Theme: Translational Neuroscience

IDENTIFYING GENETIC MODIFIERS OF *FMR1* SILENCING IN HUMAN FXS NEURAL CELLS

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Fragile X syndrome (FXS) is caused by loss of FMRP expression as a result of hypermethylation of the *FMR1* locus, induced by expansion of CGG repeats at the 5' UTR of the gene. Since core symptoms of FXS result from FMRP deficiency, restoration of FMRP expression would be expected to have the biggest therapeutic impact for FXS. As the silencing process is not fully understood, designing a candidate-based approach to modulate it is challenging. A powerful alternative is the application of unbiased, whole genome, forward genetics with the desired phenotypic outcome is the reactivation of the *FMR1* locus. In this study, we engineered reporter FXS hESC lines by CRISPR/Cas9 in which the expression of an antibiotic selection marker (Blasticidin-resistance gene, BSD^R) is driven from the endogenous mutant *FMR1* promoter (FXS-BSD^R hESC). Thus, reactivation and expression of *FMR1* is conferred by resistance to BSD in this reporter line. We differentiated these cells into neural progenitor cells and performed genome-wide CRISPR screen to identify putative genetic modulators of *FMR1* reactivation. This strategy has the potential to identify factors that can influence the epigenetic state of *FMR1* and which may be of value as drug targets.