ABSTRACT

Fragile X Syndrome (FXS) is a neurodevelopmental disorder caused by >200 repeat CGG expansion in the 5' UTR of the Fragile X mental retardation gene FMR1, leading to hypermethylation and silencing of the FMR1 transcript, thereby reducing FMRP protein. To date, there has been no quantitative assessment of the relationship between varying levels of FMR1/FMRP expression and effects on spontaneous neuronal activity.

We have developed patient-specific iPSC-derived neurons that retain the pathological repeat expansion, have decreased FMRP levels, and demonstrate increased spontaneous activity in vitro and in neuronal networks as measured by multielectrode arrays (MEA).

Using CRISPR/Cas9, we have also generated an FMR1 knockout line, as well as isogenic Fragile X cell lines with truncated numbers of CGG repeats that demonstrate increased spontaneous activity in the absence of FMRP and attenuated activity when FMRP is restored to near normal levels. We further show through FMR1 mRNA transient transfections and titration of isogenic control neurons into a Fragile X neuronal network, that low levels of FMRP (<10%) partially attenuate FMRP-induced hyperexcitability, with higher levels of FMRP (>10%) leading to full normalization of increased levels of spontaneous activity seen in Fragile X neuronal networks.

1. FRAGILE X SYNDROME (FXS)

2. FULCRUM’S APPROACH TO TARGET ID AND VALIDATION

3. HUMAN IPSC-DERIVED NEURONS

4. FXS NEURONS HAVE REDUCED FMR1/FMRP AND INCREASED ACTIVITY

5. GENERATION OF FMRP KO NEURONS

6. HOW MUCH FMRP IS NEEDED TO NORMALIZE ACTIVITY?

CONCLUSIONS

- We have developed patient-specific iPSC-derived neurons that retain the pathological repeat expansion, have decreased FMRP levels, and demonstrate increased spontaneous activity in vitro and neuronal networks as measured by multielectrode arrays.

- Using CRISPR/Cas9, we have also generated an FMR1 knockout line, as well as isogenic Fragile X cell lines with truncated numbers of CGG repeats that demonstrate increased spontaneous activity in the absence of FMRP and attenuated activity when FMRP is restored to near normal levels.

- We further show through FMR1 mRNA transient transfections and titration of isogenic control neurons into a Fragile X neuronal network, that low levels of FMRP (<10%) partially attenuate FMRP-induced hyperexcitability, with higher levels of FMRP (>10%) leading to full normalization of increased levels of spontaneous activity seen in Fragile X neuronal networks.