Zebrafish, genes, and human kidneys: gene mapping in zebrafish mutants may help uncover genetic roots of Polycystic Kidney Disease

Katie Paulich, Megan Schleusner, & Anneka Johnson ◈ Faculty Mentor: Dr. Jamie Lyman Gingerich
Biology Department ◈ University of Wisconsin-Eau Claire

The Power of AND

Introduction

- Patients with autosomal 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 (Figure 1) [1].
- Current treatments for the disease manage cyst growth but do not prevent cyst formation.
- Although the specific genes that underlie polycystic kidney disease have been identified, the intervening steps between the altered gene and the disease symptoms remain incompletely defined.
- The Lyman Gingerich lab studies polycystic kidney disease using the model organism *Danio rerio*, the zebrafish. *spinner* mutant zebrafish develop kidney cysts and may be a good model for understanding cystic kidney disease.

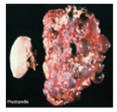

Figure 1: Comparison of a healthy human kidney to a cystic kidney.

This year, we worked as a team to identify the *spinner* gene, maintain healthy zebrafish with variant food sources, and develop new, less invasive techniques for DNA extraction with the zebrafish [2].

Establishing a stable zebrafish colony

Maintaining zebrafish:

- To maximize fish well-being, we have established a stable living environment: fish are housed at a constant 25°C, on a 12-hour light cycle, and consistently fed at the same time of day.
- To better mimic the wild-type zebrafish diet and allow the fish to eat ad lib, we tested a gel-based food. This food comes in powder form (figure 7b) that is mixed and allowed to solidify into a gel-block, which is fed to the fish.
- We found that fish initially adapted to eating at the bottom of the tank instead of the top, but that they seemed to lose interest in the food over time.

Given these results, we concluded it was best to continue the feeding of our fish with the previously used flake food.

Results

We have better resolved the span of the deletion (Figure 7). This will allow us to more precisely identify candidate genes involved in the phenotype.

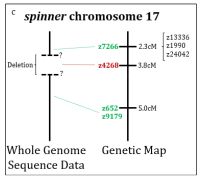

Figure 7: Identification of the boundaries of the deletion in the zebrafish genome.

Discussion

Skills acquired:

After completing another 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 RNAinterference, polycystic kidney disease, and zebrafish husbandry), we are able to design experimental procedures and determine continued directions for research.
- Through time spent in the fish room, we developed the ability to sex zebrafish and set up successful crosses.
- By continuing to work with our fish, we have reached our goal of obtaining and testing out new, less invasive methods for DNA extraction so that we can accomplish our research goals while ensuring our fish remain happy and healthy.
- We have moved to our new lab space in the Biology Molecular Suite and are enjoying the increased opportunities to interact with other researchers.

Looking forward:

Continuing to develop the new DNA extraction technique:

- We have had preliminary success with the new, less invasive swabbing method of DNA extraction. Optimization of this method of DNA extraction will allow us to use it as our primary method for DNA extraction from adult fish as an alternative to tail clipping.
- We also will continue to map the *spinner* mutation with the goal of identifying the causative gene and better understanding cystic kidney disease.

Extracting DNA using less invasive methods

A new approach:

A new, less invasive method of DNA extraction involves a skin swab of the zebrafish. We tested whether this method could yield enough DNA for PCR (Figure 2).

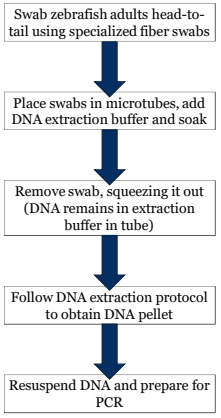

Figure 2: Flowchart of general DNA extraction and preparation.

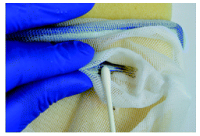

Figure 3: Fish swabbing technique; image from Breacker et al. 2017 [6].

Figure 4: Isolated PCR product obtained from skin swab protocol.

We found that with slight modification of the original protocol (e.g., greater number of swabs, ensuring fresh extraction buffer), this less invasive procedure obtains sufficient DNA for PCR.

Investigating zebrafish with cystic kidneys

Introduction:

- Our lab, in collaboration with the Mayo Clinic has begun to characterize the changes that take place during kidney cyst formation using *spinner*, a zebrafish mutant [3, 4].
- In addition to forming kidney cysts, the *spinner* mutant has a curved body morphology (Figure 5) and cilia defects in otic vesicles and the lateral line, rendering the fish deaf.
- The precise gene affected in the *spinner* mutant zebrafish remains unknown. Through deep RNA sequencing, we have identified 30 genes that are expressed in the wild-type, but not in the *spinner* mutant zebrafish, as well as a region of the genome that is missing in the mutant fish.

Figure 5: Three-day-old wild-type zebrafish and *spinner* zebrafish.

Method:

In order to determine whether the missing genomic region is the cause of the spinner mutant phenotype, we are taking a genetic mapping approach. We hypothesize that the deletion will be linked to the phenotype.

- Using a number of published markers (DNA sequence differences in different strains of zebrafish), we are localizing the mutation to a particular region of a chromosome.
- To do this, we extract DNA from zebrafish tissue, amplify the particular markers using PCR (Figure 6), and then digest the PCR products with restriction enzymes.
- Examination of the zebrafish genome sequence revealed that there are gaps in our knowledge of the specific sequence of some regions, including the region containing the deletion.
- To resolve this, we searched the genome sequence for each of the markers near the deletion.

Figure 6a: Gel electrophoresis set up separating PCR products by size.

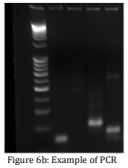

Figure 6b: Example of PCR products following gel electrophoresis.

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Acknowledgements

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