Zebrafish, *C. elegans* and Human Polycystic Kidney Disease

Identifying potential disease biomarkers through comparative analysis

Student Researcher: Samantha Meyer
Faculty Mentor: Dr. Jamie Lyman-Gingerich
Biology Department

The Power of AND | University of Wisconsin Eau Claire

Polycystic kidney disease is a genetic disease with no known cure

The focus of this project is to identify the downstream effects of mutations in the causative genes (PKD1 and PKD2) of polycystic kidney disease (ADPKD). ADPKD is responsible for 5% of all end-stage renal disease and is characterized by large, fluid-filled cysts forming in kidney tubules and collecting ducts that disrupt the normal functioning of the kidneys. It is currently thought that primary cilia (1) and different signaling pathways may play a major role in the progression of ADPKD.

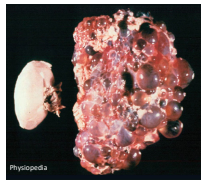


Figure 1. Comparison of a healthy human kidney to a cystic kidney.

Currently there is no cure for ADPKD, but this research could aid in the understanding of symptom progression and early diagnosis.

Zebrafish and *C. elegans* as models to study ADPKD

Figure 2. Adult zebrafish (left) and a *C. elegans* (right). Images are not to scale.

Both species are useful models because they have:

- Externally developing transparent embryos
- Large clutch sizes
- Short generation time
- Availability of genome editing tools
- Conservation of human kidney-expressed genes (2)

Zebrafish and human kidneys share many similarities while primary cilia can be easily studied in C. elegans.

The Spinner Mutant develops kidney cysts

Zebrafish homozygous for a mutation in the *spinner* gene were used to better understand cyst formation and its secondary effects. The spinner mutant fish are characterized by:

- Kidney cysts
- Curvature of the spine
- Otolith defects
- Death by 5 days old

Figure 3. Three day old wild-type (top) and *spinner* (bottom) mutant fish.

Transgenic *C. elegans* can be used to visualize PKD-2::GFP localization

Previous research in the lab identified 113 genes that affect PKD-2 localization to primary cilia.

RNAseq allows for comparison of gene expression levels and patterns

wild-type | *spinner*

↓ ↓

Collect RNA from *spinner* mutant embryos and wild-type siblings

↓

Sequencing

↓

Map to reference genome

↓

Data analysis and interpretation

Wild-type and spinner fish have very different gene expression profiles

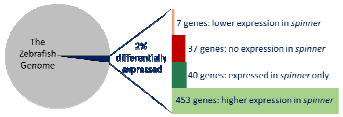


Figure 4. Of 27,207 zebrafish genes analyzed, 537 (2%) showed statistically significant (student's paired t-test, p< .001) differential expression between *spinner* mutant and sibling wild-type fish at three days old (after the onset of cyst formation.)

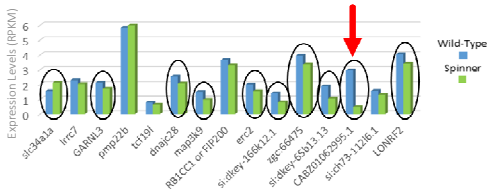
Which Gene is Affected in the Spinner Fish?

Hypothesis: If the mutation results in the *spinner* gene not being expressed, we would expect that candidate genes would only be expressed in wild-type, not mutant, fish.

- Of the 37 genes expressed in wild-type, but not in *spinner* fish, 10 genes are linked to regions of the genome previously associated with the *spinner* mutation while 8 genes are associated with kidneys, cysts, or cilia.
- Combining these two sets yields 3 promising candidate genes:
  1. *ccr6b* – encodes a G-protein coupled receptor
  2. *thbs1* – encodes a matricellular protein with known roles in kidney disease
  3. *chrm5a* – encodes a receptor found in a ciliated cell-type of the eye

Can we identify different patterns of gene expression in wild-type versus spinner fish?

Hypothesis: By identifying the genes with differential expression, we can identify the pathways involved in cystogenesis or the downstream effects of these gene.



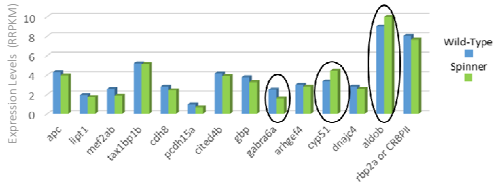


Figure 5. A selection of genes showing significant differential expression between wild-type and *spinner* mutant fish, that are also related to cysts, cilia or kidneys. p<0.001 (paired t-test)

- The circled genes in Figure 5 show the genes with the largest expression differences. For some genes, expression is higher in wild-type, while in other genes expression is higher in *spinner*. Assignment of these genes to pathways may allow us to draw conclusions about how these pathways interact.
- The gene CABZ01062995.1 (indicated by the red arrow) shows the most dramatic difference in expression levels of any of the genes analyzed. Currently, the function of this gene is unknown. In the future, we plan to identify this gene product's function and localization.
- Literature review reveals that many of the differentially expressed genes can be categorized according to common function or subcellular localization.

Is there overlap between the genes identified as differentially expressed during zebrafish cystogenesis and those that affect PKD2 localization in *C. elegans*?

Hypothesis: If primary cilia structure and function are related to kidney cyst formation, genes that affect cilia (assessed by PKD2 localization) may also be differentially expressed during cystogenesis.

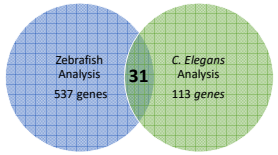


Figure 6. There are 537 differentially expressed genes in zebrafish and 113 genes affecting PKD2 localization in *C.elegans*. Comparison of these two datasets showed 31 common genes.

Conclusions and future directions

We have identified and analyzed 537 differentially-expressed genes in a zebrafish cystic kidney mutant using RNAseq. This work lays the groundwork for future research including identification of the specific nature of the *spinner* causative gene, further definition of the pathways involved in cyst formation and associated disease symptoms, and characterization of the functions and expression patterns of uncharacterized genes.

References

1. Adams, M. (2010) *Nature Education* 3(9):54
2. Howe, Kerstin et al. "The Zebrafish Reference Genome Sequence and Its Relationship to the Human Genome." *Nature* 496.7446 (2013): 498–503. *PMC*. Web. 24 Mar. 2017.
3. Qian, X. et al. (2014. *OMICS, 18*(2), 98-110.

Acknowledgements

- University of Wisconsin – Eau Claire Office of Research and Sponsored Programs (funding)
- Dr. Noriko Umemoto, Alaa Koleilat, and Dr. Stephen Ekker, Mayo Clinic
- Dr. Caroline Sussman, Dr. Peter Harris and Dr. Vicente Torres, Mayo Translational Polycystic Kidney Disease Center

We thank the Office of Research and Sponsored Programs for supporting this research, and Learning & Technology Services for printing this poster.