REVIEW ARTICLE

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Applications of CRISPR/Cas9 for Gene Editing in Hereditary Movement Disorders

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ABSTRACT

Gene therapy is a potential therapeutic strategy for treating hereditary movement disorders, including hereditary ataxia, dystonia, Huntington's disease, and Parkinson's disease. Genome editing is a type of genetic engineering in which DNA is inserted, deleted or replaced in the genome using modified nucleases. Recently, clustered regularly interspaced short palindromic repeat/CRISPR associated protein 9 (CRISPR/Cas9) has been used as an essential tool in biotechnology. Cas9 is an RNA-guided DNA endonuclease enzyme that was originally associated with the adaptive immune system of *Streptococcus pyogenes* and is now being utilized as a genome editing tool to induce double strand breaks in DNA. CRISPR/Cas9 has advantages in terms of clinical applicability over other genome editing technologies such as zinc-finger nucleases and transcription activator-like effector nucleases because of easy *in vivo* delivery. Here, we review and discuss the applicability of CRISPR/Cas9 to preclinical studies or gene therapy in hereditary movement disorders.

Key Words
CRISPR/Cas9; gene editing; gene therapy; hereditary movement disorders.

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INTRODUCTION

In the early 21st century, the Human Genome Project was successfully completed, revealing the entire sequence of the human genome.[1] This success has accelerated the rate of genomic research, which addresses the function of genes and their resultant translated proteins. Over the last decade, due to the advances in next-generation sequencing, a rapidly increasing number of pathogenic variants and mutations has been discovered.[2] Additionally, over last 5 years, genomic engineering technologies (that is, the modification of the genome at precise, predetermined loci) have achieved huge technical improvements that are now being utilized as valuable tools in preclinical research that may eventually give aid to patients suffering from intractable diseases.[3]

An increasing number of genetic mutations that cause hereditary movement disorders presenting with ataxia, dystonia, parkinsonism, chorea or spastic paraparesis have been identified.[4-6] Although various pathogenic mechanisms such as protein aggregation, mitochondrial dysfunction, oxidative stress, apoptosis and autophagy have been identified from these genes, disease-modifying treatments for neurodegenerative disorders or hereditary movement disorders are lacking.[7-9] Novel chemical drugs, stem cell therapies, and gene therapies have been suggested as promising new therapies for these disorders. The currently available drugs for these disorders are for symptomatic treatment; however, they fail to cure the disease or reverse disease progression. Although the theory behind stem cell therapies is promising, there are still many technical obstacles to be solved. Moreover, a large amount of data from preclinical studies and clinical trials as well as data about safety are needed before the broad application of these therapies to patients.[10,11] Gene therapies using genome editing technologies are another potentially powerful therapeutic strategy for the disease-modifying treatment of hereditary movement disorders or neurodegenerative disorders. Here, we discuss the applicability of the newest genome engineering method, the clustered regularly interspaced short palindromic repeat/CRISPR associated protein 9 (CRISPR/Cas9) system, to hereditary movement disorders.

GENE THERAPY METHODS: GENE SILENCING AND GENE EDITING

Gene therapy refers to the introduction of defined genetic material to specific target cells or tissues of a patient for the final purpose of curing or altering particular disease symptoms.[12] This has long fascinated clinicians and scientists because it has the potential to ultimately cure a disease. Gene therapy can be classified into two categories: gene silencing and gene editing (Table 1).[13,14] Gene silencing is a general term used to describe the suppression of gene expression. RNA interference, antisense oligonucleotides and microRNAs are all gene silencing technologies and were the 'gold standards' for the knockdown of genes and studying gene function *in vitro* and *in vivo* for many years.[15,16] Double-stranded RNA (dsRNA) is a key molecule in gene silencing; dsRNAs are processed into small interfering RNAs (siRNAs) by the endonuclease Dicer, and these siRNAs are loaded into the RNA-induced silencing complex complex that pairs with the messenger RNA (mRNA) through base-pairing, causing the mRNA to be subsequently degraded.[17,18] Gene editing was developed to improve the limitations of gene silencing. Genome editing inserts, deletes or replaces target DNA sequences in the genome using engineered nucleases such as zinc-finger nucleases (ZFNs), tran-

