Expression of GABAergic neurotransmission related genes in epileptic zebrafish models

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INTRODUCTION

The major clinical feature of many inborn errors of metabolism (IEMs) is epileptic encephalopathy. Seizures are caused by the accumulation of toxic intermediates or substrates of a defective enzyme. Pyridoxine-dependent epilepsy (PDE) is a metabolic epilepsy in which seizures are only attenuated by treatment using pyridoxine (vitamin B6). Using CRISPR/Cas9, we generated two zebrafish models with loss-of-function for the defective genes in PDE: *aldh7a1* and *plpbp*. These fish displayed epilepsy and deficiency in pyridoxal-5' phosphate (PLP) (active form of vitamin B6). To better understand the underlying molecular mechanisms of seizure occurrence in PDE, GABAergic neurotransmission was analyzed within *plpbp* homozygous mutant zebrafish.

METHODOLOGY

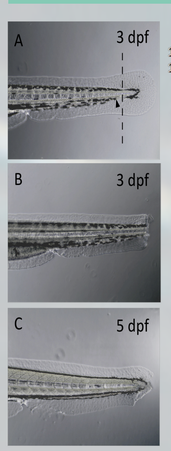


Figure 1. Larval fin clip. (A) Non-severed larval fin at three days post-fertilization (dpf). The line represents the site of transection, located within the pigment gap. The black arrowhead demonstrates the limit of the caudal blood circulation. (B) Larva following fin clipping procedure at three dpf. (C) Larval zebrafish fin regrowth at five dpf. Fin regeneration from a blastemal formation is observed only two days post-fin transection.

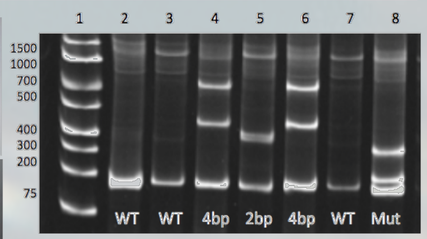


Figure 3. Genotyping of *plpbp* compound heterozygous (2bp deletion /4bp deletion) from laval fin biopsies. (B) PCR amplification of *plpbp* larval fin clippings. A 1 kb ladder (Thermo Scientific, SM1343) is shown in lane 1. Lanes 2-10 each represent a single fin biopsy. PCR results identify mutant genotypes (compound heterozygous, lanes 2, 4, and 8), heterozygous genotypes (lanes 3, 6, 7, 9 and 10) and WT genotypes (lane 5).

Figure 2. Genotyping of *aldh7a1* heterozygous cross from laval fin biopsies. PCR amplification of *aldh7a1* larval fin clippings. A 1 kb ladder (Thermo Scientific, SM1343) is shown in lane 7. Lanes 1-6 each represent a single fin biopsy. PCR results identify mutant genotypes (lane 1), heterozygous genotypes (lanes 3, 4 and 5) and WT genotypes (lanes 2 and 6).

1. Genotyping procedure of zebrafish larvae, 3 dpf. Fin biopsy and PCR
2. RNA extraction from larvae tissue
3. qPCR studies targeting a panel of GABA-related genes

RESULTS

GABAergic gene expression in *plpbp* mutants

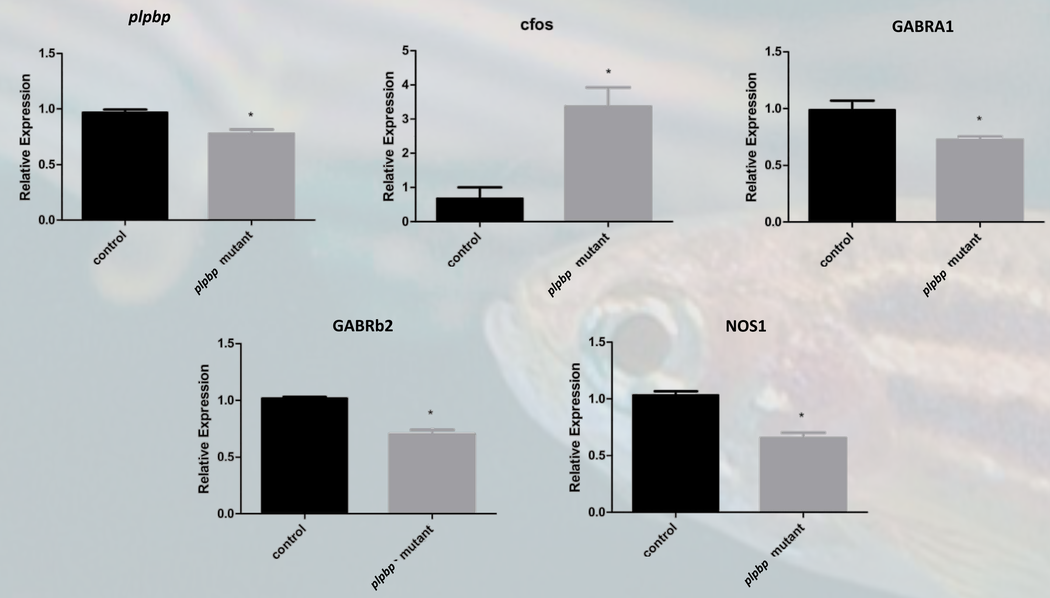


Figure 4. GABAergic gene expression in *plpbp* mutants compared to expression in control WTs. Decreased expression of *plpbp,* GABRA1, GABRb2 and NOS1 is observed in mutants compared to WT. Increased *c-Fos* expression is also observed.

