Laying the groundwork for investigating the link between genes and polycystic kidney disease

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Introduction

Our lab studies human polycystic kidney disease using zebrafish and C. elegans as models.

- Patients with autoulosomal dominant polycystic kidney disease (ADPKD) suffer from disrupted kidney function due to large, fluid-filled cysts that form in the collecting ducts and kidney tubules (a)(i). Current treatments for the disease manage cyst growth but do not prevent cyst formation.
- Although the specific mutations that underlie polycystic kidney disease have been identified, the intervening steps between the altered gene and the disease symptoms remain unclear. We are particularly interested in the link between primary cilia and kidney cysts.
- This year, we worked to amplify target zebrafish genes, maintain healthy zebrafish and nematode populations, and study the effects in C. elegans of downregulating cilia-related genes through RNA interference (RNAi)(ii).

Investigating zebrafish with cystic kidneys

The zebrafish mutant, spinner, forms kidney cysts and has cilia defects thus making it a good model to study how cysts arise.

- In collaboration with the Mayo Clinic (Rochester), we aim to:
  - Characterize the gene expression changes that take place during kidney cyst formation using deep RNA-seq.
  - Characterize additional symptoms found in spinner mutant zebrafish(iii).
  - Identify the specific change in the DNA that underlies the mutant phenotypes.

The precise gene affected in the spinner mutant zebrafish remains unknown. Through deep RNA-seq, we have identified 10 genes that are expressed in the wild-type, but not in the spinner mutant zebrafish – these are ideal candidates for the cause of the spinner phenotype. In addition, whole genome sequence data revealed a deletion of part of chromosome 17.

In order to localize the mutation within the genome, we are using known markers to "map" the mutation.

![Mapping Strategy](image)

- Deletion of chromosome 17 is located somewhere between 2.3cM and 1.3cM.
- The spinner mutation is tightly linked to this deletion.
- The whole genome sequence data in this region is incorrectly assembled, thus candidate gene identification will require additional steps.

We plan to more precisely identify the boundaries of the deleted region and test for the presence of candidate gene sequences within the spinner mutant DNA.

![figure 1](image)

Dealing with cilia gene roles

Analysis of genes related to cilia structure and function may lead to insights into the underlying pathology of ADPKD.

ADPKD is a ciliopathy, a disease with symptoms caused by dysfunctional cilia.

- The gene PKD-2 encodes the protein PKD-2 which is found in the primary cilia of both C. elegans and humans. The PKD-2 protein must be targeted to the ciliary membrane for proper cilia function in both humans and nematodes(iv).
- Prior work in our lab has established an efficient pipeline for screening candidate PKD-2 localization factors in C. elegans. The lab screened 2,571 genes by RNA interference (RNAi) and identified 113 putative PKD-2 localization factors.

We aim to:
- Further characterize these PKD-2 localization factors.
- Assess the effects of interfering with translation of localization factors in strains with enhanced sensitivity to RNAi.

![figure 2](image)

Looking forward

Now that we have established both zebrafish and C. elegans models, future work in our lab will focus on developing a more nuanced understanding of the connections between mutations, cilia and disease symptoms.

Characterization of the zebrafish spinner mutant:

- Analyze heart asymmetry.
- Assess liver development and look for presence of cysts.
- Identify, clone, and characterize the causative gene.

Factors affecting PKD-2 localization:

- Analyze cilia integrity and function to determine whether putative PKD-2 localization factors affect PKD-2 localization directly or as a secondary consequence.
- Identify zebrafish orthologs of C. elegans localization factors to investigate gene function in a vertebrate.

Zebratfish Stripes and Pigmentation:

During the introduction of several populations to our zebrafish facility, we observed darkening of the stripes and overall pigmentation.

- Investigate if the pigment darkening is due to water pH, light cycles, or food type (and thus may be useful as a measure of fish acclimation).

Skills developed during our time in the lab

After completing an academic year of research in the Lyman Gingerich lab, we have developed several scientific research skills:

- Through analysis and discussion of primary literature on several topics (including RNA interference, polycystic kidney disease, and zebrafish husbandry), we can design experimental procedures and determine directions for research.
- Through time spent at the microscope and in the fish room, we developed the ability to sex both C. elegans and zebrafish and set up successful crosses.
- By developing a plan to compare different types of growth media (necessary for maintaining C. elegans), we learned about experimental design and the proper use of equipment, such as the autoclave.
- By running a number of different protocols (PCR, gel electrophoresis, dye-filling assays), we learned how to use microscopes, centrifuges, and balances. We also developed skills to interpret results and ask the next research questions.

Establishing a stable zebrafish colony

When using live organisms for research, like zebrafish and C. elegans, it is important to maintain healthy sustainable populations.

- C. elegans are relatively easy to maintain while the zebrafish require more intensive care.
- To maximize fish well-being we have established a feeding and husbandry schedule and their room is kept at a constant 25°C.
- Breeding the fish is essential for continued research. During early development, the fish are transparent making it easy to observe the internal organs. We have had success obtaining several clutches of embryos.

![figure 3](image)

References

6. Amack lab; University of New York, Upstate Medical University
7. Wormatlas; Introduction to male C. elegans anatomy

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