Table 1. Gene silencing vs. gene editing

	Gene silencing	Gene editing
Approach	RNAi, ASO, miRNA	ZFNs, TALENs, CRISPR/Cas9 (RGENs)
Molecular target	RNA	DNA
Modulation of targeting	Knock out	Knock out or knock in
Method of delivery	Nanoparticles, viral vectors, bioconjugates	ZFNs, TALENs: viral vectors CRISPR/Cas9: viral vectors, electroporation, PEI-mediated transfection, nanoparticles
Off-target risk	High	Low or moderate

RNAi: RNA interference, ASO: antisense oligonucleotides, miRNA: microRNA, ZFNs: zinc-finger nucleases, TALENs: transcription activator-like effector nucleases, CRISPR/Cas9: clustered regularly interspaced short palindromic repeat/CRISPR associated protein 9, RGENs: RNA-guided engineered nucleases, PEI: polyethylenimine.

scription activator-like effector nucleases (TALENs), and Cas9.[3,19] Gene editing has low off-target effects, shows an ease of multiplexing and has greater target specificity compared to gene silencing.

There are three common requirements for any effective gene therapy modality: 1) the identification of the target gene that is mutated in the disease; 2) a delivery system for the genes or materials; and 3), an approach for regulating the expression of the target gene. Delivery tools for the genetic material in gene therapy are divided into viral and non-viral categories. Adeno-associated viruses (AAVs) and lentiviruses are commonly used for movement disorders.[20-22] AAVs and lentiviruses have the capability to infect both dividing and non-dividing cells, and the latter can integrate into the genome of host; however, the former does not. However, they are still not safe to apply to clinical trials even though their viral genomes have been modified to remove virulence genes, including those that are self-replicating.[23] Naked plasmid DNA and cationic lipid methods belong to the non-viral set of delivery tools. Unfortunately, non-viral delivery tools are not sufficient for the treatment of chronic neurodegenerative conditions because they create only transient modification in gene expression. New delivery systems that induce permanent effects safely are thus required for clinical application.

GENE EDITING

Gene silencing has helped researchers achieve the knockdown of specific gene targets cheaply, simply, and quickly. However, it has critical limitations, including incomplete gene silencing, temporary effects, and off-target errors, which limit its broader clinical application.[24] In the past decade, a new strategy has emerged that enables researchers to manipulate practically any gene in cells and tissues. This core methodology is referred to as gene editing, which is a type of genetic engineering in which DNA is inserted in, deleted from or replaced in a genome using site-specific nucleases, which enable the precise modification of genes by introducing double strand breaks (DSBs) at the target location in the genome. These programmable nucleases include ZFNs and TALENs, which create site-specific DSBs at target locations.[25,26] Distinct from these site-specific nucleases, CRISPR/Cas9 is an RNA-guided engineered nuclease (RGEN) system, in which a synthetic guide RNA (gRNA) introduces a DSB at a specific location in the target genome.[27-29] Below is a brief review regarding the key features of these three types of programmable nucleases–ZFNs, TALENs and the CRISPR/Cas system (Table 2).[30,31]

A ZFN consists of a Fok1 cleavage domain and a zinc-finger binding domain. ZFNs recognize specific target DNA through protein-DNA interactions. Because the cleavage of DNA strands occurs after Fok1 dimerization, zinc-finger proteins need to be designed to recognize unique left and right half-sites.[32] ZFN target sites consist of two zinc-finger binding sites separated by a spacer sequence. Although theoretically ZFNs can recognize specific 9-bp sequences, the recognition efficiency can be decreased because of interference between recognition modules.[33]

Similar to ZFNs, TALENs are chimeric proteins comprised of a Fok1 cleavage domain and a DNA binding domain from the transcription factor of *Xan-*

Table 2. Comparison of different programmed nucleases

	ZFNs	TALENs	CRISPR/Cas9 (RGENs)
DNA targeting specificity determinant	Zinc-finger proteins	Transcription activator-like effectors	CRISPR RNA of sgRNA
Nucleases	*Fok*I	*Fok*I	Cas9
Restriction in target site	G-rich	Start with T and end with A	End with NGG or NAG (lower activity) sequence (PAM)
Ease of engineering	Difficult	Moderate	Easy
Ease of multiplexing	Low	Moderate	High
Off-target effects	Moderate	Low	Variable
Cytotoxicity	Variable to high	Low	Low
Ease of *in vivo* delivery	Moderate: viral vectors	Moderate: viral vectors	Moderate: viral vectors, nanoparticles, PEI-mediated transfection
Cost	High	Moderate	Low

