Validation of the zebrafish mutant *lessen* as an experimental model to study Hirschsprung's disease

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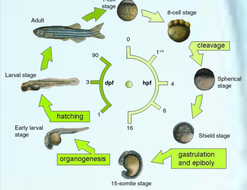
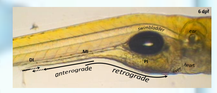
Introduction

Zebrafish emerged as a model organism in experimental research, including studies of congenital gastrointestinal (GI) diseases like Hirschsprung's disease (HD). HD is characterized by aganglionosis of the distal intestine. The zebrafish mutant, *lessen,* expressing HD characteristics, is suggested to be an experimental model to unravel HD developmental mechanisms. This study aims to compare neurochemical content of enteric neurons and GI motility between wild type and mutant zebrafish to further validate this model.





Zebrafish wild type morphology and general outline of GI motility patterns

Methods

Using immunological staining methods on isolated intestines from wild type (*wt)* and mutant embryonic (3 to 6 dpf) zebrafish, we demonstrated and quantified the expression of different neurochemical markers in the three functional intestinal segments: the proximal, middle and distal intestine (PI; MI; DI). An antibody against Hu was used as a pan-neuronal marker.

Contractile activity of the GI tract was filmed and frequency and direction of contractions in each intestinal region were analyzed.

wild type (A: wt) vs mutant lessen (B: *lsn*)

Abbreviations

5HT: serotonin; CB: calbindin; CR: calretinin; DI: distal intestine; dpf: days post-fertilization; ENS: enteric nervous system; GI: gastrointestinal; HD: Hirschsprung's disease; hpf: hours post-fertilization; IR: immunoreactivity; lsn: lessen; MI: middle intestine; nNOS: neuronal nitric oxide synthase; PACAP: pituitary adenylate cyclase activating peptide; PI: proximal intestine; VIP: vasoactive intestinal peptide; wt: wild type.

Bars = 50 µm

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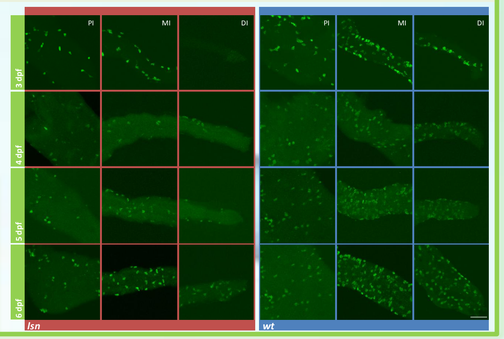
Immunohistochemistry

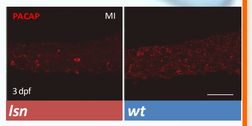
Neurons

The total amount of neurons for *wt* and *lessen* (*lsn*) increases in time, but total numbers in mutants are decreased compared to *wt*. The number of neurons was significantly reduced in the DI (even absent at 3 dpf) and the MI, but less in the PI at each embryonic stage.



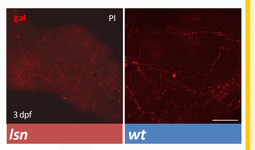
PACAP and VIP

Mutant fish only show slight immunoreactivity (IR) in the PI at 3 dpf, compared to the whole intestine in *wt*. 4 dpf, PACAP and VIP IR is found in the MI as well and at 5 and 6 dpf also the DI shows some IR in the enteric nervous system (ENS). Mucosal cells show PACAP and VIP IR over the whole intestine from 3 dpf.



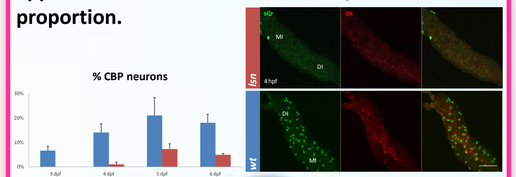
Galanin

Galanin IR is restricted to the PI and the first part of the MI in *lsn* at 3 dpf compared to the whole intestine in *wt*. IR in the DI starts from 5 dpf.



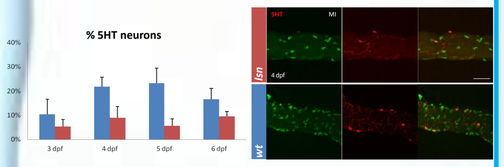
Calbindin and calretinin

In *wt* first IR is present at 3 dpf in the MI. In *lsn*, neurons start to show IR for both CB and CR around 4 dpf in the PI and MI. At 5 dpf, the amount of IR for the CBP was 80% less compared to *wt*. At 6 dpf no apparent increase can be observed, in number nor proportion.



