Understanding the function of OPTN gene in neurons using TALEN facilitated mutagenesis

Alicia Manning

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What is Amyotrophic Lateral Sclerosis (ALS)?

- Neurodegenerative disease involving death of motor neurons
- Reduced signaling to muscles leads to muscle weakness
- Ultimately ending in little to no muscle movement
Symptoms and Progression

- Progression symptoms: difficulty speaking, reduced limb use, struggling with swallowing breathing
- Life expectancy: 2-5 years
- Familial 5-10% vs. Sporadic 90-95%
- No ethnic, racial or socioeconomic boundaries
Primary Open-Angle Glaucoma (POAG)

- One of the leading causes of blindness
- Difficult to detect
  - Painless
  - Increase intraocular pressure
- Thought to be due to defect in eye’s drainage system
Glaucoma

- Generally diagnosed in mid to later stages of vision lost
- Causes permanent damage to optic nerve
- Irreversible vision loss
Understanding Optineurin (OPTN) gene

- Little known about its cellular function
- Speculations:
  - Association with clearance of protein aggregates in neurons
- How to study OPTN function using zebrafish?
TALENs

Transcription activator-like effector nuclease
OPTN Introns & Exons

ATG – Start codon

TALEN target site

Stop codon
Putting It All Together

GG1 Reaction

A1  A2  A3  B1  B2  B3

A = RVD 1-10
B = RVD 11-15

GG2 Reaction

1  2  3  4

A1  A2  A3  B1  B2  B3
Zebrafish & Injecting

- Why use Zebrafish?
  - Ex utero embryos
  - Large clutches
  - Mature in 3 months
  - Inexpensive

- Inject RNA form of TALENs as close to 1 cell stage
F0 Data

- Band 1 control
- Band 2 & 3 OPTN from injected fish
- Band 2 shows no mutation
- Smear on band 3 shows presences of addition and deletion mutations
Next Steps & Future

- Breed F0 with wild-type to produce F1
- Heterozygote F1 could be mated together to produce homozygous mutant F2
- Mate mutants with a MNX1 GFP fish
  - GFP is found in all motor neurons and pancreas
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