

Developmental regional distribution and neurochemical coding of enteric neurons in the zebrafish mutant *lessen* compared to the wildtype zebrafish

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INTRODUCTION

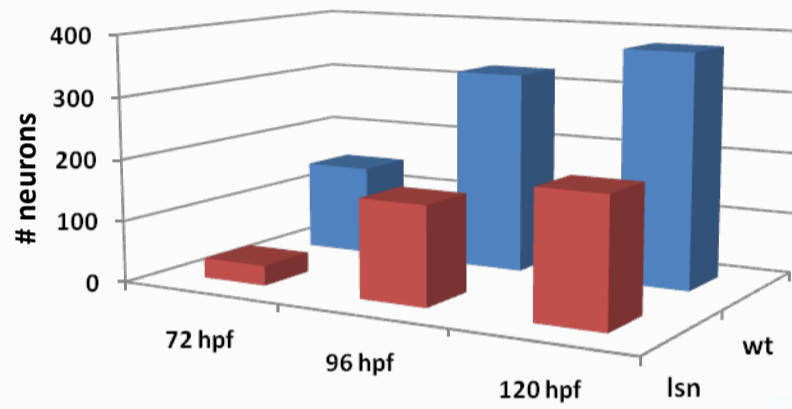
Hirschsprung's disease (HD) is a congenital disorder of the enteric nervous system characterized by aganglionosis along a variable portion of the colon. A recently developed mutant zebrafish, *lessen (lsn)*, expressing HD characteristics, is suggested to be an experimental model to unravel underlying developmental mechanisms for HD.

This study aims to compare the neurochemical coding of enteric neurons of the wildtype zebrafish with the mutant *lsn* to further validate this mutant as a suitable model for HD. Multiple immunofluorescence staining was used to detect previously characterized neurochemical markers at 72, 96 and 120 hours post-fertilization (dpf) in the proximal (PI), mid (MI) and distal intestine (DI) of both wild type and mutant animals.

RESULTS

Neurons

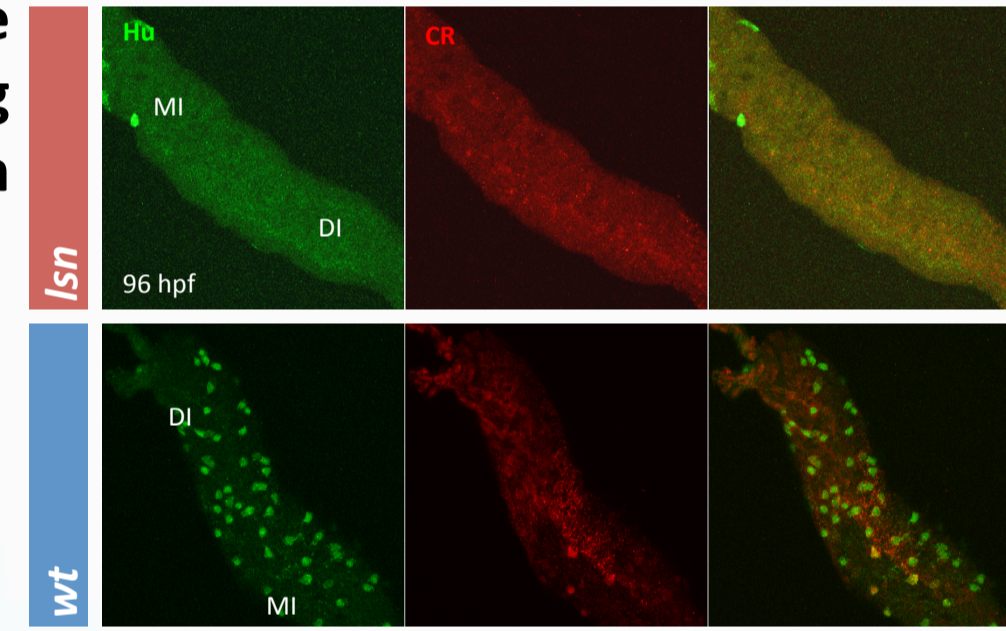
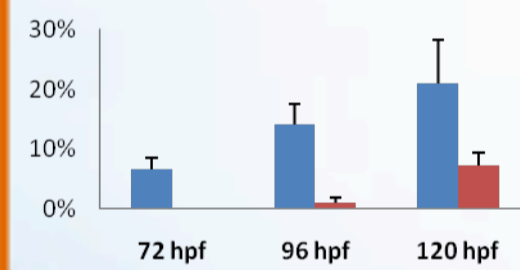
The total amount of neurons for wild type and mutant increases in time, but total numbers in mutants are decreased compared to wild type. The number of neurons was significantly reduced in DI (even absent at 72 hpf) and MI, but less in PI at each embryonic stage.



