

Understanding the function of SIGMAR1 gene in neurons using TALEN-mediated mutagenesis

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Iowa State University Symposium on Undergraduate Research
and Creative Expression

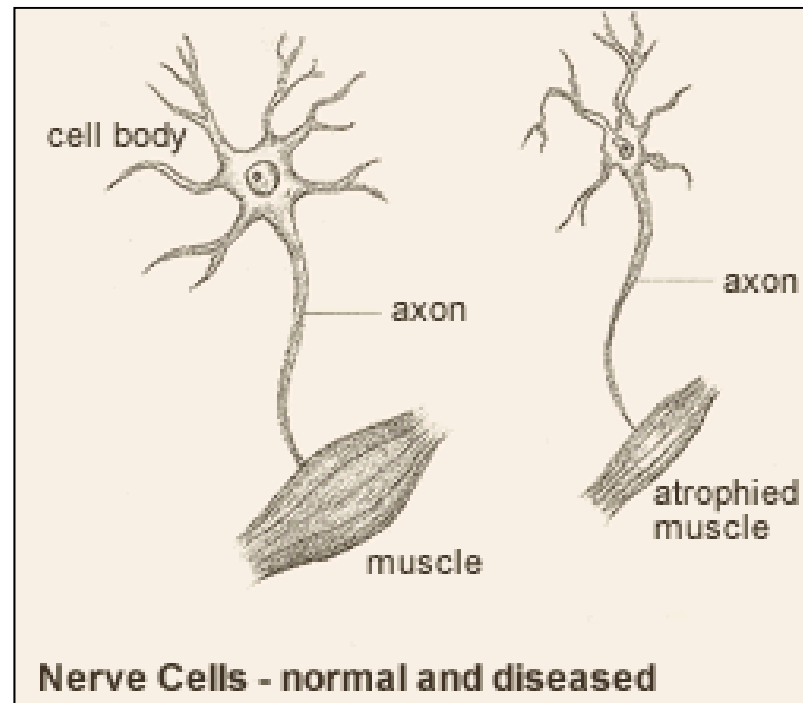
What is Amyotrophic Lateral Sclerosis?

- 5th most prevalent neurodegenerative disorder
- Loss of motor neurons
- Leads to muscle weakness, paralysis, and eventually death
- 10% of cases are familial



Symptoms of ALS

- Muscle weakness
- Fasciculation and cramping
- Thick speech
- Shortness of breath
- Difficulty breathing/swallowing
- Death in approximately 3-5 years



Cause of ALS?

- 20% of familial mutations- SOD1
- Sequence genomes to find additional mutations linked to other familial cases
 - Identified SIGMAR1
 - Is this gene causative?
 - If so, what is SIGMAR1 doing in motor neurons?

SIGMAR1

- Sigma non-opioid intracellular receptor 1
- Vesicle trafficking
- Regulates ion channels
- Ca²⁺ signaling
- Role in motor neuron function and disease?

Animal Model: Zebrafish

- Why use zebrafish?
 - Embryo is easily accessible
 - Big enough for injections
 - Mature quickly

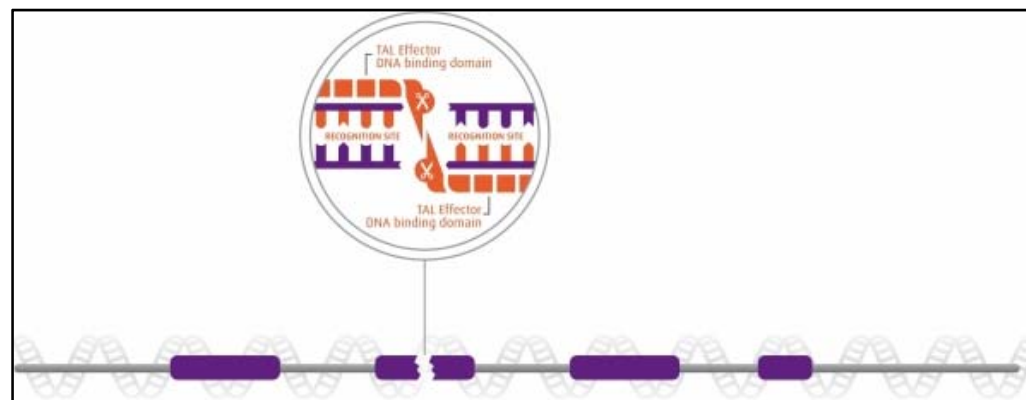
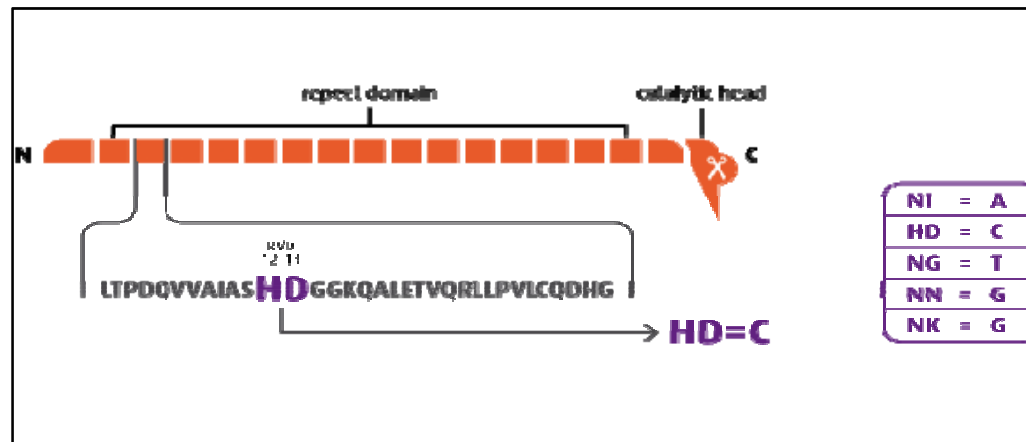


