Investigation of Novel Cys-Loop Ligand-Gated Chloride Channels in *Dirofilaria immitis*

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Background

Dirofilaria immitis is the parasitic roundworm that causes canine heartworm disease[1].

- Transmitted by mosquito vectors[1]
- Hosts
  - Dogs (Definitive host)
  - Cats
  - Humans

  Accidental Hosts

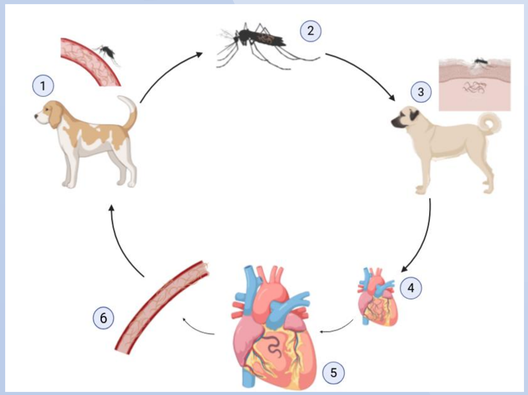


Figure1: Transmission cycle of *D. immitis*[6]

The neuromuscular system of *D. immitis* controls movement and feeding, making it a target for anthelmintic therapy.[2]

Cys-loop receptors mediate fast excitatory and inhibitory signalling in parasitic nematodes.[3,4]

Acetylcholine-gated chloride channels (ACCs) and the **dopamine-gated chloride channel** are potential drug targets because these receptor types are absent in mammals.

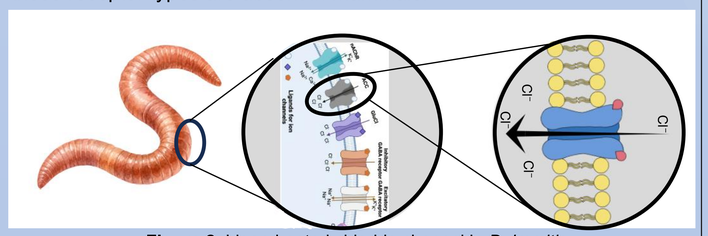


Figure 2. Ligand-gated chloride channel in *D. immitis*

Objective

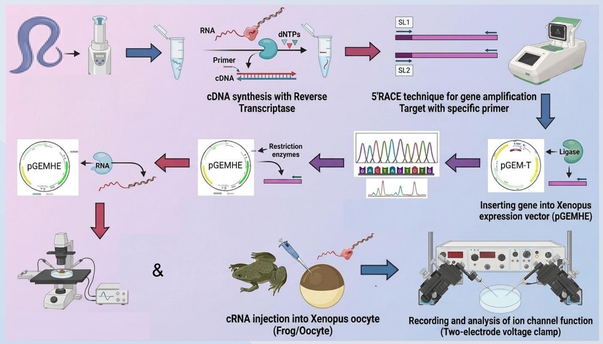
1. **Gene cloning and expression analysis**
   Isolate the full-length sequences of *lgc-46*, *lgc-41*, and *lgc-53* and compare their expression in *D. immitis* microfilariae.

2. Electrophysiological characterization
Characterize LGC-46, LGC-41, LGC-53, and the previously cloned ACC family members LGC-47, LGC-49, and ACC-1 using two-electrode voltage clamp (TEVC) in *Xenopus laevis* oocytes. Test selected co-expression combinations.

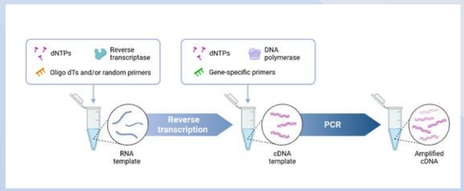
3. Effects of RNAi on parasite motility
Assess how RNAi-mediated knockdown of the target genes affects parasite motility.

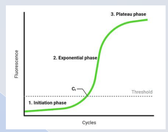
Methods

1 Gene cloning and Heterologous expression in *Xenopus laevis* oocytes[6]

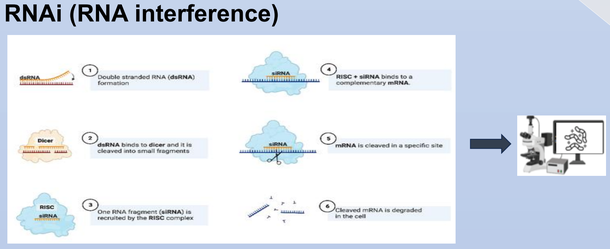


2 Gene expression analysis (qPCR)





3 RNAi (RNA interference)



Co-expression of *lgc-49* and *ACC-1:*

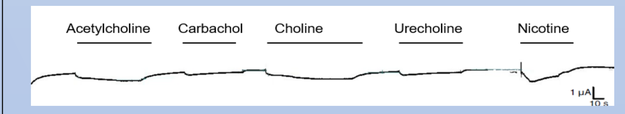


Figure 14. Agonist screening (All compounds 500 uM)

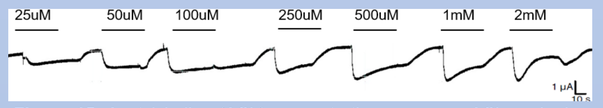


Figure 15. Acetylcholine (ACh) concentration-response: ACh-evoked currents were recorded across concentrations ranging from 25 µM to 2 mM.

Co-expression of *lgc-49* and *lgc-47:*

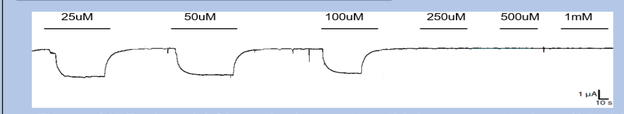


Figure 16. Reduced ACh-evoked currents at higher concentrations (25 µM–1 mM), consistent with possible desensitization.

