CRISPR/Cas9-mediated knock-in of patient mutations in the epilepsy-associated gene STXBP1

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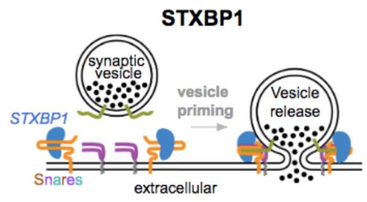
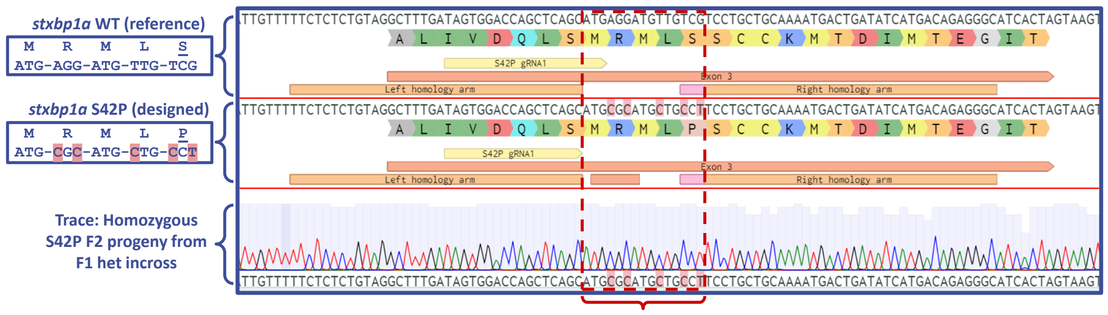
Overview: We use the nematode (*C. elegans*) and zebrafish (*Danio rerio*) as *in vivo* models to investigate the functional effects of patient-derived genetic variation. To date, many knockout models of human genetic disorders have been generated in zebrafish, but often times this fails to reflect the molecular nature of patient mutations seen in the clinic. In this example, we used precision genome editing targeting *stxbp1a* – a zebrafish ortholog of human the gene syntaxin-binding protein 1 (STXBP1) – to generate two precise knock-in models of patient variants to assess differences in phenotypes with respect to established loss of function mutants.



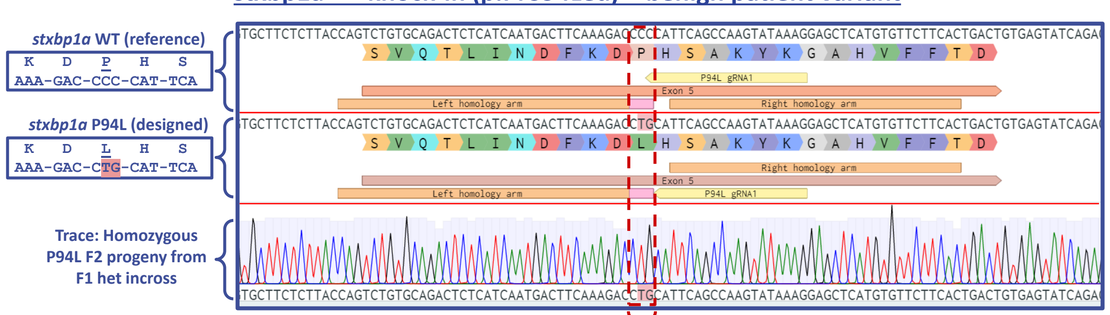
STXBP1 (also known as MUNC18-1) is a protein involved in synaptic vesicle trafficking, and mutations in this gene are implicated in childhood epilepsies and several neurodevelopmental disorders[1,2].