* Significant results with a p-value of 0.05

GABAergic gene expression in *aldh7a1* mutants

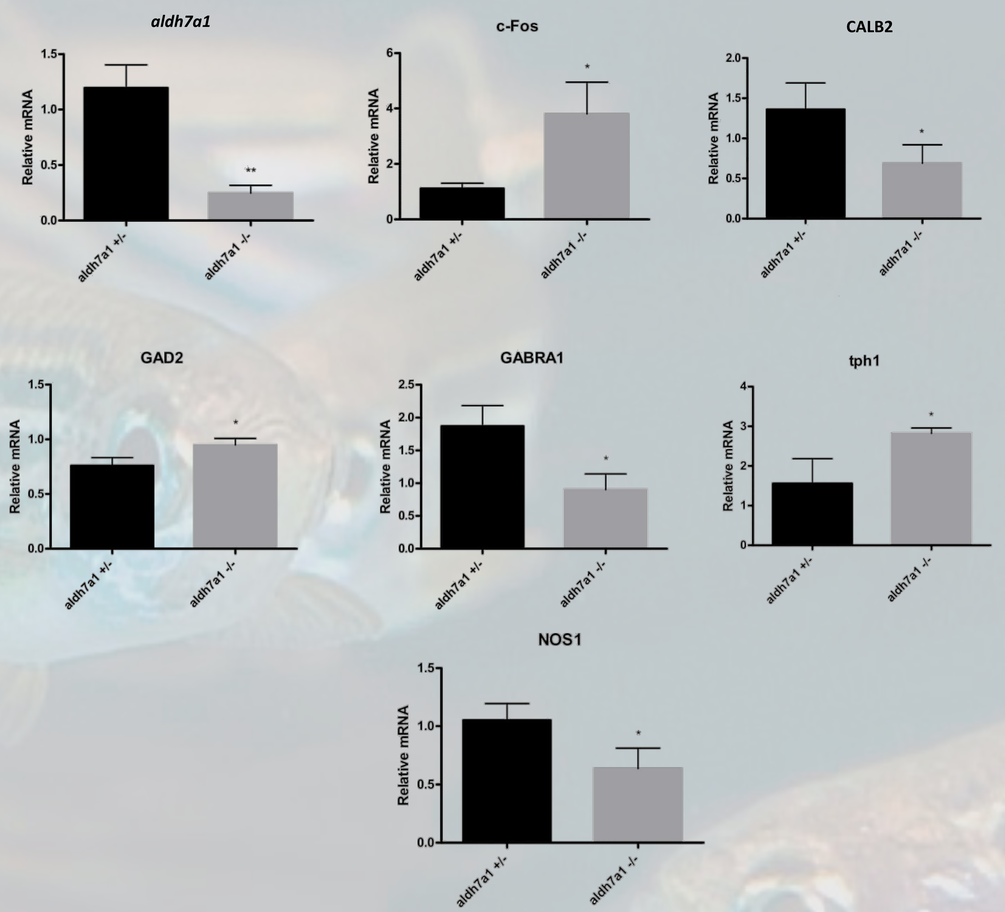
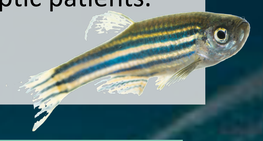


Figure 4. GABAergic gene expression in *aldh7a1* mutants compared to expression in control WTs. Decreased expression of *aldh7a1,* NOS1, CALB2, GABRA1 and increased expression of *c-Fos,* GAD2 and *tph1* is observed in mutants compared to WT.

* Significant results with a p-value of 0.05

CONCLUSION

- Deficiency in vitamin B6 (low levels of PLP, pyridoxal 5'-phosphate) was found in both *aldh7a1* and *plpbp* zebrafish[2]. PLP is cofactor of several enzymes, including the ones involved in neurotransmitter synthesis (GABA, Dopamine and Serotonin);
- **c-fos**: Increased expression indicates increased neuronal activity[4] –seizure biomarker;
- **Nitric oxide (NO_ synthase 1 (NOS1):** catalyzes production of nitric oxide, which can act as a neurotransmitter. Disappearance of NO peak may induce epileptic seizure[5].
- **GABRA1**: encodes α-subunit of GABA-receptor. Decreased expression may reduce effect of inhibiting neuronal excitability.
- **Tryptophan hydroxylase (*tph1b*):** enzyme involved in the synthesis of serotonin. Increased serotonin has been demonstrated as effective in seizure suppression[6]. Increased expression of *tph1b* may suggest a mechanism aiming to increase serotonin levels.
- **Glutamate decarboxylase 2 (GAD2) :** PLP (pyridoxal-5'-phosphate)-dependent enzyme that catalyzes the α-decarboxylation of L-glutamate into GABA. Increased expression of GAD2 may suggest a mechanism fighting to increase GABA production related genes.
- **Calretinin (CALB2):** calcium-binding protein involved in calcium signaling. Decreased expression is observed in epileptic patients.



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