Early axon guidance and synapse maturation defects in a zebrafish model of Mucopolysaccharidosis type II

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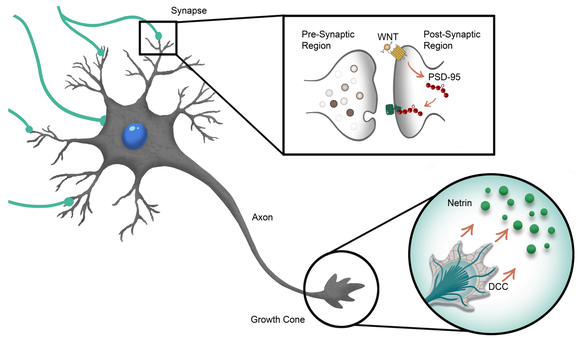
ABSTRACT

Mucopolysaccharidosis type II (MPS II) is a rare X-linked lysosomal storage disorder caused by a deficit of the lysosomal enzyme iduronate-2-sulfatase (**IDS**), a hydrolase involved in the degradation of dermatan and heparan sulfate (HS) glycosaminoglycans. Disease manifestations include progressively severe central nervous system defects resulting in **epilepsy, hyperactivity, loss of attention and aggressive behaviors**.
It is well known that HS plays a critical role in neurodevelopment by shaping the neural extracellular matrix and modulating neuroligand-receptor interactions. The goal of our investigation is to deeply characterize the mechanistic basis of MPS II-related brain defects using an established **zebrafish Ids knock out (KO) model**.
Towards this aim, we preliminarily performed a complementary approach (based on Western Blot, immunofluorescence and other techniques) to investigate **axon guidance** and **synapse maturation defects** in KO and age-matched control larvae at early developmental stages.
In the former case, we found in KO fish a consistent dysregulation of **Netrin1** and Deleted in colorectal cancer (**DCC**), two well-known molecules involved in axonal chemoattraction. Concerning the synaptic maturation, we detected an overactivation of the canonical **Wnt pathway** and increased expression of the postsynaptic density protein 95 (**PSD-95**) in Ids loss of function fish. All abnormalities have been documented before any overt HS accumulation, suggesting that a hierarchy of multiple aberrant cellular and signaling cascades, rather than the simple lysosomal engorgement, underlies the altered MPS II neurophysiology.

BACKGROUND

For a long time, the pathogenesis of MPSs has been attributed to the progressive accumulation of undegraded GAGs and inflammation; however, it is now clear that more complex lysosomal-related mechanisms may contribute to the pathological manifestations, even before evident GAGs engulfment. These include impaired autophagy, incorrect vesicular trafficking and altered signaling. During embryonic development, **heparan sulfate proteoglycans (HSPGs) display crucial roles in neurogenesis**, **axon guidance and synaptogenesis** by acting as regulators of tissue architectures and signaling-dependent cell fate decisions. Specifically, morphogens and chemotactic cues binding to HSPGs restricts their diffusion along the surface of receiving cells, modulating the interaction between ligands and transmembrane receptors. This is, for example, the case of **Netrin/DCC signaling**, one of the most important chemoattractant axis involved in axonal development.
Once reached their target tissue, axons must form appropriate connections. Here HSPGs, together with **Wnt signaling**, are key players in synapses maturation and plasticity. At this level, several Wnt ligands can directly influence **PSD-95** expression and clustering, thus regulating synaptic function.
Interestingly, previous RNA-seq analysis performed on brains of MPS II mice revealed gene expression alterations of axon guidance, synapses and Wnt singling-related mediators.



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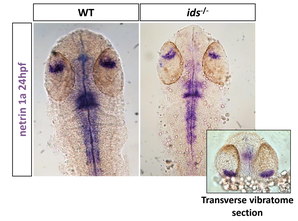
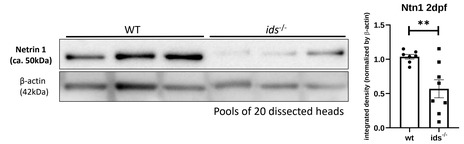
Netrin1 dysregulation in *ids*$^{-/-}$ larvae

Netrin1, one of the most known extracellular cue for axon guidance, is dysregulated in ids mutant zebrafish larvae at early embryonic stages.
In situ hybridization reveals lower Netrin1 signal in ko larvae at 24 hours post fertilization (hpf). The signal concentrates at the midline, with higher intensity at the midbrain-hindbrain boundary (MHB). Positive staining is also evident in the ventral region of the eye, as highlighted by the transverse vibratome section.

