Role of mismatch repair enzymes in GAA-TTC triplet-repeat expansion in Friedreich's ataxia induced pluripotent stem cells (iPSCs).

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Public Summary:
Over twenty human genetic diseases are caused by expansion of simple trinucleotide repeat sequences within essential genes, resulting in toxic proteins (as in the polyglutamine expansion diseases, such as Huntington's disease (HD)), toxic RNAs (as in Myotonic Dystrophy type 1), or gene repression (as in Friedreich's ataxia (FRDA) and Fragile X syndrome (FXS)). Our laboratory has generated induced pluripotent stem cells (iPSCs) from fibroblasts obtained from patients with Friedreich's ataxia (FRDA). By comparing cells before and after reprogramming, we found that triplet repeats were expanded in the FRDA iPSCs. During growth of the iPSCs in culture, the repeats continue to expand, suggesting that expansion might be linked to DNA replication in these cells. The expansion we observe in iPSCs does not occur in the fibroblast (skin cells) from which the iPSCs were derived. Similarly, on differentiation of the FRDA iPSCs into neurons (brain cells), repeat expansion stops. This observation suggests that some cellular factors necessary for expansion may be selectively expressed in iPSCs, but not in fibroblasts or neurons. Our studies have been aimed at the understanding the molecular basis underlying triplet repeat expansion/instability that we have observed during the establishment and propagation of iPSCs from disease-specific fibroblasts. Previous studies have implicated the mismatch repair (MMR) enzymes in repeat expansion in mouse models for other triplet repeat diseases. We find that silencing of the MSH2 gene, encoding one of the subunits of the MMR enzymes, impedes repeat expansion in human FRDA iPSCs. We find that components of the human mismatch repair (MMR) system are associated with the disease alleles in the FRDA iPSCs, and that silencing of these genes at the level of their messenger RNAs is sufficient to suppress repeat expansion. Moreover, we have monitored the levels of the MMR enzymes in fibroblasts, iPSCs and neurons, and as expected these enzymes are present at higher amounts in the iPSCs. This increase in abundance of these cellular enzymes might be the explanation for repeat expansion in the iPSCs.

Scientific Abstract:
The genetic mutation in Friedreich's Ataxia (FRDA) is a hyper-expansion of the triplet-repeat sequence GAA-TTC within the first intron of the FXN gene. Although yeast and reporter construct models for GAA-TTC triplet-repeat expansion have been reported, studies in FRDA pathogenesis and therapeutic development are limited by the availability of an appropriate cell model in which to study the mechanism of instability of the GAA-TTC triplet repeats in the human genome. Herein, induced pluripotent stem cells (iPSCs) were generated from FRDA patient fibroblasts after transduction with the four transcription factors Oct4, Sox2, Klf4 and c-Myc. These cells were differentiated into neurospheres and neuronal precursors in vitro, providing a valuable cellular model for FRDA. During propagation of the iPSCs, GAA-TTC triplet repeats expand at a rate of about two GAA-TTC triplet repeats per replication. However, GAA-TTC triplet repeats are stable in FRDA fibroblasts and neuronal stem cells. The mismatch repair enzymes MSH2, MSH3 and MSH6, implicated in repeat instability in other triplet-repeat diseases, are highly expressed in pluripotent stem cells compared to fibroblasts and neuronal stem cells, and occupy FXN intron 1. In addition, shRNA silencing of MSH2 and MSH6 impeded GAA-TTC triplet-repeat expansion. A specific pyrrole-imidazole polyamide targeting GAA-TTC triplet-repeat DNA partially blocks repeat expansion by displacing MSH2 from FXN intron 1 in FRDA iPSCs. These studies suggest that in FRDA, GAA-TTC triplet-repeat instability occurs in embryonic cells and involves the highly active mismatch repair system.