*Nature Reviews Neuroscience* | Published online 8 Mar 2018; doi:10.1038/nrn.2018.29


NEURODEVELOPMENTAL DISORDERS

# A targeted rescue

> the *FMR1* promoter in edited FXS iPSCs exhibited an increase in the presence of markers of active chromatin and a reduction of repressive marks

The cause of fragile X syndrome (FXS) has been shown to be the epigenetic silencing of the *FMR1* gene in individuals carrying disease-causing genetic mutations, suggesting that manipulations that can relieve this repression could reverse the neurological deficits associated with the disorder. In their new study, Jaenisch and colleagues show that a tool that allows targeted demethylation of specific nucleotide sequences can be used to reactivate *FMR1* and to reverse some phenotypic abnormalities in cells derived from individuals with FXS.

In FXS, the silencing of *FMR1* is associated with the presence of an expanded and hypermethylated CGG trinucleotide repeat-containing region in the 5′ untranslated region of the gene. To target this region for DNA methylation editing, the authors infected an induced pluripotent stem cell (iPSC) line derived from an individual with FXS with two viral vectors. One vector expressed a fusion protein composed of a catalytically inactive form of the nuclease Cas9 and an active form of the methylcytosine dioxygenase TET1, which mediates DNA demethylation; the other vector encoded an RNA that binds specifically to Cas9 and to the CGG expansion, to facilitate accurate targeting.

In 'edited' iPSCs, the methylation of the *FMR1* CGG expansion was drastically reduced, when compared with non-edited cells, and *FMR1* expression was restored to 90% of its level in healthy human embryonic stem cells. When postmitotic neurons generated from FXS iPSCs were infected with the vectors, the effects were similar (although less robust), suggesting that this approach can also reactivate *FMR1* expression in differentiated cells. Furthermore, when neural precursor cells derived from edited FXS iPSCs were injected into the brains of mice, the cells differentiated into neurons that maintained *FMR1* activation several months after injection, showing that *FMR1* reactivation can be sustained in vivo.

The hypermethylation of the *FMR1* CGG expansion is thought to promote the formation of heterochromatin, a highly compacted form of chromatin characterized by the presence of repressive epigenetic marks and inaccessibility to RNA polymerase, at the *FMR1* promoter. The authors found that the *FMR1* promoter in edited FXS iPSCs exhibited an increase in the presence of markers of active chromatin and a reduction of repressive marks, suggesting that demethylation of the CGG expansion drives the *FMR1* promoter to adopt an active conformation that enables gene expression.

To investigate the effects of reversing the hypermethylation of the *FMR1* CGG repeat on neuronal function, the authors differentiated edited and non-edited FXS iPSCs into neurons and examined their electrophysiological properties. In keeping with the hyperexcitability that has been observed in neurons from individuals with FXS, neurons derived from non-edited iPSCs displayed higher than normal firing rates; however, firing rates were restored to control levels in neurons derived from edited iPSCs. Electrophysiological properties were similarly rescued to control levels in neurons in which demethylation was induced after differentiation.

This study demonstrates the utility of DNA demethylation editing as a tool to investigate the contribution of DNA methylation to disease and suggests possible avenues for the rescue of neuronal function in FXS through the reversal of the DNA methylation-driven silencing of *FMR1*.

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**ORIGINAL ARTICLE** Liu, X. S. et al. Rescue of fragile X syndrome neurons by DNA methylation editing of the *FMR1* gene. *Cell* **172**, 979–992 (2018)