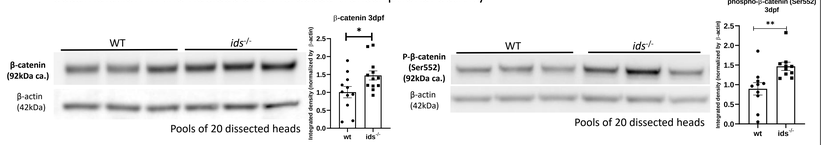


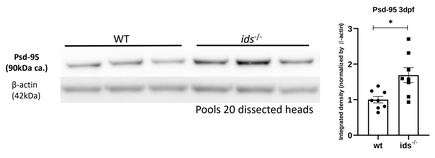
The same type of downregulation is also traceable through western blot analysis on *ids* mutant and control dissected heads at 2 days post fertilization (dpf).



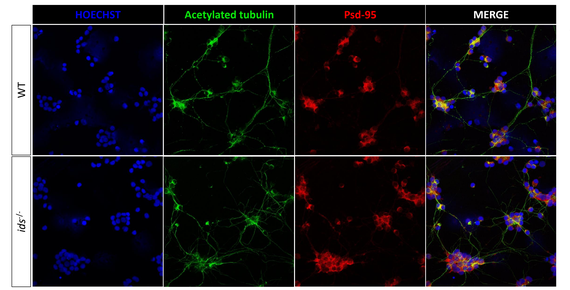
WNT pathway mediators and PSD-95 dysregulation in *ids*$^{-/-}$ larvae

Western blot revealed increased levels of the canonical Wnt pathway transducers β-catenin and phospho-β-catenin (Ser552) at 3dpf in ids mutant dissected heads. β-catenin phosphorylation at serine 552 induces its accumulation in the nucleus and increased transcriptional activity.

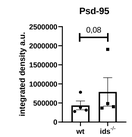




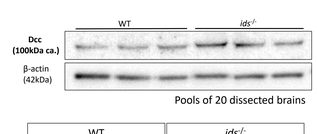
Increased protein levels of Psd-95, a fundamental scaffold protein of the postsynaptic compartment and known Wnt pathway target, are detected in head lysates from 3dpf *ids*$^{-/-}$ zebrafish larvae when compared to age-matched controls.

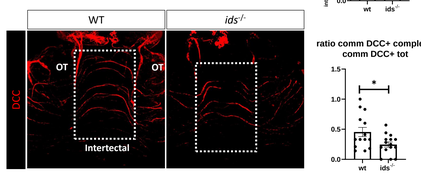


Immunofluorescence on primary neuronal cell culture at 2 DIV shows increased Psd-95-associated fluorescent signal in *ids*$^{-/-}$ derived cells compared to controls.



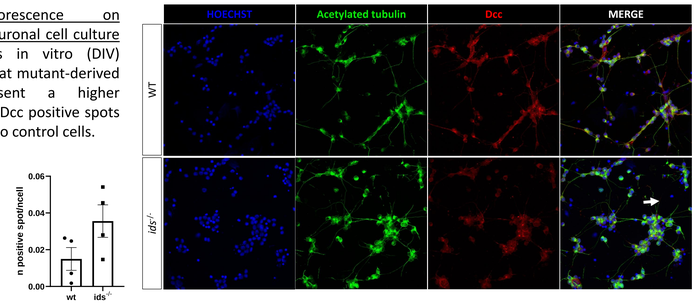
Dcc dysregulation in *ids*$^{-/-}$ larvae





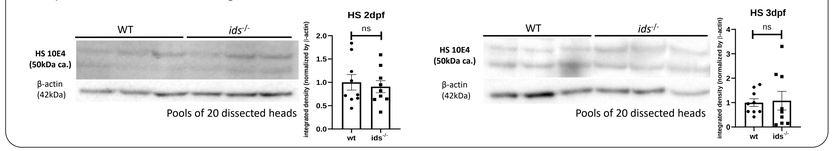
Dcc, one of the main Netrin1 receptors involved in axon chemoattraction, is dysregulated in mutant ids larvae at early developmental stages.
In particular, western blot analysis on dissected heads and brains reveals Dcc upregulation in 2dpf KO larvae when compared with age matched controls.
Dcc whole mount immunofluorescence on isolated brains at 6dpf suggests a different Dcc patterning on the intertectal commissures at the level of the optic tectum (OT).

Immunofluorescence on primary neuronal cell culture at 2 days in vitro (DIV) suggests that mutant-derived cells present a higher number of Dcc positive spots compared to control cells.



Total HS levels are not affected in *ids*$^{-/-}$ larvae

Western blot analysis reveals that at both 2 and 3dpf, total heparan sulphate levels of *ids* mutant larvae are comparable with the ones of age-matched controls



CONCLUSIONS

Zebrafish model for MPSII, bearing a loss of Ids function, show defects in axon guidance related molecules (Netrin-Dcc axis) and Wnt pathway modulators during embryonic stages. This suggests that, together with impairment of correct guidance mechanisms, there could be problems in synapse formation and plasticity. Interestingly, these alterations are manifested before HS evident accumulation.