C	c.646C → T	6	1/1009	0/793
p.Gly 223ins	*c.667insGGC	6	0/1009	2/793
p.Gly 223_224ins	*c.667insGGCGGC	6	0/1009	1/793
p.Gly 223del	*c.679_681delGGC	6	2/1009	0/793
p.Gly 223_Gly224del	*c.679_684delGGCGGC	6	4/1009	0/793
p.G225V	c.674G → T	6	1/1009	0/793
p.G226S	c.676G → A	6	0/1009	1/793
p.Gly 227_Gly230del	c.680_691delGGCGGCGGTGGT	6	1/1009	0/793
p.Gly 227_Gly229del	c.679_687delGGCGGCGGT	6	1/1009	1/793
p.Gly 228_Gly229del	c.682_687delGGCGGT	6	1/1009	0/793
p.Gly 229_230del	*c.685_690delGGTGGT	6	0/1009	1/793
p.G230C	c.688G → T	6	1/1009	0/793
p.R234C	c.700C → T	6	1/1009	0/793
p.G507D	c.1520G → A	14	2/1009	0/500
p.R521C	c.1561C → T	15	2/1009	0/538

Fig 3. R521C nucleotide change: C->T