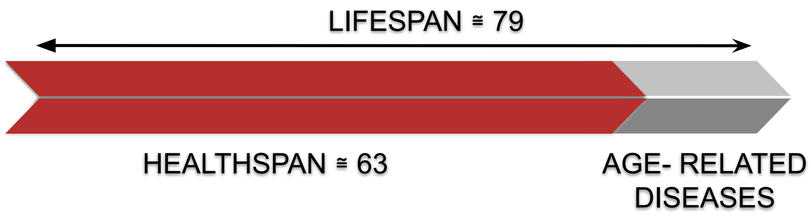
Understanding the Molecular Basis of Neural Ageing in *C. elegans*

Ahsen Konaç[1,2], Sebiha Çevik[1,2], Oktay I. Kaplan[1]

1-Rare Disease Laboratory, School of Life and Natural Sciences, Abdullah Gul University, Kayseri, Turkey

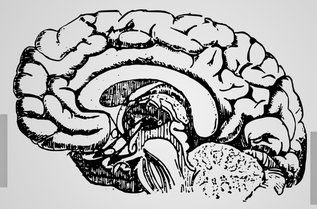
2- Department of Molecular Biology and Genetics, Faculty of Life and Natural Sciences, Abdullah Gül University, Kayseri, Turkey

INTRODUCTION



Expanded lifespan does not necessarily equate with improved healthspan and neuronal ageing is one of the keystones of unhealthy ageing.

HALLMARKS OF NEURONAL AGEING



- Dysregulated calcium homeostasis
- Oxidative damage
- Mitochondrial dysfunction
- Glial cell activation & inflammation
- Aberrant neuronal network activity
- Impaired DNA repair and molecular waste disposal

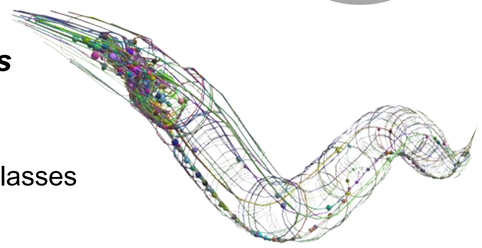
EXAMPLES OF NEURONAL AGEING ASSOCIATED DISEASES

- Alzheimer's Disease
- Parkinson's Disease
- Amyotrophic Lateral Sclerosis

As a model organism:

Caenorhabditis elegans

- 302 Neurons
- 56 Glial Cells
- >118 Different Neuron Classes
- Lifespan 12 to 18 days



AIM OF THE STUDY

This study aims to understand the molecular mechanisms behind neural ageing and to test the involvement of particular genes on neuronal ageing in *C. elegans.*

METHODOLOGY

1. Establishment and optimization of fluorescence-based assays to be able to evaluate the neuronal expression of candidate genes over the *C. elegans* lifetime.
2. Determination of candidate genes based on the literature: Yuan et. al (2020).
3. Generation of mutant *C.elegans* models: CRISPR-Cas9.
4. Imaging / analysis.

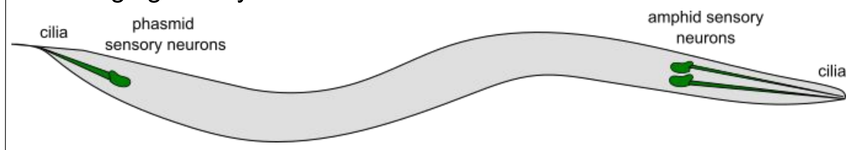
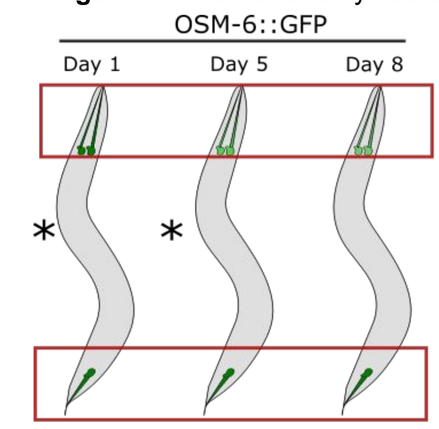


Figure 1: Ciliated sensory neurons in *C. elegans.*



Measurement of relative GFP intensities in amphid and phasmid cilia.

Figure 2: Representation of optimization of fluorescence-based assay. Since there is a significant loss of fluorescence signal in 5-day wild type worms compared to the 1-day worms, the rest of the experiment established on comparison of 1 and 5 days old worms.