RGENs: RNA-guided engineered nucleases, ZFNs: zinc-finger nucleases, TALENs: transcription activator-like effector nucleases, CRISPR: clustered regularly interspaced short palindromic repeat, Cas9: CRISPR associated protein 9, sgRNAs: single-guide RNAs, PAM: protospacer adjacent motif, PEI: polyethylenimine.

thomonas.[34,35] TALENs recognize specific target DNA through protein-DNA interactions. A TALEN target site consist of two TALE binding sites separated by a spacer. The DNA-binding domain of a TALEN is composed of multiple repeats and can recognize 33–35 nucleotides.[35] Although there was a problem with low efficiency during the early stages of development, platinum TALENs have high efficiency in mammalian cells. Additionally, the most advantageous feature of TALENs is that they can be designed to target almost any given DNA sequence because the cutting of target DNA sequences with TALENs is achieved by Fok1, which is linked to complementary DNA sequences.[3]

CRISPR/CAS9 SYSTEM

The CRISPR/Cas9 system is categorized as an RGEN that recognizes a target specific sequence with a 23-bp length, and the mechanism of action is different from that of ZFNs and TALENs.[19,28] Unlike ZFNs and TALENs, CRISPR/Cas9 uses gRNA instead of a protein-DNA interaction to recognize genomic DNA and utilizes Cas9 as a nuclease.[35,36] The gRNA can recognize approximately 20-bp nucleotides and requires a protospacer adjacent motif (PAM), which can recruit Cas9.[36] Cas9 is guided by specific sequences of gRNA that are related to a *trans*-activating crRNA (tracrRNA) and form the complementary DNA target sequence, resulting in a site-specific DSB.[28,29,37,38] CRISPR/Cas9 has an ability to disrupt multiple genes simultaneously, so it can be more useful for studying genetic interactions and making models of multigenic disorders than ZFNs and TALENs. More recently, Cpf1, which is a single-RNA guided nuclease that does not use tracrRNA for genome editing, has been described.[39] Different Cas proteins are able to target specific DNA sequences easily by controlling the short specific part of the gRNA, which can be achieved in one simple cloning step. Another major advantage of Cas proteins is that dual-guide RNAs or single-guide RNAs can be designed and generated easily.[36,40,41] Meanwhile, one major problem is the presence of off-target effects, which involve the nonspecific recognition and digestion of non-targeted DNA regions. The methods for avoiding off-target effects need further investigation for the effective application of CRISPR/Cas9 to human disease.

The efficiency and delivery methods are the remaining issues to be resolved in gene editing. The efficiency of gene editing has to be verified and studied further in polygenic diseases, as many gene editing therapeutic studies have been investigated in the treatment of monogenic diseases.[42-45] A viral vector is required for the delivery of gRNA and Cas9 of CRISPR/Cas9 into the mammalian central nervous system *in vivo*. Safe and efficient delivery methods should be developed for the application of CRISPR/Cas9 in *in vivo* systems because the vector itself may cause insertional mutagenesis.[46]

APPLICATIONS OF CRISPR/CAS9 SYSTEMS IN HEREDITARY MOVEMENT DISORDERS

Why is CRISPR/Cas9 applicable for hereditary movement disorders or neurodegenerative disorders?

Many genes have been identified to be critically involved in the pathogenesis of hereditary movement disorders or neurodegenerative disorders; hence, these are potential targets for the CRISPR/Cas9 system to develop disease modifying treatment strategies. Huntington's disease (HD) is a prototype disease among several trinucleotide repeat disorders, in which the expansion of a polyglutamine region stretches beyond a certain threshold and causes disease. Among autosomal dominant cerebellar ataxia, spinocerebellar ataxia types 1, 2, 3, 6, 7, and 17 are trinucleotide repeat disorders in which the accumulation of abnormal proteins with an expanded polyglutamine track is a common pathogenic mechanism in neurodegeneration.[47] Although most cases of Parkinson's disease (PD), Alzheimer's disease and amyotrophic lateral sclerosis are sporadic onset and associated with multifactorial etiological factors, the accumulation of abnormal misfolded proteins is a common pathological feature.[8,48-50] Genome engineering to modify abnormal protein production and prevent their accumulation appears to be effective in these diseases.

Some hereditary movement disorders occur in an autosomal recessive pattern, which is caused by loss-of-function mutation of certain genes.[51,52] Given that CRISPR/Cas9 can knock in a specific transgene,[53] these autosomal recessive movement disorders can also be good targets for the application of CRISPR/Cas9.