Calcium binding proteins: calbindin and calretinin

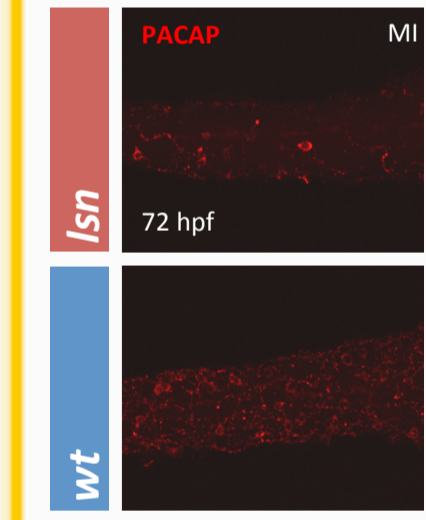
In wild type first IR is present at 72 hpf in the MI. In mutants, neurons start to show IR for both CB and CR around 96 hpf in the PI and MI. At 120 hpf, the amount of IR for the CBP was 80% less compared to wild type, being most pronounced in the DI, followed by the MI, and showing almost no difference in the PI.

% CBP-neurons



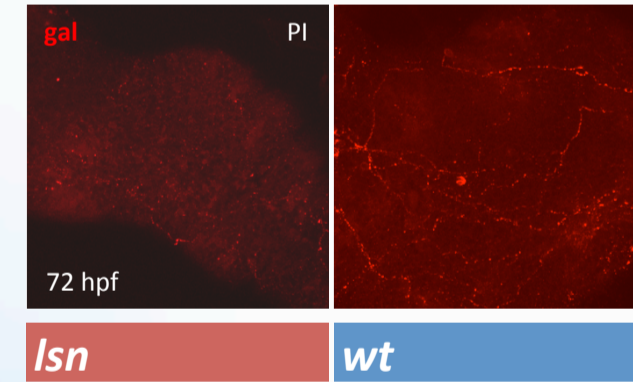
PACAP

Mutant fish only show slight IR in the PI from ENS at 72 hpf, compared to the whole intestine in wild type. 96 hpf, PACAP IR is found in the MI as well and at 120 hpf also the DI shows some IR in the ENS. Mucosal cells show PACAP IR over the whole intestine from 72 hpf.



