Modeling Rare Diseases in *Caenorhabditis elegans*

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Abstract

There are approximately 7,000 rare diseases in humans, ~80% of which are monogenic. A rare disease is defined as affecting less than one in 1,500 people. Combined, these rare diseases affect nearly 1 in 10 Americans (25 to 30 million people), and treatments only exist for around 5% of these diseases. Thanks to the advent of whole genome sequencing, the gene(s) responsible for many rare diseases are known, opening the door for more comprehensive studies. Around 60% of the more than 20,000 protein-coding genes in *Caenorhabditis elegans* are estimated to have human counterparts. We can therefore study worm phenologs of human disease, or the distinct phenotypes in worms which, while different from the human phenotypes, stem from mutations in a homologous gene. The CRISPR-Cas9 system also allows us to mutate individual nucleotides analogous to those implicit in the human diseases to mimic the patient alleles. Presented here are examples of three ongoing projects designed to uncover cellular interactors and potential drug targets for improving and expanding the treatments available to patients suffering from a rare disease.

R01B10.6 - Seipin *(Berardinelli-Seip Syndrome)*

Rare mutations in the seipin gene BSCL2 are associated with the autosomal recessive congenital generalized lipodystrophy, or Berardinelli-Seip Syndrome (BSCL). This disease manifests primarily as a near complete loss of adipose tissue and severe insulin resistance. The yet-unnamed worm ortholog of seipin sits at the R01B10.6 locus and has 97.1% homology to the human isoform 1 of seipin. Seipin is required on the ER to form lipid droplets. *S. cerevisiae* data suggest a genetic interaction between null alleles of Seipin and MCTP-1 (another ER-localized, lipid-droplet-synthesis-involved protein). Our preliminary data supports this for *C. elegans* as well (Figure 1).

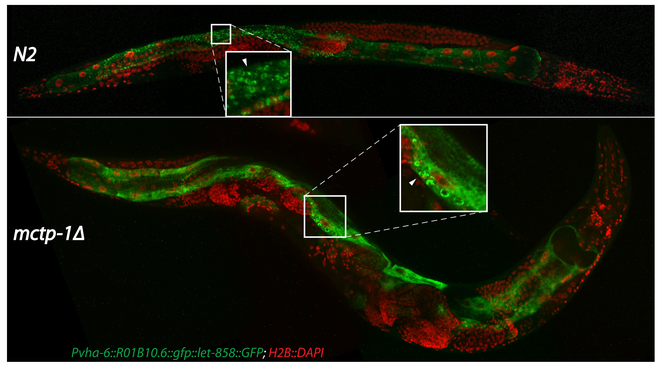

Figure 1: Seipin::GFP localization throughout intestine. *mctp-1Δ* homozygotes do not form as many lipid droplets as *N2* (white arrowheads, (n=4). *mctp-1Δ* mutants also appear to have an enlarged intestinal lumen (n=4). *Special thanks to Hi Yi Mak, Hong-Kong University of Science and Technology for the seipin gfp transgene.*

lpd-8 - Iron-Sulfur Cluster Scaffold *(Multiple Mitochondrial Dysfunctions Syndrome)*

The iron-sulfur cluster scaffold protein NFU1 is associated with the autosomal recessive Multiple Mitochondrial Dysfunctions Syndrome, which is characterized by encephalopathy, hypotonia, and psychomotor delay, as well as a lactic acidosis and a failure to thrive. Reconstructed human patient alleles (Figure 2) in the *C. elegans* NFU1 ortholog *lpd-8* (90.5% homology) are sterile (Figure 3). We are generating a new balancer for this region so that we can carry out suppressor screens.

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  10 individuals C
Human   203 KLKMQGSCTGC
             LK QGSCT C
C.eleg  161 QLKLQGSCTSC
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Figure 2: Annotated Conserved Iron-Sulfur Binding Region. The Fe-S Binding Motif CxxC is highlighted in orange. The patient allele (G208C) is highlighted in red. This allele has been recapitulated in *C. elegans* and is denoted *lpd-8(av56)*. As in the full knockout of this gene, this point mutant displays a late larval arrest or sterility phenotype (Figure 3).

