InVivo Biosystems Platform Case Study:

STXBP1 Humanization

Early-infantile epileptic encephalopathy (EIEE, also known as Ohtahara Syndrome) is a rare autosomal-dominant epileptic condition, noted to afflict at least 200 individuals worldwide; the exact population prevalence of this form of epilepsy is yet unknown.[2] The causative gene of EIEE is now understood to be *STXBP1*, which encodes syntaxin-binding protein 1 (STXBP1).[7] The STXBP1 protein functions in chaperoning syntaxin to the synapse, priming synaptic vesicles for coordinated neurotransmitter release, and orchestrating the assembly of protein complexes responsible for membrane fusion.[6] Mutations in the *STXBP1* gene result in a more thermolabile protein product and impact protein binding to syntaxin, altering neuronal activity and culminating in seizures.[2,7]

To date, at least 85 mutations in STXBP1 have been directly linked to EIEE forms of epilepsy; however, nearly 100 mutant variants are currently categorized as having "conflicting [significance] interpretations" or ultimately being "[variants of] uncertain significance" (VUS).[1,2] As most cases of EIEE are linked to de novo mutations in *STXBP1*, it becomes imperative to accurately understand the true pathogenicity status of differing variants.[2]

Using Clinical Avatar tools, InVivo Biosystems examined three independent variants in human *STXBP1*, towards the two concurrent goals of (1) better understanding each variant's status as pathogenic or benign, and (2) outlining a phenotype-based model of the variant landscape. Three different humanized *C. elegans* strains were generated, each containing one of the human *STXBP1* gene variants under analysis – two known pathogenic variants (R406H and R122X) and one VUS (Y75C). Mis-sense substitution R406H reflects a well-established pathogenic allele of STXBP1, and variant R122X is a non-sense mutation leading to protein truncation.[3,5,8,9] Currently, VUS Y75C is predicted to be deleterious based upon the combination of its location in a critical and evolutionarily conserved region of the protein and *in silico* analyses.[4] A fourth humanized strain was also created, containing the normal-functioning wild type *hSTXBP1* gene, to serve as an experimental control; these animals are referred to as the "gene-swap" strain.

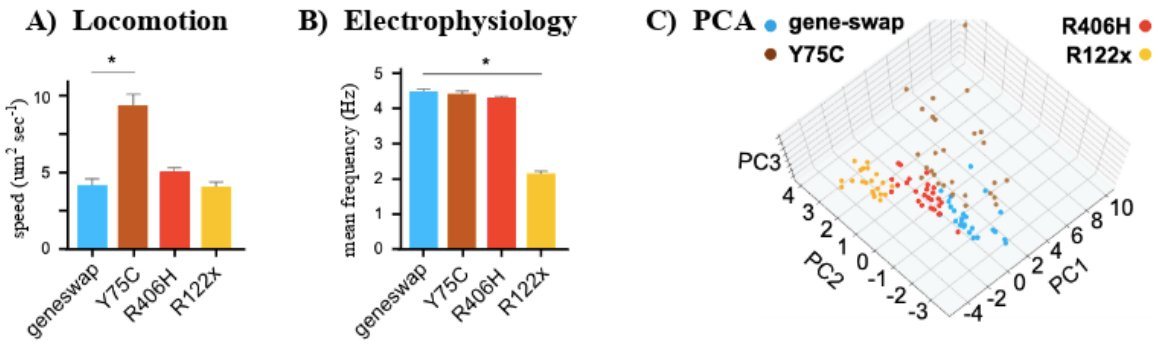

Figure 1. InVivo Biosystems analysis of STXBP1 humanized strains. *Locomotion analysis (A) and electrophysiology response (B) results indicate differing functional consequence of known pathogenic variants (R406H, R122X) and VUS (Y75C); (*p < 0.05). Principle component analysis (C) of 21 locomotion parameters results in distinct functional clustering groups for all four humanized strains.*

Two parameters were considered in the initial analysis – locomotion and pharyngeal pumping frequency. *C. elegans* locomotion was measured as animal velocity across an agarose surface, derived from automated analysis of video data recording worm movement. Pharyngeal pumping frequency was

examined by monitoring the electrophysiological output of the worm pharynx via NemaMetrix ScreenChip technology, which measures the current cycles generated by the worm pharyngeal muscle upon contraction and relaxation.

Locomotion analysis of humanized control animals and *hSTXBP1* variant strains served to underscore prior interpretations of reported allele pathogenicity, and revealed a previously unknown phenotype of VUS Y75C. Humanized animals expressing STXBP1^{R406H} exhibited minor deviation from "gene-swap" humanized controls (Fig. 1A). As variant R406H is reported and understood to be pathogenic, these animal model results tracked well with an expected outcome of STXBP1^{R406H} deviant behavior. Conversely, while STXBP1^{R122X} humanized animal locomotion was observed to mimic controls, humanized VUS strain STXBP1^{Y75C} exhibited a statistically significant hyperactivity (Fig. 1A, $p < 0.05$). This observation opens up the distinct possibility of (1) slightly differing molecular mechanisms for variant mis-function, and (2) differing categories of phenotypic outcomes depending on protein variant specifics.

Electrophysiological analysis of humanized control animals and *hSTXBP1* variant strains highlighted an additional phenotypic outcome that may serve to differentiate possible classes of *STXBP1* variants. Mean pharyngeal pumping frequency of VUS strain STXBP1^{Y75C} and known pathogenic variant strain STXBP1^{R406H} did not deviate from the gene-swap humanized control (Fig. 1B). Alternatively, truncated protein variant strain STXBP1^{R122X} exhibited a more than two-fold decrease in pharyngeal pumping frequency, again indicating the possibility of differing mechanism(s) of action among different *STXBP1* gene variants.

Finally, principal component analysis (PCA) results likewise emphasize the complexity of biological variants. A PCA performed on the three humanized variant strains and the humanized gene-swap control considered 21 different locomotion parameters; results of the multi-parametric analysis demonstrate that each strain occupies a unique clustering space (Fig. 1C). This indicates the necessity of considering multiple unique endpoints in order to adequately capture the spectrum of deficiencies present in pathogenic variants.

The deep phenotyping methods developed and implemented in the InVivo Biosystems Platform detect the subtle functional consequences of gene variants. Observable and quantifiable biology is partnered with machine-learning algorithms to compile high-dimensional phenotypic data into defined clusters of benign and pathogenic groups, all within clinically relevant timeframes.

References

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