The application of CRISPR/Cas9 for the generation of model system for hereditary movement disorders

The CRISPR/Cas9 system is accelerating the development of biological research and enabling targeted genetic interruption in almost any cell type. Although CRISPR/Cas9 has an off-target problem, it has opened the door to the development of new *in vitro* and *in vivo* model systems for studying the complexities of the nervous system in regards to hereditary movement disorders, including applications for the study of synaptic and neural circuit function,[54,55] neuronal development,[56,57] and genetic neurological diseases.[58]

Genome editing using CRISPR/Cas9 is possible in various cell lines, including human induced pluripotent stem cells, which can be utilized as a valuable *in vitro* tool for the investigation of specific mutations in the pathogenesis of various disorders.[59] For example, Vannocci et al.[60] developed a novel cellular model of Friedreich's ataxia, which is an autosomal recessive ataxia caused by reduced levels of *frataxin*, using CRISPR/Cas9 to stably introduce the disease frataxin gene into cells.

Traditionally, transgenic experimental model systems using species such as mice, flies, fish and cells have provided neuroscientists with important and valuable information about the molecular pathology of many hereditary disorders.[61,62] Transgenic mouse models are widely used because, in addition to knockouts, the genomes of mice can be modified to create pathologies based on gain-of-function mutations using a versatile set of genetic tools. However, rodent models are not sufficient to recapitulate the full range of pathological phenotypes when compared to patients with hereditary movement disorders. The ability to investigate genetically modified large animals, such as pigs, dogs, and non-human primates, has the potential to significantly enhance our understanding of the complex pathological process of the human disease. Large animal models are more capable of confirming therapeutic effects that cannot be adequately modelled in rodents. However, the transgenic modification of genes in large animals using traditional gene targeting technology is generally less successful due to the lack of available embryonic stem cell lines.

Recently, CRISPR/Cas9 was successfully used to generate the precise disruption of single and multiple genes in pigs[63] and non-human primates,[64] which can be used as large animal models of hereditary movement disorders or neurodegenerative disorders. Recently, Holm et al.[65] suggested the use of CRISPR-mediated pig models for neurodegenerative disorders, including HD and PD. However, off-target effects and mosaic mutations are problems that need to be solved during the CRISPR/Cas9-mediated generation of large animal models. Although off-target mutations will be diluted quickly over generations in small animal models with short breeding times, this can be a serious problem in large animal models, such as monkeys, which have longer periods between generations. Moreover, somatic mosaicism and allele complexity can occur during CRISPR/Cas9-mediated mutagenesis through zygote injection.[66] The generation of large animal models using CRISPR/Cas9 will be improved by reducing the off-target effects and mosaic mutations.

CRISPR/Cas9-mediated preclinical therapeutic applications for hereditary movement disorders or neurodegenerative disorders

Several approaches for gene therapy, including gene silencing and virus-mediated gene delivery, in hereditary movement disorders have been pursued both in preclinical studies[67,68] and in early phase clinical trials.[21,69-73] Meanwhile, CRISPR/Cas9-mediated gene editing is still in the early preclinical phase. Dr. Nicolas Merienne and his colleagues performed research to reduce mutant huntingtin aggregation by using CRISPR to delete the open reading frame of the *HTT* gene, leading to the loss of mHtt expression.[74] In these studies, CRISPR/Cas9 reduced the aggregation of mutant huntingtin in the mouse striatum, demonstrating the potential of the CRISPR/Cas9 system as a gene therapy modality for hereditary movement disorders. Recently, Chen et al.[75] showed that the CRISPR-mediated knock in of designer receptors exclusively activated by designer drugs (DREADDs) enables the precise regulation of human pluripotent stem cell (hPSC)-derived neurons by chemical compounds. When the hPSC-derived human midbrain dopaminergic neurons were transplanted into a PD mouse model, their motor function was able to be reversed or enhanced by DREADD ligands. Further, in June 2016, the US National Institutes of Health approved a pro-

posal to use CRISPR/Cas9 in the first human clinical trial to edit the genome of T cells to augment cancer therapies,[76] which will be the starting point for subsequent CRISPR clinical trials in various human diseases. CRISPR-mediated gene therapies in HD, PD, dystonia, and hereditary ataxias can be challenging, but will be a feasible therapeutic option in the near future.

CONCLUSION

Although there are still many problems to be solved, such as off-target effects, delivery system, efficacy, safety concerns, and ethical issues, CRISPR/Cas9 is quickly being applied as an essential tool in biotechnology and will be applied to clinical practice sooner or later. CRISPR/Cas9-mediated preclinical research and clinical trials should be encouraged and performed in hereditary movement disorders or neurodegenerative disorders.