11th Place: Alzheimer Zebrafish, stained for Tau (red), neurons (green), and pathologic Tau (blue) (10x) by Dominik Paquet, Ludwig-Maximilians-University, Munich, Germany

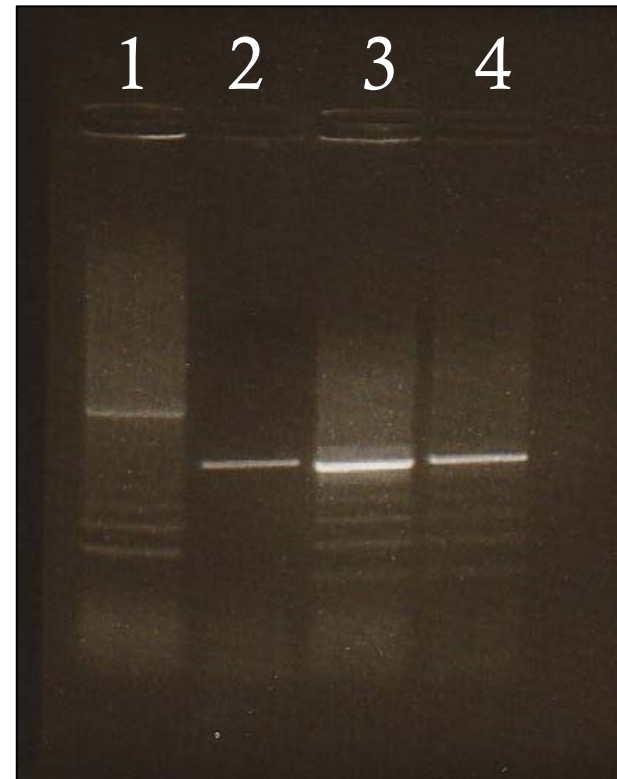
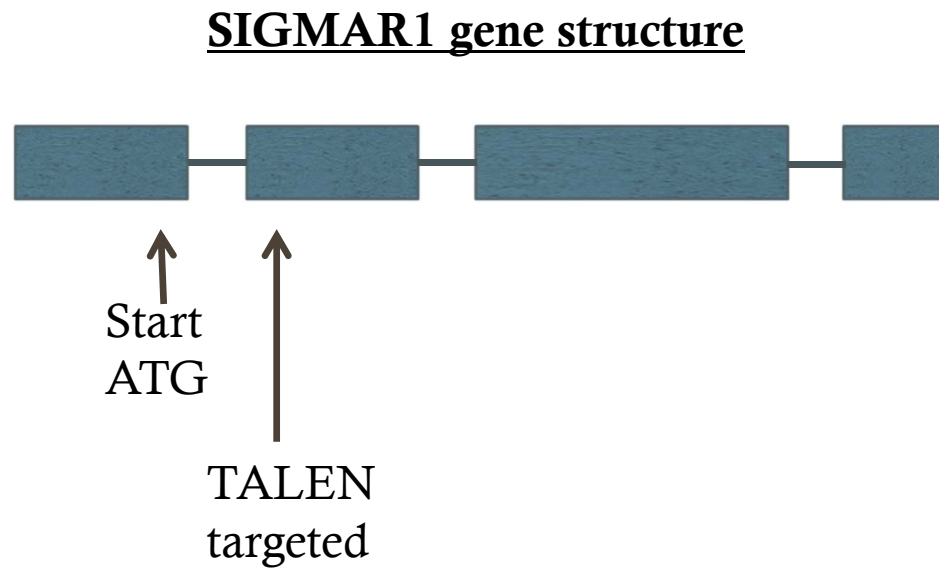
TALENs

- Transcription activator-like effector nucleases
- TAL effectors found in plant pathogenic bacteria
- Functionalized proteins
- Targets specific sequence in gene of interest
- Induces double stranded breaks
- Repair leads to mutations

What are TALENs?

