Zebrafish modeling for the clinic: Rapid *in vivo* functional testing of patient variants for clinical applications.

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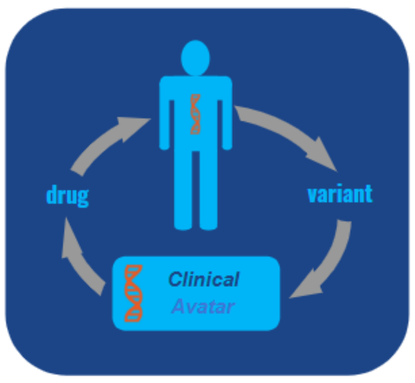
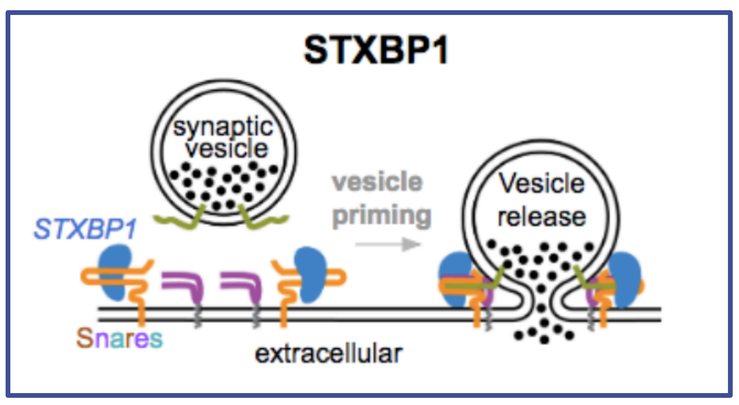
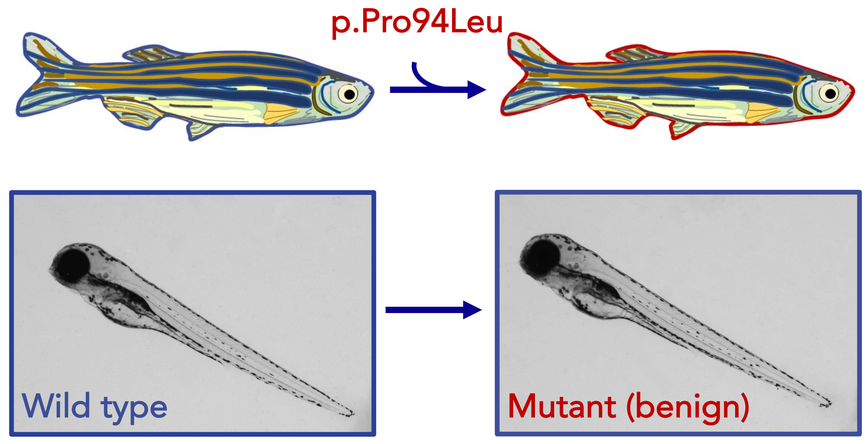
Synopsis: We use the zebrafish (*Danio rerio*) as an *in vivo* model to measure the functional effects of patient-derived genetic variation. In this way, human genetic variants identified in the clinic are quantitatively and qualitatively connected to model animal phenotypes. As a proof of concept study, we used precision gene editing and transient knockdown approaches targeting *Stxbp1a* – a zebrafish ortholog of human gene syntaxin-binding protein 1 (*STXBP1*) – to model variant genetic contributions to pediatric epilepsy and encephalopathy.





STXBP1 (also known as MUNC18-1) is a protein involved in synaptic vesicle trafficking, and mutations in this gene are implicated in childhood epilepsies and several neurodevelopmental disorders[1,2].

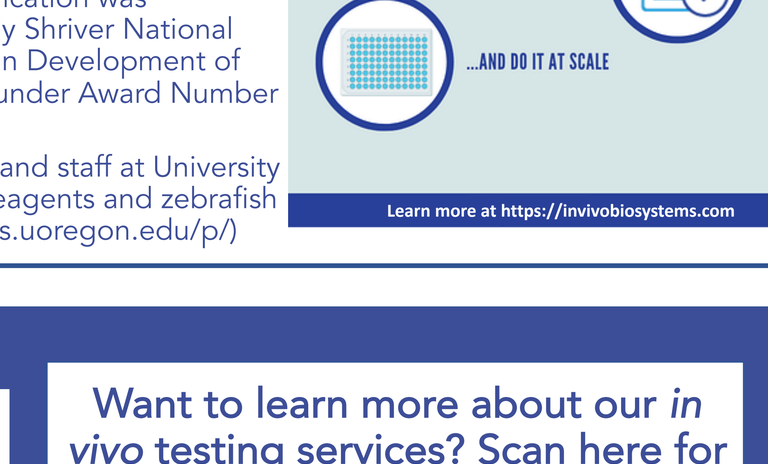
Assessing Stxbp1a patient variants with precision gene editing