Conflicts of Interest

The authors have no financial conflicts of interest.

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REFERENCES

1. International Human Genome Sequencing Consortium. Finishing the euchromatic sequence of the human genome. Nature 2004;431:931-945.
2. Jacob HJ. Next-generation sequencing for clinical diagnostics. N Engl J Med 2013;369:1557-1558.
3. Kim H, Kim JS. A guide to genome engineering with programmable nucleases. Nat Rev Genet 2014;15:321-334.
4. Olgiati S, Quadri M, Bonifati V. Genetics of movement disorders in the next-generation sequencing era. Mov Disord 2016;31:458-470.
5. Kumar KR, Lohmann K, Klein C. Genetics of Parkinson disease and other movement disorders. Curr Opin Neurol 2012;25:466-474.
6. Jarman PR, Wood NW. Genetics of movement disorders and ataxia. J Neurol Neurosurg Psychiatry 2002;73 Suppl 2:II22-II26.
7. Katsuno M, Tanaka F, Sobue G. Perspectives on molecular targeted therapies and clinical trials for neurodegenerative diseases. J Neurol Neurosurg Psychiatry 2012;83:329-335.
8. Morimoto RI. Proteotoxic stress and inducible chaperone networks in neurodegenerative disease and aging. Genes Dev 2008;22:1427-1438.
9. Filosto M, Scarpelli M, Cotelli MS, Vielmi V, Todeschini A, Gregorelli V, et al. The role of mitochondria in neurodegenerative diseases. J Neurol 2011;258:1763-1774.
10. Lindvall O, Kokaia Z. Stem cells in human neurodegenerative disorders--time for clinical translation? J Clin Invest 2010;120:29-40.
11. Lindvall O, Kokaia Z, Martinez-Serrano A. Stem cell therapy for human neurodegenerative disorders-how to make it work. Nat Med 2004;10 Suppl:S42-S50.
12. O'Connor DM, Boulis NM. Gene therapy for neurodegenerative diseases. Trends Mol Med 2015;21:504-512.
13. McManus MT, Sharp PA. Gene silencing in mammals by small interfering RNAs. Nat Rev Genet 2002;3:737-747.
14. McMahon MA, Rahdar M, Porteus M. Gene editing: not just for translation anymore. Nat Methods 2011;9:28-31.
15. Elbashir SM, Harborth J, Lendeckel W, Yalcin A, Weber K, Tuschl T. Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. Nature 2001; 411:494-498.
16. Hommel JD, Sears RM, Georgescu D, Simmons DL, DiLeone RJ. Local gene knockdown in the brain using viral-mediated RNA interference. Nat Med 2003;9:1539-1544.
17. Fire A, Xu S, Montgomery MK, Kostas SA, Driver SE, Mello CC. Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. Nature 1998; 391:806-811.
18. Tijsterman M, Ketting RF, Plasterk RH. The genetics of RNA silencing. Annu Rev Genet 2002;36:489-519.
19. Gaj T, Gersbach CA, Barbas CF 3rd. ZFN, TALEN, and CRISPR/Cas-based methods for genome engineering. Trends Biotechnol 2013;31:397-405.
20. Duque S, Joussemet B, Riviere C, Marais T, Dubreil L, Douar AM, et al. Intravenous administration of self-complementary AAV9 enables transgene delivery to adult motor neurons. Mol Ther 2009;17:1187-1196.
21. Marks WJ Jr, Bartus RT, Siffert J, Davis CS, Lozano A, Boulis N, et al. Gene delivery of AAV2-neurturin for Parkinson's disease: a double-blind, randomised, controlled trial. Lancet Neurol 2010;9:1164-1172.
22. Federici T, Taub JS, Baum GR, Gray SJ, Grieger JC, Matthews KA, et al. Robust spinal motor neuron transduction following intrathecal delivery of AAV9 in pigs. Gene Ther 2012;19:852-859.
23. Naldini L. Gene therapy returns to centre stage. Nature 2015;526:351-360.
24. Heidenreich M, Zhang F. Applications of CRISPR-Cas systems in neuroscience. Nat Rev Neurosci 2016;17:36-44.
25. Urnov FD, Rebar EJ, Holmes MC, Zhang HS, Gregory PD. Genome editing with engineered zinc finger nucleases. Nat Rev Genet 2010;11:636-646.
26. Carroll D. Genome engineering with zinc-finger nucleases. Genetics 2011;188:773-782.
27. Barrangou R, Fremaux C, Deveau H, Richards M, Boyaval P, Moineau S, et al. CRISPR provides acquired resistance against viruses in prokaryotes. Science 2007;315:1709-1712.
28. Deltcheva E, Chylinski K, Sharma CM, Gonzales K, Chao Y, Pirzada ZA, et al. CRISPR RNA maturation by trans-encoded small RNA and host factor RNase III. Nature 2011; 471:602-607.
29. Garneau JE, Dupuis MÈ, Villion M, Romero DA, Barrangou R, Boyaval P, et al. The CRISPR/Cas bacterial immune system cleaves bacteriophage and plasmid DNA. Nature 2010; 468:67-71.
30. Horvath P, Barrangou R. CRISPR/Cas, the immune system of bacteria and archaea. Science 2010;327:167-170.