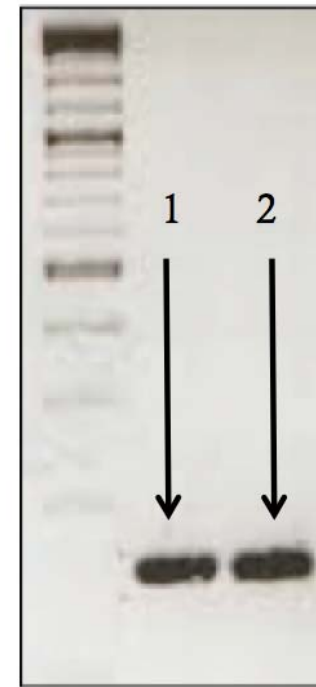


Construction of SIGMAR1 TALEN



SIGMAR1 Mutation in Fish

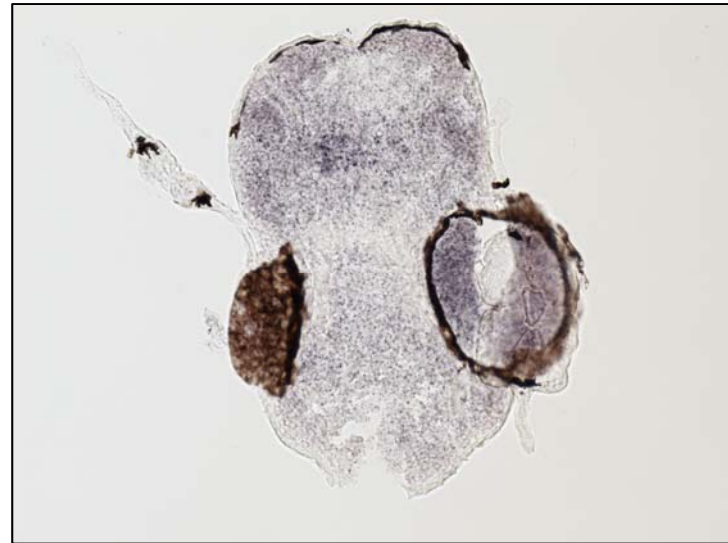
- Made RNA from DNA
- SIGMAR1 TALEN injected at 25 ng/ul
- Results showed the fish did not have mutations



1: SIGMAR1 control
2: SIGMAR1

Where is SIGMAR1 expressed during zebrafish development?

In Situ Hybridization

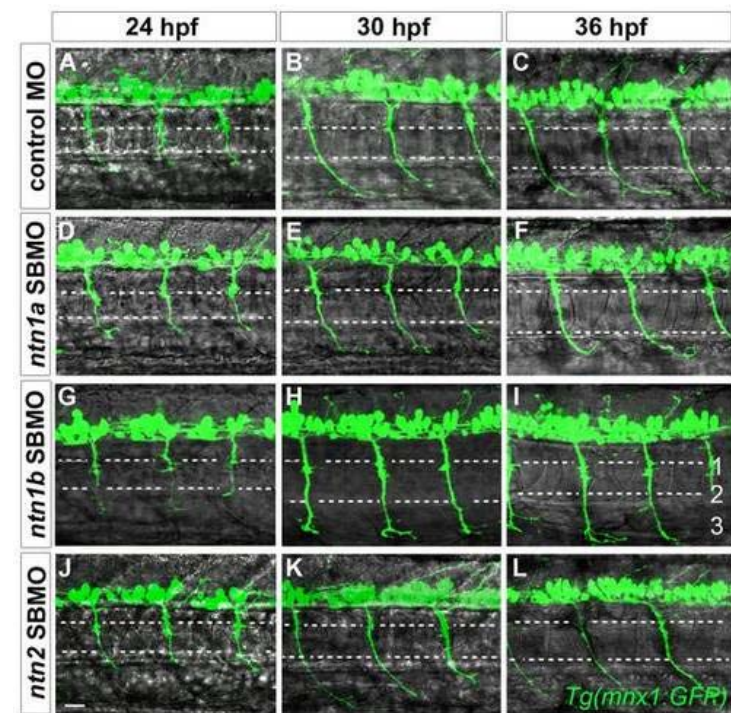


Summary

- ALS is characterized by the loss of motor neurons in the brain and spinal cord.
- Mutations in SIGMAR1 receptors have been found in ALS patients.
- TALENs have the potential to mutate genes like SIGMAR1.

Future Directions

- Create SIGMAR1 CRISPR
- Mate mutant fish with MNX1 GFP zebrafish
- Evaluate motor neuron growth and function
 - Swimming test
- Use ALS-specific mutation fish for drug screening



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