Gene-editing therapy for neurological disease

Moira A. McMahon and Don W. Cleveland

Guide RNA-mediated CRISPR–Cas nucleases are a powerful technology for the engineering of mammalian genomes. CRISPR–Cas9-dependent editing of mutated genes that cause Huntington disease and fragile X syndrome was recently achieved in cell-based models, heralding the first step towards developing this technology into viable therapeutics for neurological diseases.

CRISPR–Cas is an RNA-guided nuclease system that is capable of site-specific gene modification, and was originally discovered in bacteria as part of a natural defence against invading pathogens. This system has recently been adapted for editing of mammalian genomes, either by disrupting the expression of genes containing disease-causing mutations or — in a more sophisticated form — by editing those genes to correct the mutation. One exciting aspect of CRISPR–Cas nuclease technology is its potential to ameliorate or even cure diseases that currently have no treatment options, including many neurological diseases. Genetic disorders of the nervous system fall into two general categories: the nucleotide (usually trinucleotide) repeat expansion diseases, which account for over two dozen neurological diseases, and diseases resulting from missense or nonsense mutations in coding regions of genes. In a series of recent studies, CRISPR–Cas9-based gene editing has been tested in cell-based models of two nucleotide repeat expansion diseases: fragile X syndrome (FXS) and Huntington disease (HD).

For mammalian gene editing, a 102-nucleotide single-guide RNA (sgRNA) is most commonly used to direct the nuclease to a precise sequence in the genome. When bound through the guide RNA to the specific target DNA, the sgRNA–nuclease complex is activated to induce a site-specific double-stranded DNA break. Repair of this break by non-homologous end joining is error-prone, frequently adding or removing short nucleotide stretches, thereby creating gene-inactivating mutations. Alternatively, precise replacement of the mutant DNA sequence with a corrected one can be achieved by homologous recombination in the presence of an appropriate user-designed DNA repair template.

FXS is characterized by a CGG-repeat expansion in the 5′-untranslated region of the fragile X mental retardation 1 (FMR1) gene, which results in epigenetic silencing, as measured by DNA hypermethylation, chromatine changes, and decreased levels of FMR1 mRNA and FMR1 protein. No therapies are currently available for this condition.

In an initial proof-of-principle study, Park et al. expressed CRISPR-Cas9 nucleases, along with an sgRNA targeting the FMR1 gene upstream of the CGG repeat, in induced pluripotent stem cells (iPSCs) from a patient with FXS. Although the single nuclease cut typically generated small deletions, in one case it produced a larger change, resulting in the removal of CGG repeats and reactivation of FMR1. The edited FXS iPSC clones were then successfully differentiated to mature neurons, suggesting that the procedures required for gene editing and deletion of the CGG repeat did not alter the differentiation potential of the cells. In a second study, Xie et al. went one step further by co-expressing two sgRNAs that flanked the CGG repeat to induce two double-stranded breaks (Fig. 1a). Subsequent recombination resulted in more precise (and more efficient) removal of the repeat than was achieved by Park et al. In both studies, removal of the CGG repeat resulted in increased expression of FMR1.

An even more impressive achievement with CRISPR–Cas nuclease technology has recently been reported in the context of HD, an adult-onset, fatal degenerative disease caused by a dominant CAG-repeat expansion within the coding sequence of the huntingtin gene. In cells derived from a patient with HD, Shin et al. demonstrated that the allele containing the CAG-repeat expansion could be selectively inactivated without altering the normal allele. Like Xie et al., the authors used two sgRNAs that flanked the repeat, which were chosen on the basis of patient-specific nucleotide polymorphisms that distinguished the expanded allele from the wild-type allele. Use of these sgRNAs induced deletion of ~44 kb of DNA, including the CAG repeat (Fig. 1b), the promoter region and the transcription start site, thereby creating a null allele. This report represents the first example of ‘allele-specific’ editing in a trinucleotide repeat expansion disease, and the approach might be broadly applicable to other conditions in which disease-related haplotypes have been identified.

