# Polycystic Kidney Disease and Protein Localization: Analysis of the role of gar-3 in protein localization to cilia in *Caenorhabditis elegans*

The Power of AND

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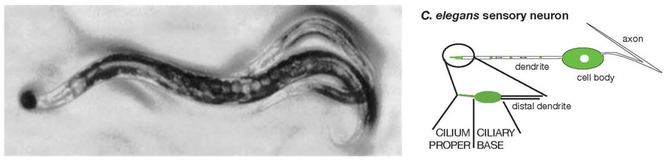
## Introduction

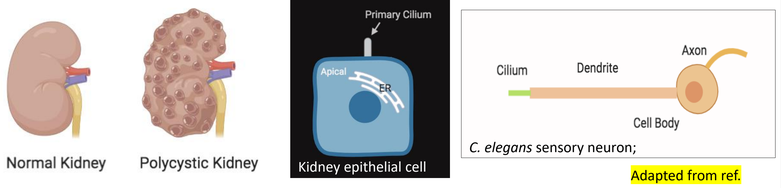
### Polycystic Kidney Disease-2 (PKD-2) protein localization is conserved between humans and *C. elegans*




Primary cilia are found on cell surfaces of many different eukaryotic cells, including human kidney epithelial cells. *C. elegans* are used as a model organism for studying polycystic kidney disease (PKD) because the PKD-2 protein localizes to the primary cilia of some of their sensory neurons; this is similar to PKD-2 protein localization to cilia of human kidney epithelial cells.

### Mislocalization of PKD-2 results in symptoms in both humans and *C. elegans*

In the human kidney, primary cilia extend from the apical surface of the epithelium. In *C. elegans,* primary cilia extend from dendritic endings of sensory neurons. Mislocalization of the PKD proteins contributes to polycystic kidney disease in humans and mating behavior defects in *C. elegans.*




### The Gar-3 Gene encodes an acetylcholine receptor that may play a role in localization of PKD2 to cilia

In zebrafish that develop kidney cysts, expression of an acetylcholine receptor (CHRM5A) is absent (our own unpublished data). To investigate whether this receptor is specifically involved in PKD2 localization, we turned to our *C. elegans* model. We asked whether loss of *gar-3* (the ortholog of CHRM5A) expression in *C. elegans* would affect PKD2 localization and/or primary cilia.

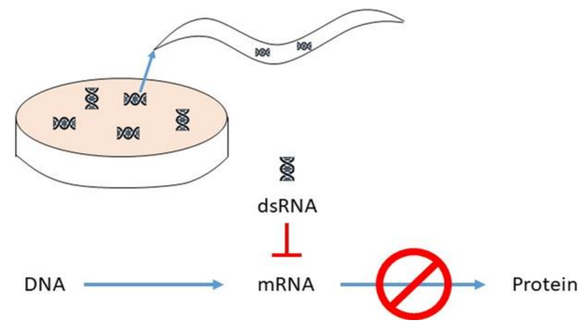
## Hypothesis

If *gar-3* has a role in cilia structure, function or receptor localization, I would predict that reduction of *gar-3* expression would lead to cilia and PKD-2 protein localization defects.

## Approaches/ Methods

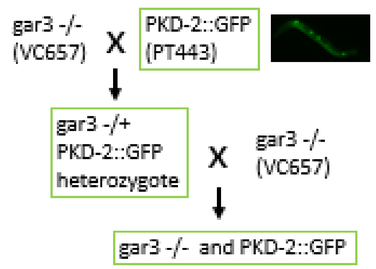
We are taking two approaches to reduce *gar-3* expression in *C. elegans* and examine PKD-2::GFP localization. First, we are decreasing the amount of *gar-3* transcript using RNAinterference. We are also using a genetic approach to produce worms that are homozygous mutant for *gar-3* and carry the PKD-2::GFP transgene. We can then assess cilia structure using a dye-filling assay and PKD-2::GFP ciliary localization.

### 1. RNAinterference against the *gar-3* gene



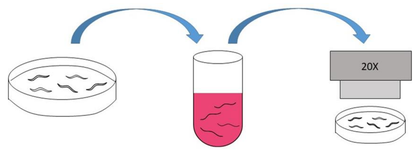

*C. elegans* are fed bacteria containing double stranded RNA that binds with the *gar-3* mRNA, which lowers the total amount of protein made by the *gar-3* gene.

### 2. Using genetic crosses to produce a worm that is homozygous mutant for gar-3 and expresses PKD-2::GFP



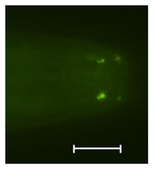

PT443 male worms are crossed with VC657 hermaphrodites in two subsequent genetic crosses to produce male progeny that are homozygous mutant for the *gar-3* gene.

### 3. Assessment of ciliary structure and PKD-2::GFP localization

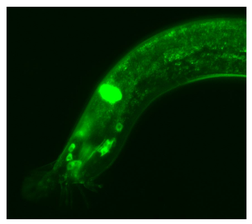


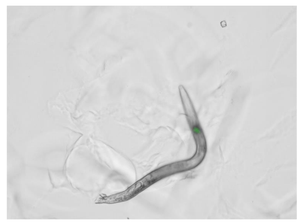

Intact neurons fill with a lipophilic fluorescent dye (DiI) when *C. elegans* are soaked in the dye solution allowing us to assess amphid and phasmid neuron integrity.

Fluorescence microscopy reveals the pattern of PKD-2::GFP in male-specific neurons.



## Preliminary Results and Future Directions





Initial results from the genetic crosses suggest that we have produced *C. elegans* homozygous mutant for *gar-3* and containing PKD-2::GFP. Of the worms that were homozygous mutant, PKD-2::GFP localized to the amphid neurons only, were longer than normal, and had more dramatic head movements (represented above).

Ongoing and future experiments include:

1. Downregulation of *gar-3* expression using RNAinterference and assessment of effects on cilia structure and protein localization.
2. Assessment of phasmid and amphid neuron integrity via dye-filling in *gar-3* genetic mutants.
3. Assessment of PKD-2::GFP localization in *gar-3* genetic mutants.

## References

Bae Y.-K., J. Lyman Gingerich, M.M. Barr, and K.M. Knobel. "Identification of Genes Involved in the Ciliary Trafficking of C. Elegans PKD-2." *Developmental Dynamics : an Official Publication of the American Association of Anatomists*, U.S. National Library of Medicine, Aug. 2008, www.ncbi.nlm.nih.gov/pmc/articles/PMC3118579/.

"Gar-3 (Gene) - WormBase : Nematode Information Resource." *WormBase*, wormbase.org/species/c_elegans/gene/WBGene00001519#0-9f71b3e-10.

Parnell, Stephen C, et al. "Polycystin-1 Activation of c-Jun N-Terminal Kinase and AP-1 Is Mediated by Heterotrimeric G Proteins." *The Journal of Biological Chemistry*, U.S. National Library of Medicine, 31 May 2002, www.ncbi.nlm.nih.gov/pubmed/11912216.

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