stxbp1a^{S42P} knock-in (p.Ser42Pro) – putative pathogenic patient variant



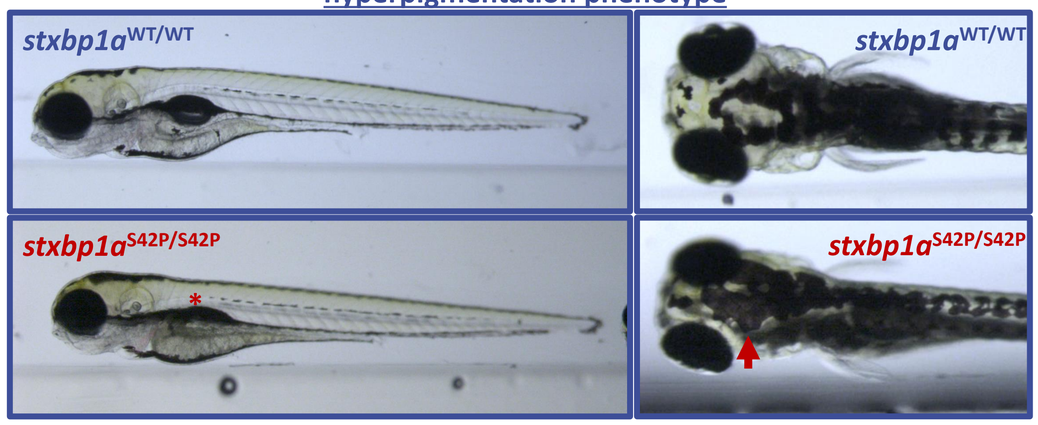
Description:
Sequence alignment (Benchling screen capture) of wild-type reference sequence (top), edited mutant sequence (middle), and single *stxbp1a*^{S42P} F2 embryo homozygous sequence trace (bottom). Patient variant details: NM_001032221.6(STXBP1):c.124T>C (p.Ser42Pro); ClinVar variant interpretation: Criteria provided, conflicting interpretations of pathogenicity (Pathogenic, Uncertain significance); accession VCV000280467.5.

stxbp1a^{P94L} knock-in (p.Pro94Leu) – benign patient variant



Description:
Sequence alignment (Benchling screen capture) of wild-type reference sequence (top), edited mutant sequence (middle), and single *stxbp1a*^{P94L} F2 embryo homozygous sequence trace (bottom). Patient variant details: NM_001032221.6(STXBP1):c.281C>T (p.Pro94Leu); ClinVar variant interpretation: Criteria provided, no conflicting interpretations of pathogenicity (Benign, Likely benign); accession VCV000207405.9

stxbp1a S42P homozygous mutants partially phenocopy the *stxbp1a* loss of function hyperpigmentation phenotype

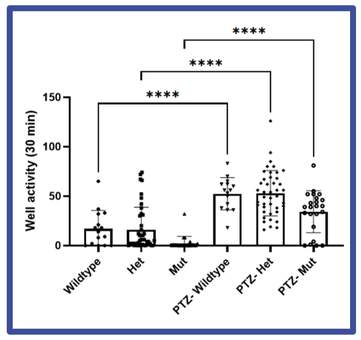


Description:
Homozygous *stxbp1a* S42P mutants partially reproduce published *stxbp1a* loss of function hyperpigmentation phenotype, with diffuse and overlapping melanophores in the head region compared to wild-type siblings (red arrow)[1]. Nursery survival rates suggest that the homozygous genotype is lethal by 10 dpf, supported by the lack of apparent swim bladder inflation by 5 dpf in all mutant larvae (red asterisk). Images taken in brightfield at 5 dpf.

stxbp1a S42P homozygous mutants partially phenocopy the *stxbp1a* loss of function reduced swimming activity phenotype

Description:
Homozygous *stxbp1a* S42P mutants partially reproduce published *stxbp1a* loss of function movement phenotype, as suggested by single larval recordings in a 96-well plate locomotion assay where homozygous S42P mutant larvae trend towards reduced activity in baseline and PTZ-treated conditions (right panel; PTZ = pentylenetetrazole).

Compared to loss of function mutants, which fail to hatch and generally do not move, the movement phenotype presented here would suggest a less severe loss of function in *stxbp1a*.[1] The P94L line presents with no detectable movement defects (not shown).



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.

Acknowledgements:

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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/)