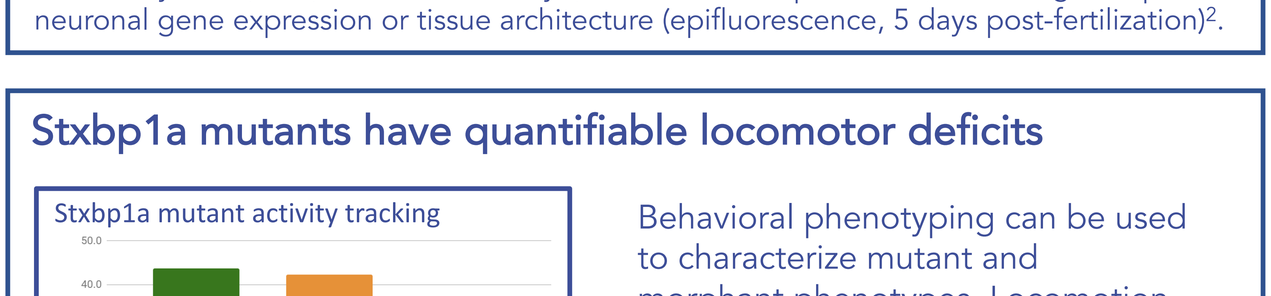
Stxbp1a is a highly conserved zebrafish ortholog of human STXBP1 (87% identity). Using CRISPR/Cas9 technology, we were able to precisely generate a benign patient mutation at the conserved amino acid residue (CCC>CTG, p.P94L).

Pathogenicity annotations track with Stxbp1a crispant survival rates

Following CRISPR injections to generate patient variants at conserved residues, annotated pathogenic mutations show decreased survival to adulthood compared to benign mutations.

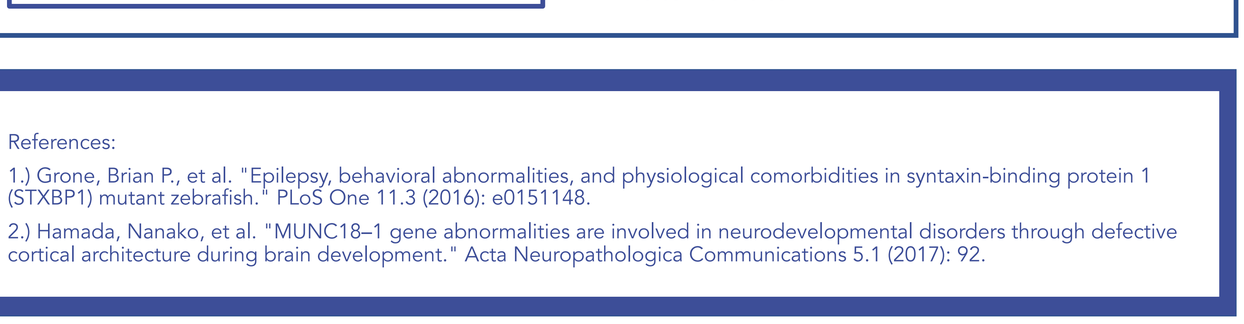


Transient Stxbp1a knockdown recapitulates mutant phenotypes



Translation blocking morpholinos (AUG-MO) reproduce published Stxbp1a null mutant phenotypes, including hyperpigmentation and failure to hatch (brightfield, 5 days post fertilization)[1].

Neuronal gene expression is altered in Stxbp1a mutants & morphants



Wholemount immunofluorescence imaging reveals that expression of the neuronal marker Pax2 is decreased in both Stxbp1a mutants (center) and morphants (right) in central nervous system (CNS) tissues like the eye, hindbrain and spinal cord, indicating disrupted neuronal gene expression or tissue architecture (epifluorescence, 5 days post-fertilization)[2].

Stxbp1a mutants have quantifiable locomotor deficits

Behavioral phenotyping can be used to characterize mutant and morphant phenotypes. Locomotion tracking data acquired with the NemaMetrix wMicroTracker shows that Stxbp1a mutants do not swim, consistent with published Stxbp1a mutant data[1].

Conclusions: The result of these functional studies is a tailored toolkit in zebrafish for connecting patient-specific phenotypes with conserved morphological, molecular, and behavioral phenotypes. This platform can be scaled for high throughput pipelines and custom phenotyping assays to suit your research needs.

Next steps: Experiments are now in progress to couple **crispant**, **morphant** and **mutant** models of disease with **transient rescue experiments** and **compound testing** targeted at assessing variant function as well as drug toxicity and efficacy in a rapid experimental timeline.

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2.) Thanks to Dr. Timothy Mason and staff at University of Oregon AqACS Facilities for reagents and zebrafish husbandry services. (https://aqacs.uoregon.edu/p/)

References:

1.) Grone, Brian P., et al. "Epilepsy, behavioral abnormalities, and physiological comorbidities in syntaxin-binding protein 1 (STXBP1) mutant zebrafish." PLoS One 11.3 (2016): e0151148.

2.) Hamada, Nanako, et al. "MUNC18–1 gene abnormalities are involved in neurodevelopmental disorders through defective cortical architecture during brain development." Acta Neuropathologica Communications 5.1 (2017): 92.