What is the next step? The successful translation of these proof-of-principle studies to in vivo gene editing is eagerly anticipated, but several challenges remain. One major challenge is delivery of the sgRNA and nuclease to target cells. Current approaches are focused on delivery of these two components either by adeno-associated virus (AAV) or as ready-assembled sgRNA–nuclease complexes. Viral delivery of gene-modifying components has a long history, and has already been shown to be effective for gene delivery throughout all rodent (and most nonhuman primate) nervous systems. One example is AAV-mediated delivery of a gene encoding a short hairpin RNA to induce catalytic destruction of the mRNA encoding superoxide dismutase 1 (SOD1). Dominant mutations in the SOD1 gene cause the fatal
paralytic disease amyotrophic lateral sclerosis (ALS). AAV-induced repression of mutant SOD1 protein synthesis slowed disease progression and extended survival\(^1\). In principle, AAV could be used to deliver genes encoding both a CRISPR–Cas nuclease and an activating sgRNA to inactivate target genes, including mutant SOD1 in inherited ALS.

Several pitfalls lie ahead for therapy with AAV-mediated gene editing. Although the replication-incompetent AAV genome does not integrate into the cellular genome, in the versions of AAV currently in use, expression of the genes on the AAV vector continues indefinitely. Also, the consequences of a possible undesirable immune response resulting from chronic production of a bacterially derived CRISPR–Cas nuclease have not been tested. A further safety concern arises from the recognition that chronic expression of an active CRISPR–Cas nuclease will inevitably lead to cleavage and editing of potential ‘off-target’ sites.

A potentially more promising approach for \textit{in vivo} editing of genes known to carry neurological disease-causing mutations is transient activation of CRISPR–Cas nuclease. One possible strategy would involve AAV delivery of the gene encoding the nuclease and separate infusion of a chemically modified synthetic CRISPR RNA (scrRNA) to provide nuclease activation and DNA-targeting specificity\(^2\). Wide dissemination throughout the CNS of chemically modified RNAs of a similar size to scrRNA has already been demonstrated\(^3\), providing evidence to support the feasibility of transient nuclease activation to target disease-causing genes. One major advantage of this system is that the levels of scrRNA can be controlled by dosage, and its gradual degradation over time can act as an inherent switch to turn off the CRISPR–Cas system, resulting in a transiently active gene-editing complex. That said, efficacy of infusion of synthetic CRISPR RNAs into the nervous system has not been established.

A final major challenge for extension of gene-editing approaches to applications within the nervous system is the efficiency of gene silencing or correction: can the changes be made in sufficient numbers of cells to alter the disease course? Although the percentages are not clearly stated, a pessimist would note that Xie \textit{et al.}\(^4\) were only able to isolate five FXS iPSC clones with deletion of the CGG repeat, and FMR1 mRNA and protein levels were only restored in one of these clones. Together taken with the numbers of clones characterized by Park \textit{et al.}\(^1\) and Shin \textit{et al.}\(^5\), these observations indicate that the percentage of gene-edited cells \textit{in vitro} is likely to be far below the number needed \textit{in vivo} to reverse disease. Strategies to address this challenge will include optimization of delivery methods, identification of the most active sgRNAs, and — potentially — identification of additional, more active Cas nucleases. What seems certain is that continued development of genome-editing technology to target neurological diseases is likely to provide a greater understanding of the diseases themselves and, hopefully, will one day form the basis of a successful therapy.

Figure 1 | CRISPR-Cas9-based gene editing. This schematic figure illustrates gene-editing schemes for two trinucleotide repeat expansion diseases. \textbf{a} | Fragile X syndrome. Xie \textit{et al.}\(^1\) used the CRISPR–Cas9 system to excise the expanded CGG repeat in the fragile X mental retardation 1 (FMR1) gene, resulting in reactivation of the gene. \textbf{b} | Huntington disease. Shin \textit{et al.}\(^6\) used the CRISPR–Cas9 system to excise the expanded CAG repeat in the huntingtin gene, thereby preventing production of mutant huntingtin protein. sgRNA, single-guide RNA.


Correspondence to D.W.C.
dcleveland@ucsd.edu
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