Rapid phenotypic assessment for mutations involved in human diseases: a novel model for cardiac arrhythmia in the nematode *C. elegans*.

Yoanne M. Clovis[1], Alexis Webb[1], Carl Turner[1], Kathryn McCormick[1], Shawn Lockery[1]
[1] NemaMetrix Inc., 44 W 7th Avenue, Eugene, United States

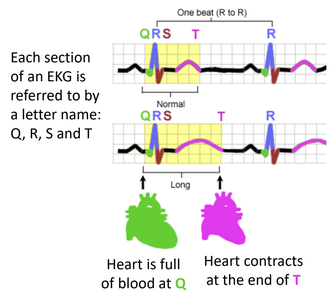
NemaMetrix

Background and rationale

Epilepsy, deafness and cardiac arrhythmias such as long-QT syndrome (LQTS), can be associated with KCNQ1, a member of the KCNQ family of potassium channels. Symptoms of LQTS include fainting, seizures, **abnormal rate and rhythm of the heartbeat** (arrhythmia) and sudden death. Meanwhile, mutations in KCNQ2 and KCNQ3, are associated with benign neonatal epilepsy.

The pharynx of nematodes is a rhythmic muscular pump involved in feeding that bears many similarities with the heart of vertebrates. In particular, both are rhythmic muscular pumps that do not require nervous system input and rely on similar types of ion channels. Homologs of KCNQ genes are found in a wide range of model organisms, including flies and mice, where they often have functions analogous to those in humans.

Q = starts ventricular depolarization/ contraction
T = ends ventricular repolarization/ starts relaxation



LQTS = prolonged heart contraction

We investigated the effect of mutations in the *C. elegans* KCNQ-like genes on the electrical excitability of the pharynx. Two such genes, *kqt-1* and *kqt-3*, are orthologous to the human genes KCNQ2 to KCNQ5, and KCNQ1 respectively. *kqt-1* is mainly expressed in the muscles of the pharynx, whereas *kqt-3* is present in mechanosensory and chemosensory neurons, which can regulate feeding behavior.

We hypothesized that mutations in *kqt-1* and *kqt-3* induce abnormalities in pharyngeal pumping that are reminiscent of cardiac arrhythmias.

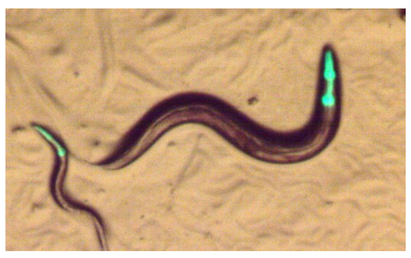
Material and methods

Mutations

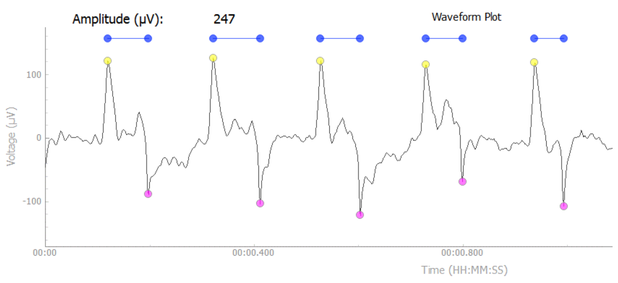
kqt-1(aw3) mutants have a 620 bp deletion of the 2.4 Kb long *kqt-1* gene. *kqt-3(aw1)* mutants have deletions of 1674 bp of the 3.1 Kb long *kqt-3* gene.

Recording electropharyngeograms (EPGs) and data analysis

Worms were pre-incubated for in M9 buffer containing 10 mM serotonin to induce pumping. EPGs were recorded using the NemaMetrix ScreenChip System. Recordings were analyzed using NemAnalysis software which automatically identifies individual pumps and extracts mean pump duration, mean inter-pump interval (IPI) and the mean pump frequency for each worm.



C. elegans worms expressing GFP in the pharyngeal muscle



EPG trace highlighting various parameters of interest

- E peak: Excitation/contraction phase
- R peak: Relaxation phase
- E to R: 1 pump

Results

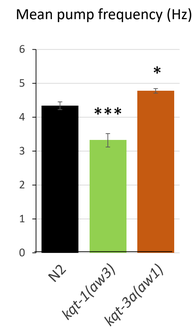
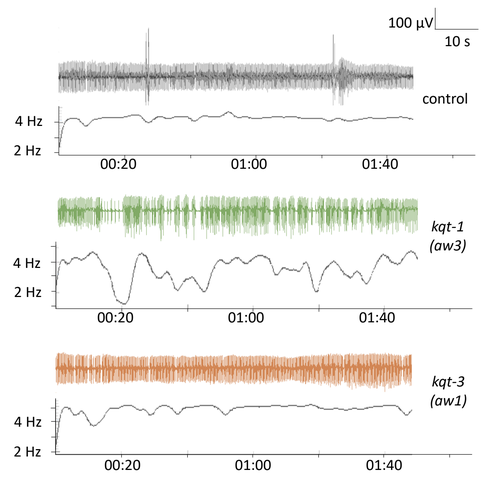


Fig. 1: Mutations in *kqt-1 and kqt-3* cause abnormal pumping rate.
*p<0.05; ***p<0.01
(*n* ≥ 21 worms for each strain)

Fig. 2: Lack of functional *kqt-1* but not *kqt-3* leads to arrhythmia.
EPGs (upper panels) and frequency over time (lower panels) are showed for one worm per strain. In control (grey) and *kqt-3(aw1)* (red) worms, pumps occur at regular intervals, whereas in *kqt-1(aw3)* worms (green), pumps occur at irregular intervals, shown by the sudden drops in frequency.