Results

1- Gene Cloning

***lgc-53*: Restriction digestion confirmed a cloned insert of the expected size**

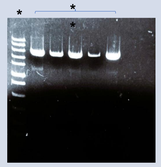


Figure 3. Agarose gel of digested pGEM-T/*lgc-53* construct with EcoRI
* DNA ladder
**Lane 1: pGEM-T vector, Lane 2: insert corresponding to *lgc-53*.

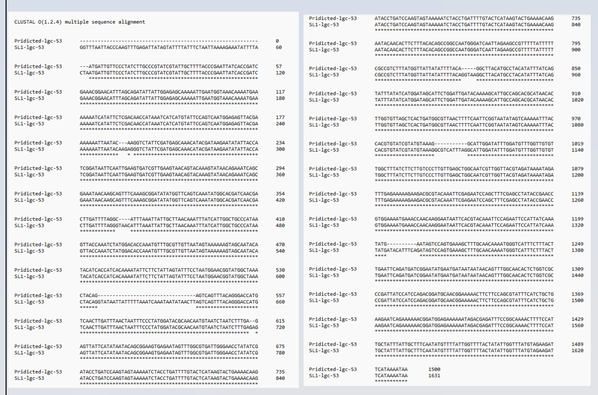


Figure 4 and 5. SL1- *lgc-53* and predicted *lgc-53* CDS alignment

***lgc-46*: Alternative splicing of *lgc-46* produces multiple transcripts**

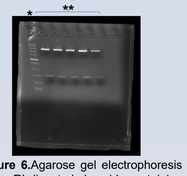


Figure 6. Agarose gel electrophoresis of EcoRI-digested plasmids containing the *lgc-46* insert amplified using the SL2 primer, confirming successful cloning of the amplified fragment.
*DNA ladder
**pGEM-T/*lgc-46* recombinant plasmids amplified using the SL2 primer.



Figure 7. Alternative spliced-leader attachment sites in *lgc-46*. The arrow indicates the SL2 attachment site.

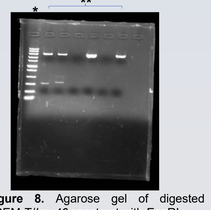


Figure 8. Agarose gel of digested pGEM-T/*lgc-46* construct with EcoRI
* DNA ladder
**pGEM-T/*lgc-46* recombinant plasmids amplified using the SL1 primer

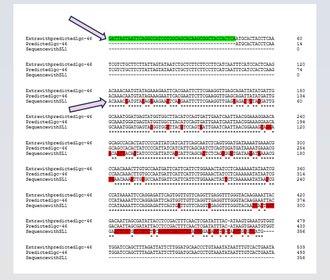


Figure 9. Alternative spliced-leader attachment sites in *lgc-46*. Arrows indicate two alternative SL1 attachment sites: one upstream of an additional sequence (green) and one within the predicted *lgc-46* sequence. Red nucleotides indicate nucleotide polymorphisms relative to the predicted reference sequence.

2- Electrophysiological characterization of LGC-49 and several other ACC subunits

***lgc-49* expressed alone:**

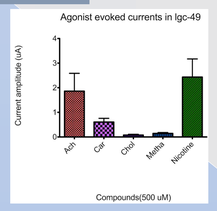


Figure 10. Agonist-evoked current amplitudes in LGC-49-expressing oocytes (500 µM, mean ± SEM, n = 6). Nicotine and acetylcholine elicited the largest mean currents.

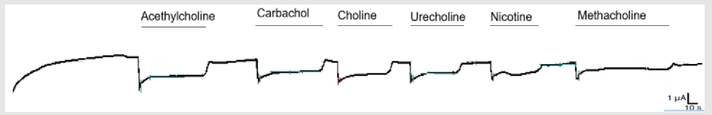


Figure 11. Agonist screening (All compounds 500 µM)

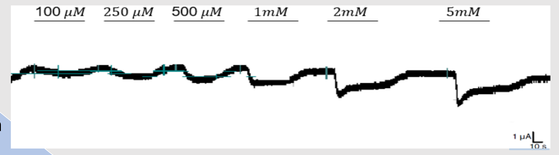


Figure 12. Dose-response with Methacholine

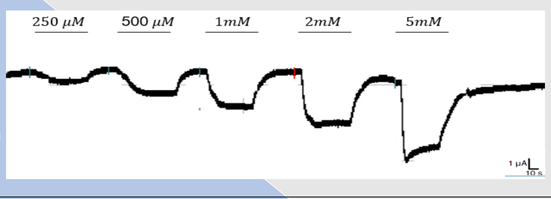


Figure 13. Dose-response with Carbachol

Future Work

Full-length *lgc-46*, *lgc-41*, and *lgc-53* coding sequences will be cloned and expressed in *Xenopus laevis* oocytes for electrophysiological characterization. LGC-46 will also be co-expressed with other ACC subunits to investigate potential receptor combinations. RNAi will be used to assess the roles of these receptors in parasite motility, survival, and neuromuscular function.

Acknowledgment

I would like to thank my supervisor, Dr. Sean Forrester, for the opportunity to pursue this research and for his guidance and support throughout the project. I also thank the members of the Forrester Lab for their encouragement and support.

References

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4. Wolstenholme, A. J., & Rogers, A. T. (2005). Glutamate-gated chloride channels and the mode of action of the avermectin/milbemycin anthelmintics. *Parasitology*, 131(S1), S85-S95.
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