VCP mediated mutagenesis in *Danio rerio* to model motor neuron degeneration in ALS

Stephen Jones
ALS  Amyotrophic lateral sclerosis

- Neurodegenerative disease characterized by the death of motor neurons
  - Muscles waste away
  - Resulting in paralysis and eventually death.
  - Death within 2-5 years

- Two primary forms of ALS exist:
  - Familial -- 5-10%
    - A family member has had ALS
  - Sporadic -- 90-95%
    - No family member has ever had ALS

http://www.healthworks.my/als/
Valosin Containing Protein (VCP)

- Ubiquitin-dependent degradation
- Vesicle transport and fusion
- Proteasome function
- Peroxisome assembly
- Associated with clathrin and a heat shock protein (Hsc70), forming a complex
- Involved in mitosis
  - Homolytic membrane fusion
  - Spindle pole body formation
VCP and ALS

- **SOD1** is the most commonly studied gene
  - mutations found in 20% of familial cases
  - very few mutations in sporadic cases

- **Mutations in VCP** are associated with a genetic disease **IMBPFD**
  - Inclusion body myopathy with early-onset Paget disease and frontotemporal dementia
  - Muscle weakness, bone pain, and dementia
  - 10% of patients showed hallmark features of ALS

- 1-2% of familial cases of ALS have mutations in VCP
TALEN  Transcription Activator Like Effector Nucleases

- Engineered proteins
- Target specific sequence in gene of interest
- Amino acids comprising TALEN bind to DNA and induce DSB through the use of an endonuclease (Fok1)
- NHEJ repairs DNA and makes minor insertions/deletions which we can view on a gel

http://www.cellectis-bioresearch.com/genome-customization/genomic-scissors/talen/what-are-tal-effector-nucleases
VCP TALEN

- Mutate the VCP gene in zebrafish
  - Transparent
  - Easy access to embryos
  - Inexpensive
  - 3 month generation time
  - Large clutches

VCP TALEN construct

- The VCP gene has 17 exons
- Start ATG is in the first exon
- TALEN is targeted for the 3rd exon
VCP TALEN results

- The TALEN was constructed
- There was trouble transcribing the TALEN from the plasmid to RNA
- There was no RNA to inject

a: successful TALEN
b: unsuccessful TALEN
c: successful TALEN
d: unsuccessful TALEN
CRISPR Clustered regularly interspaced short palindromic repeats

- Determine the gene of interest
- Construct gRNA that is specific to that gene’s sequence
- The gRNA recruits Cas9 which cuts the genomic DNA

![Diagram of CRISPR process]
CRISPR construct

- Targeted the CRISPR for the third exon
- CRISPR is generated
- Still need to synthesize the guide RNA
- That RNA will be injected

Diagram:

- a: CRISPR injected fish
- b: CRISPR injected fish
- c: Control

Diagram showing the CRISPR construct with arrows indicating Start ATG and CRISPR positions.
MNX1 GFP

- GFP is expressed in motor neurons
- Compare motor neuron development in controls and CRISPR mutants

Swim Test

- Evaluates motor function of the zebrafish
- In collaboration with Johanna Kowalko

Figure 15-2 Methods of Behavior Analysis in Neuroscience, 2nd edition. (© Taylor & Francis Group, LLC. 2009)
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