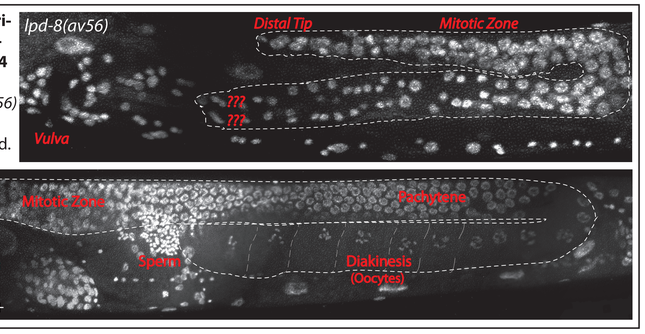

Figure 3: Anterior Hermaphrodite Gonads, 24 hours after L4 stage. *lpd-8(av56)* gonads remain underdeveloped.

kqt-3 - Voltage Gated Potassium Channel *(Long-QT Syndrome)*

Long-QT Syndrome (LQT) is an autosomal dominant class of arrhythmia characterized by an extended time of repolarization of the heart after each beat (>440ms instead of the normal 350-440ms) and can lead to sudden cardiac arrest. One of the human genes most closely associated with LQT is the alpha-subunit of the potassium channel KvLQT1, or KCNQ1. The *C. elegans* ortholog of KCNQ1 is *kqt-3* (87.9% homology) and deletion alleles of this gene display arrhythmic pumping in the pharynx (NemaMetrix, Eugene, OR). *C. elegans* themselves have genes for around 70 potassium channels and most cells express a complex mix of different channels. We are examining causative patient alleles in *kqt-3* in worms to study their affects on pharyngeal pumping, and will attempt suppressor, enhancer, and drug screens to try to reverse or enhance these phenotypes.

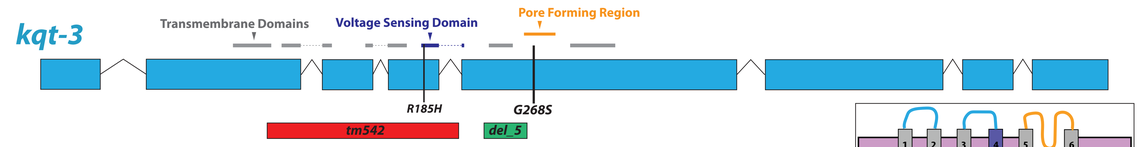

Figure 4: Gene (above) and Protein (right) Structure for *kqt-3*. The six transmembrane domains sit within exons two through five. The pore-forming domain both recognizes extracellular potassium and allows it to be pumped into the cell when the channel is activated. The pore-forming domain between transmembrane domains five and six is host to the highest density of mutations in patients. The region that is deleted in each mutant is annotated by a colored box. Two conserved point mutants implicit in LQT1, *R185H* and *G268S*, are currently being made.

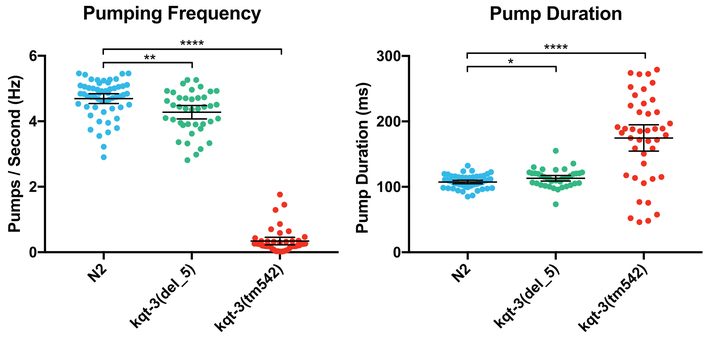

Figure 5: Pumping Frequency and Duration. The frequency of pumps per second varies significantly between wildtype and both *kqt-3* mutants (*kqt-3(del_5)* Hz p=0.0015 (**), duration p=0.0252 (*); *kqt-3(tm542)* Hz p=<0.001 (****), duration p=<0.001 (****)).

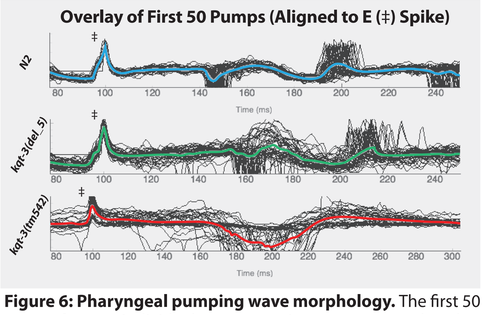

Figure 6: Pharyngeal pumping wave morphology. The first 50 pumps from an individual, representative worm are overlayed, with the average waveform indicated by the colored line for each strain. The variation in both *kqt-3* alleles is easily visualized.

Acknowledgements

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