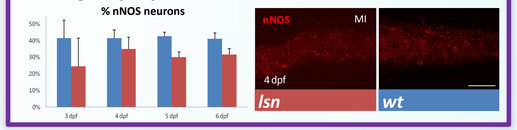
Serotonin (5HT)

Serotonin IR in neurons is absent in the first part of the intestine at 3 and 4 dpf. There is a marked decrease in both number and proportion of 5HT(+) neurons compared to *wt*.



neuronal Nitric Oxide Synthase (nNOS)

nNOS IR is decreased both in number and proportion. At 3 dpf only a very small amount of nNOS IR neurons can be found, mainly in the PI. Reductions are the most pronounced in the DI. Though there is a decrease, nNOS(+) neurons remain the largest proportion in the ENS.

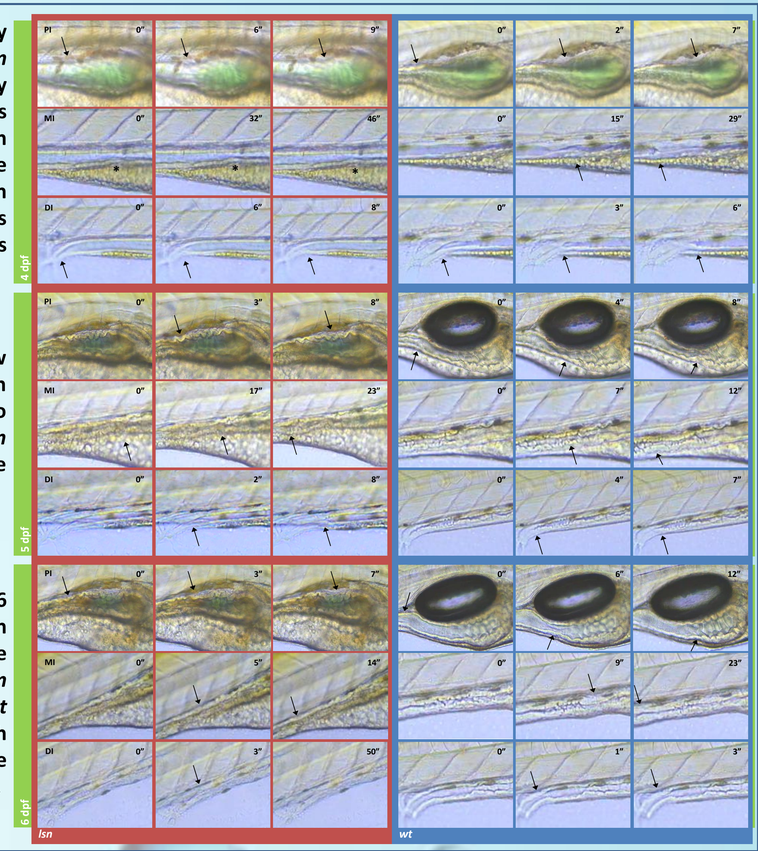


Motility

At 4 dpf, defined motility patterns are found in *lsn* and *wt*. *lsn* show relatively less retrograde contractions in PI and DI. While *wt* fish display already some anterograde contraction waves in MI, *lsn* only has spontanious contractions (*) in MI.

At 5 dpf, *lsn* show anterograde GI contraction in MI, but less compared to *wt*. Also, PI and DI of *lsn* display less retrograde contractions.

Compared to 5 dpf, *wt* at 6 dpf show no change in contraction frequency. The contraction frequency in *lsn* for PI, MI and DI match *wt* frequency, though contractions seem to be perturbed and less distinct.



Conclusion

Present study reveals abnormalities in the number and relative frequency of neurons expressing various neurochemical markers at each embryonic stage. These results are similar as data obtained in the intestine proximal to the aganglionic segment and the aganglionic segment of the *lethal spotting* mutant mice, an experimental HD model. Furthermore, the development of GI motility is retarted comparing wild type to *lsn* and the contractility is also perturbed mostly in MI and DI in *lsn.*

→ This study lends further support to previous studies that *lsn* is a suitable model for HD research.