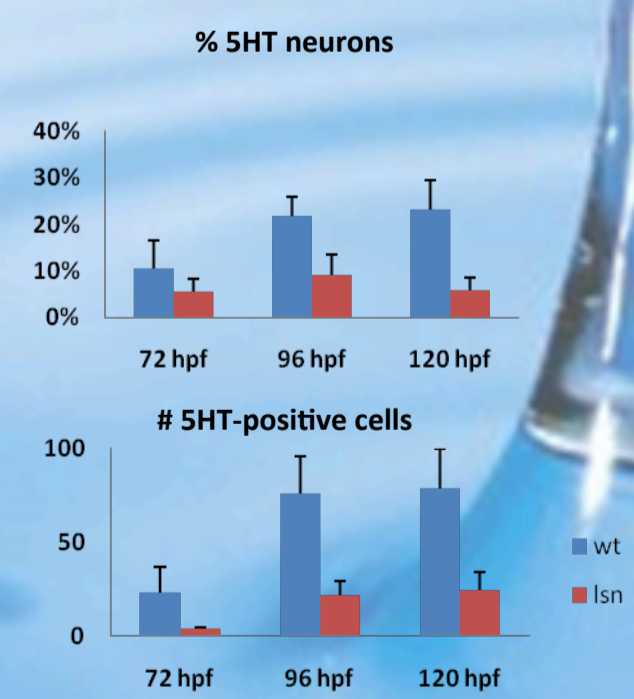
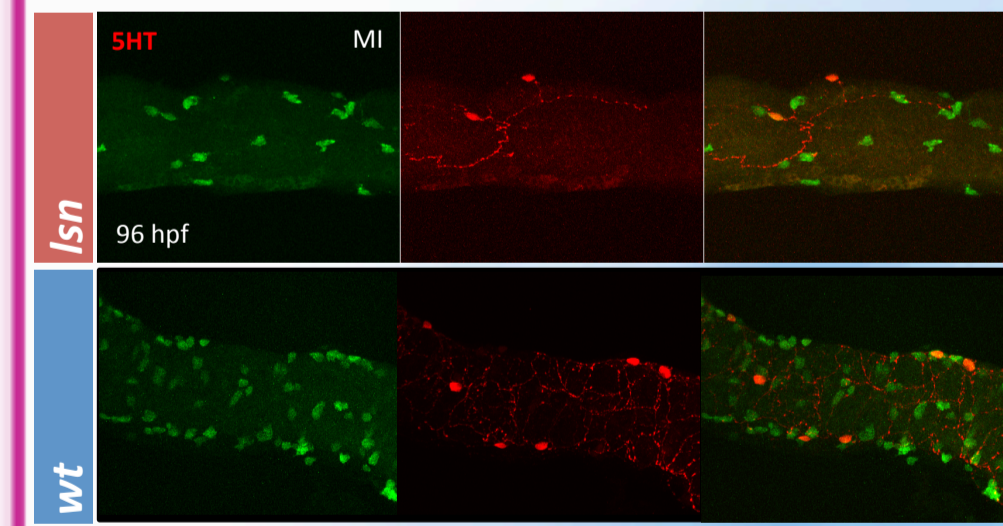
Galanin

Galanin IR is restricted to the PI and first part of the MI in *lsn* at 3 hpf compared to the whole intestine in wild type. IR in the DI starts from 120 hpf.



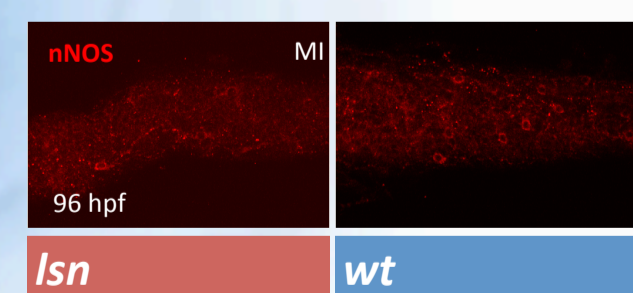
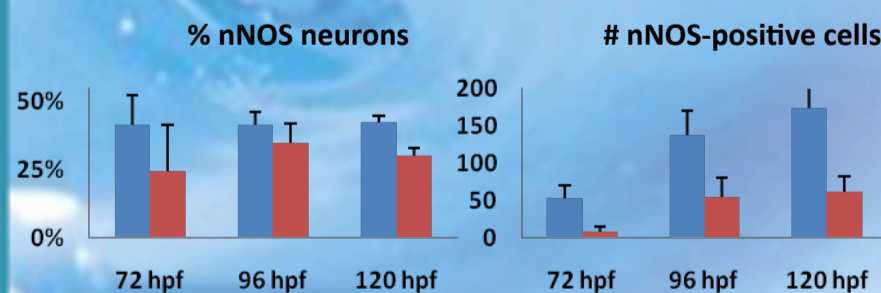
Serotonin (5HT)

5HT IR in neurons is absent in the first part of the intestine at 72 and 96 hpf. There is a marked decrease in both number and proportion of 5HT IR neurons compared to wild type.



Neuronal nitric oxide synthase (nNOS)

nNOS IR is decreased both in number and proportion. At 72 hpf 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 IR neurons remain the largest proportion in the ENS.



CONCLUSION

This study shows 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 *lsn* mutant mice, an experimental HD model, indicating that the *lessen* mutant is a suitable model to study HD.

List of abbreviations:

5HT: serotonin; CB: calbindin; CBP: calcium binding proteins; CR: calretinin; DI: distal intestine; ENS: enteric nervous system; HD: Hirschsprung's disease; hpf: hours post fertilization; IR: immunoreactivity; *lsn*: lessen; MI: mid intestine; nNOS: neuronal nitric oxide synthase; PACAP: pituitary adenylate cyclase activating peptide; PI: proximal intestine

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