9-1-2022

# Screening for evolutionary conserved genes specific to myelin sheath targeting

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We designed and conducted a screen of gene candidates that we thought may influence axon specific developmental myelination. Our candidates were determined by eliminating all non-coding and housekeeping genes in the zebrafish pre-oligodendrocyte transcriptome (provided by Bruce Appel, University of Colorado). We ran a ven analysis, cross referencing this against the mouse transcriptome (provided by Steven Sloan, Emory University). The conservation of these genes indicated evolutionary importance to survival and cell function. With the remaining candidates, we enriched for those involved in cell adhesion and post synapse by using Gene ontology's Amigo Tool 2, producing 90 candidates. Using literature, we were able to narrow this selection down to 17 high interest candidates.

We screened our candidates using a transgenic zebrafish line that used GFP to label two axon subtypes that were anatomically different. This line also labeled oligodendrocytes and myelin with RFP. We injected the embryos at the single cell stage using standard CRISPR techniques. The spinal cords of the injected embryos and their corresponding controls were imaged using a spinning disk florescent confocal microscope. Candidates screened were anks1b, strn, iqsec1, cyfip2, dtnbp1a, dtnbp1b, and ncam1a.

Our screen indicated that anks1b may be responsible for axon guidance and direction of myelination on the CoPA axon. In addition, experimental data found a possible sheath on the dendrite of the CoPA axon, indicating that anks1b may be responsible for restricting sheath formation to axons. We found that strn CRISPR injected zebrafish had abnormal CoPA trajectory and decreased myelination of the CoPA axon, indicating it may regulate the quantity of myelin produced in addition to axon guidance. In our screen for iqsec1, we observed bifurcating CoPA axons and growth cone misguidance. Manipulation of cyfip2 caused demyelination of the CoPA axon, indicating it may be responsible for regulating myelination of the dorsal region. The screen for dtnbp1a indicated that it may manipulate myelin distribution ventrally and dorsally and direct normal axon guidance in the ventral region. The screen indicated that ncam1a may cause excess dorsal and ventral myelin and an overall increase in oligodendrocyte cell bodies.

Overall, our candidate screens resulted in promising results, prompting further investigation. Using the same experimental design and lab techniques, we will further characterize the role of our candidates in developmental myelination.