PRIMARY RESULTS

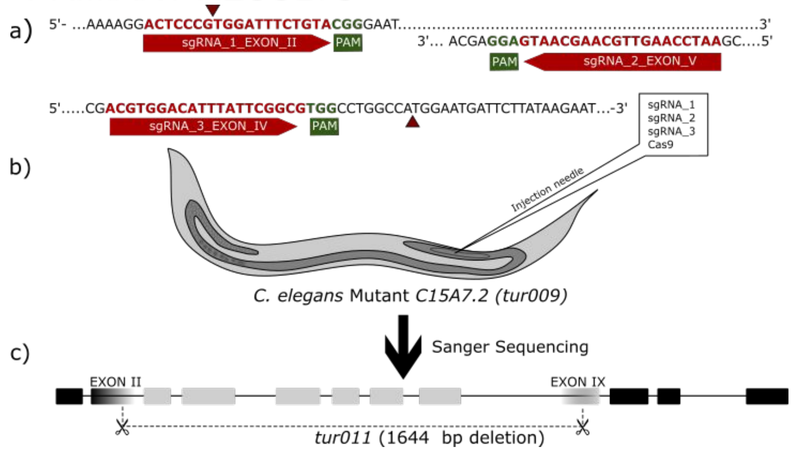


Figure 3: Generation of mutant *C.elegans* models by CRISPR-Cas9.
a)Three different guide RNAs are designed to knock out the target gene *T04F8.2.* b) sgRNAs and Cas-9 plasmid was injected to another mutant strain *tur009* that was generated in our lab. c)Knock out of target gene was confirmed by Sanger sequencing.

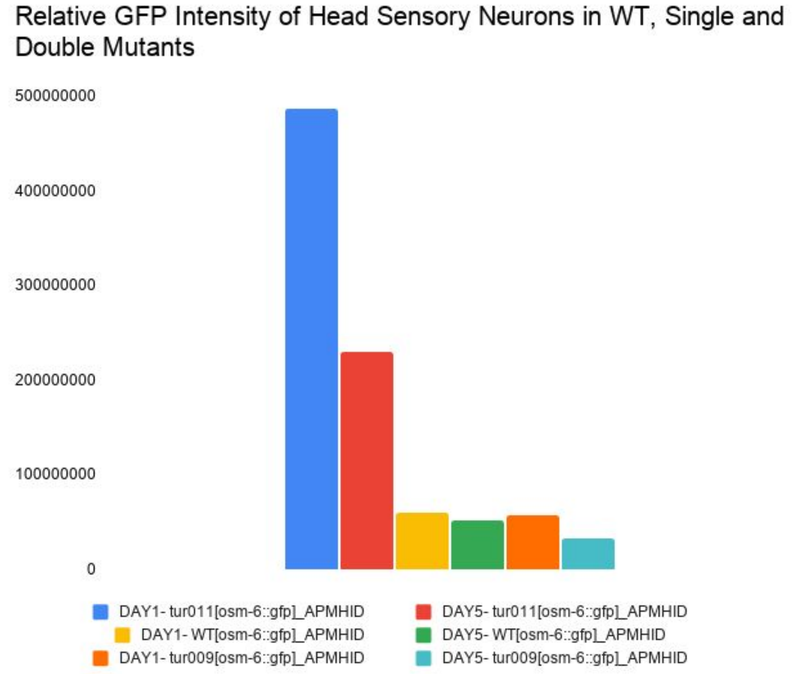


Figure 4: Relative GFP Intensity of Head Sensory Neurons in Wild Type, Single and Double Mutant *C. elegans* models.

CONCLUSION & NEXT STEPS

We have generated mutant models according to a RNA-interference-based screening study to test if neuronal ageing might be retarded if ageing-related genes are knocked out. As presented in *Figure 4,* sensory neurons of double mutant worms show significantly higher fluorescent signal compared to single mutant and wild type worms at the same age, especially in early development.

Ongoing and next steps include; Lifespan assays, increasing sample size, RT-qPCR, RNA interference (RNAi), analysis of variety of mutant strains and transgenics.

CONTACT: ahsen.konac@agu.edu.tr

REFERENCE

Yuan, J., Chang, S., Yin, S., Liu, Z., Cheng, X., Liu, X., . . . Cai, S. (2020). Two conserved epigenetic regulators prevent healthy ageing. *Nature, 579*(7797), 118-122. doi:10.1038/s41586-020-2037-y