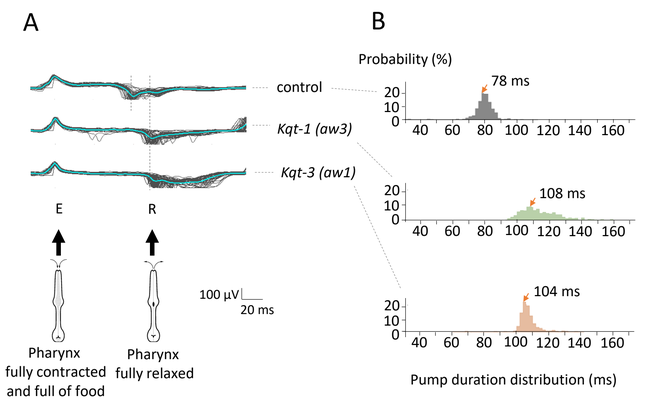
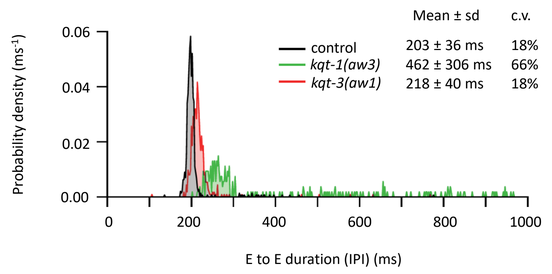


Fig. 3: Mutations in *kqt-1* and *kqt-3* prolong the pharyngeal action potential.
A) Overlay of the first 50 pumps as recorded with EPG in 1 worm per strain. The average pump shape is in green.
B) Pump Duration distribution for 1 worm per strain over a 2-min recording.
The duration of pharyngeal action potentials is prolonged. Cardiac action potentials are also prolonged in human LQTS patients.

Fig. 4: Lack of functional *kqt-1* leads to abnormally long and irregular pauses.
The IPI in control (black) and *kqt-3* (red) worms is stable around 200ms.
In *kqt-1* worms, more than 75% of the IPIs are longer than 250 ms.
(n ≥ 21 worms for each strain)



Conclusion

We present evidence that two *C. elegans* potassium channel genes, *kqt-1* and *kqt-3* are necessary for normal pharyngeal pumping.
The pumping defects observed in these mutants are consistent with the well-known role of KCNQ potassium channel mutations in generating cardiac arrhythmias in humans and model organisms. Both genes are required for normal pump duration and pump frequency as elicited by serotonin. *kqt-1* in particular, is required for normal latency between pumps and regular pumping rhythm.

We propose that the strains tested here, and the recording methodology used, could be the basis of future **screens to identify pharmacological agents** to mitigate certain arrhythmias.

Taken together, these data demonstrate the feasibility of using *C. elegans* to identify candidate genes for heart disease and to assess the effects of new therapeutic agents in **high-volume, whole-animal screens** in an **unbiased** manner.

Future directions

The present study focuses on using *C. elegans* as a model for cardiac arrhythmias [10], but *C. elegans* can also be used as a model for other KCNQ-related phenotypes, including **epilepsy** [8,9]. *C. elegans* epilepsy models exhibit localized or whole-body contractions which can silence pharyngeal pumping [8,9,11].

The methodology described in this study makes pharyngeal pumping easier to quantify than body contraction, providing a novel means of screening for compounds that might prevent epileptic convulsions.

References and acknowledgements:

1. Singh et. al, Nat. Genet. (1998).
2. Singh et al., Brain 126, 2726–37 (2003).
3. Goldman, A. M. et al. , Sci. Transl. Med. 1, 2ra6 (2009).
4. Ocorr, K. et al., Proc. Natl. Acad. Sci. U. S. A. 104, 3943–8 (2007).
5. Wei et al., J. Biol. Chem. 280, 21337–21345 (2005).
6. Wei et al. Nucleic Acids Res. 30, e110 (2002).
7. NemaMetrix ScreenChip System User Guide - nemametrix.com
8. Williams et al., Hum. Mol. Genet. 13, 2043–59 (2004).
9. Petersen et al., PNAS (2004).
10. Schüler et al., Sci. Rep. (2015).
11. Pandey et al., Seizure (2010).

The mutant worms kqt-1 (aw3) and kqt-3 (aw1) were kindly donated by Dr. Aguan Wei.

Booth #

Come try the ScreenChip system yourself during our live worm demo

Contact
Yoanne M. Clovis, PhD
Yoanne.clovis@nemametrix.com