

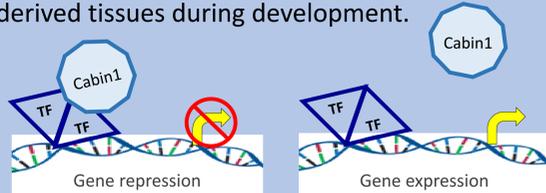
# Assessment of Auditory and Vestibular Function in Cabin1 Knockout Larval Zebrafish

Jacob E. Exline<sup>\*1</sup>, Sarah C. Sarich<sup>1</sup>, Dena R. Hammond-Weinberger<sup>1</sup>, Brian A Link<sup>2</sup>, Ava J. Udvadia<sup>1</sup>  
 UWM Biological Sciences<sup>1</sup>, MCW Cell Biology, Neurobiology & Anatomy<sup>2</sup>



## How does **Cabin1** gene expression affect auditory and vestibular function in developing zebrafish?

- ❖ Functional auditory and vestibular systems are responsible for hearing and balance; and critical for an organism's survival.
- ❖ **Cabin1** is expressed in the ear and neurons of the head.
- ❖ **Cabin1** encodes a calcium-dependent transcriptional repressor and is expressed in neural and neural crest derived tissues during development.



(Replogle et al., *in progress*)

- ❖ Preliminary results suggest that reduction in **Cabin1** gene expression in developing zebrafish impaired escape response. (Hammond-Weinberger, 2012)
- ❖ The current study tries to validate the previous studies using animals with **Cabin1** gene knock-out.

## Experimental Approach

- ❖ 3 day old larval zebrafish with targeted knockout of **Cabin1** gene.
  - CRISPR gene editing used to induce 5-base pair deletion in coding sequence.
  - Deletion results in premature truncation of the **Cabin1** protein, eliminating its function.
  - The progeny of two heterozygous parents are used, resulting in a **1:2:1 ratio** of homozygous: heterozygous: wild type fish.
- ❖ Wildtype zebrafish elicit escape responses when neurons of the head, tail, or ear stimulated.



(Bhandiwad, 2013)

- ❖ The ability of the fish to maintain itself in an upright position requires functionality of the vestibular system.

## Hypothesis

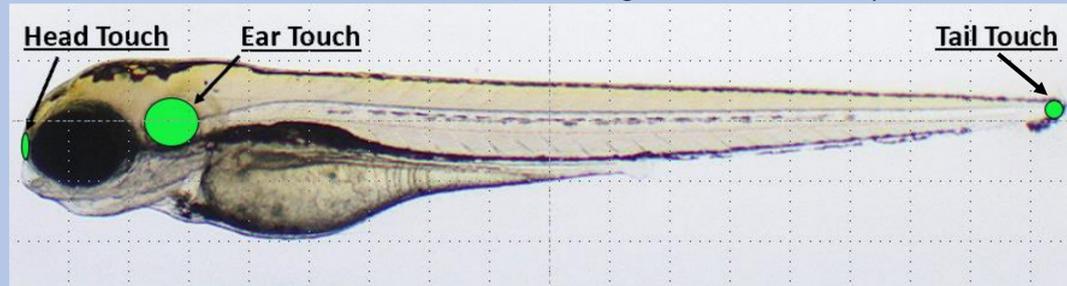
**Cabin1** expression is necessary for proper function of the auditory and vestibular systems.

## Response to Touch

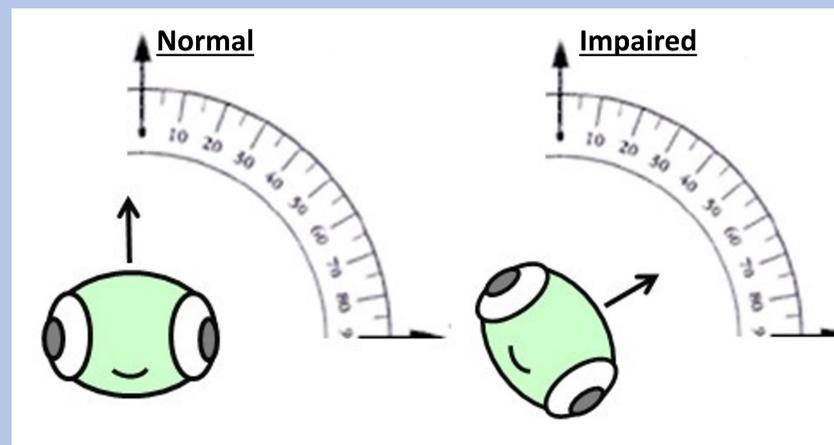
- ❖ By comparing the touch response of the otic vesicle (ear) to that of the head and tail, auditory response deficits can be differentiated from general motor deficits.

- ❖ 3Dpf larval zebrafish are evaluated with four behavioral assays:

- 1. Ear Touch** assay stimulates Cranial Nerve VIII (sensory) and the Mauthner neuron (motor output) to assess auditory escape response.
  - Light pressure to the otic vesicle activates Cranial Nerve VIII; exclusively activating the Mauthner neuron, which mediates the motor portion of escape.
  - Impaired ear touch response with reduced **Cabin1** expression; no significant impairment observed with head and tail touch. (Hammond-Weinberger, 2012)
- 2. Head Touch** evaluates function of the peripheral sensory neurons which are neural crest derived, and the Mauthner neuron.
- 3. Tail Touch** evaluates motor neuron function, to distinguish between sensory and motor defects.



## Zebrafish Posture

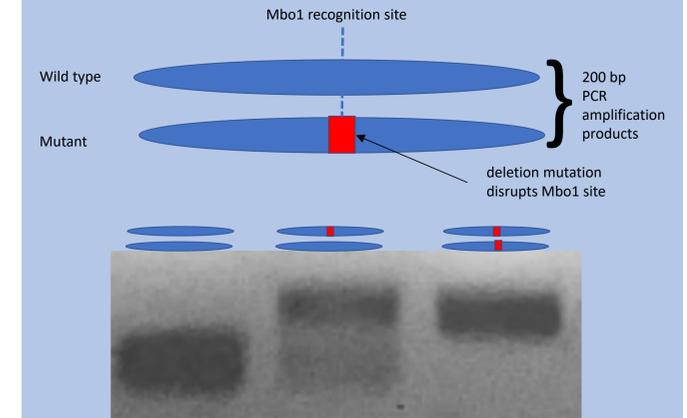


(Hammond-Weinberger, 2012)

- 4. Larval Posture** assesses vestibular function, and is observed before and after the escape response assays.
  - Upright (0°) is normal, lying down (90°) is impaired.

## Genotyping

- ❖ PCR amplification of genomic DNA and subsequent Restriction Digest with Mbo1 enzyme.



- Allows identification of larvae in which one, both, or neither chromosomes contain the **Cabin1** mutation.

## Research in Progress

- ❖ No larval zebrafish have exhibited impaired auditory escape response. (n=32)
- ❖ Preliminary vestibular assessment is inconsistent between touch assays.
  - Fish start out upright but lay down after elicited escape response, or vice versa.
  - Some fish use pectoral fin to prop themselves upright.

## Future Research

- ❖ Determine a method of delivering consistent touch pressure to target area.
- ❖ Increase sample size.
- ❖ Determine whether use of pectoral fin in upright posture is correlated with **Cabin1** genotype.

## Acknowledgements

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