31. Marraffini LA, Sontheimer EJ. CRISPR interference: RNA-directed adaptive immunity in bacteria and archaea. Nat Rev Genet 2010;11:181-190.
32. Beerli RR, Barbas CF 3rd. Engineering polydactyl zinc-finger transcription factors. Nat Biotechnol 2002;20:135-141.
33. Liu Q, Segal DJ, Ghiara JB, Barbas CF 3rd. Design of polydactyl zinc-finger proteins for unique addressing within complex genomes. Proc Natl Acad Sci U S A 1997;94:5525-5530.
34. Boch J, Scholze H, Schornack S, Landgraf A, Hahn S, Kay S, et al. Breaking the code of DNA binding specificity of TAL-type III effectors. Science 2009;326:1509-1512.
35. Moscou MJ, Bogdanove AJ. A simple cipher governs DNA recognition by TAL effectors. Science 2009;326:1501.
36. Mali P, Yang L, Esvelt KM, Aach J, Guell M, DiCarlo JE, et al. RNA-guided human genome engineering via Cas9. Science 2013;339:823-826.
37. Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E. A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. Science 2012;337:816-821.
38. Gasiunas G, Barrangou R, Horvath P, Siksnys V. Cas9-crRNA ribonucleoprotein complex mediates specific DNA cleavage for adaptive immunity in bacteria. Proc Natl Acad Sci U S A 2012;109:E2579-E2586.
39. Zetsche B, Gootenberg JS, Abudayyeh OO, Slaymaker IM, Makarova KS, Essletzbichler P, et al. Cpf1 is a single RNA-guided endonuclease of a class 2 CRISPR-Cas system. Cell 2015;163:759-771.
40. Hsu PD, Scott DA, Weinstein JA, Ran FA, Konermann S, Agarwala V, et al. DNA targeting specificity of RNA-guided Cas9 nucleases. Nat Biotechnol 2013;31:827-832.
41. Cong L, Ran FA, Cox D, Lin S, Barretto R, Habib N, et al. Multiplex genome engineering using CRISPR/Cas systems. Science 2013;339:819-823.
42. Gaspar HB, Cooray S, Gilmour KC, Parsley KL, Adams S, Howe SJ, et al. Long-term persistence of a polyclonal T cell repertoire after gene therapy for X-linked severe combined immunodeficiency. Sci Transl Med 2011;3:97ra79.
43. Howe SJ, Mansour MR, Schwarzwaelder K, Bartholomae C, Hubank M, Kempski H, et al. Insertional mutagenesis combined with acquired somatic mutations causes leukemogenesis following gene therapy of SCID-X1 patients. J Clin Invest 2008;118:3143-3150.
44. Joyce PI, Fratta P, Fisher EM, Acevedo-Arozena A. SOD1 and TDP-43 animal models of amyotrophic lateral sclerosis: recent advances in understanding disease toward the development of clinical treatments. Mamm Genome 2011;22:420-448.
45. Kara E, Tucci A, Manzoni C, Lynch DS, Elpidorou M, Bettencourt C, et al. Genetic and phenotypic characterization of complex hereditary spastic paraplegia. Brain 2016;139(Pt 7):1904-1918.
46. Cox DB, Platt RJ, Zhang F. Therapeutic genome editing: prospects and challenges. Nat Med 2015;21:121-131.
47. Kordasiewicz HB, Stanek LM, Wancewicz EV, Mazur C, McAlonis MM, Pytel KA, et al. Sustained therapeutic reversal of Huntington's disease by transient repression of huntingtin synthesis. Neuron 2012;74:1031-1044.
48. Dobson CM. Protein folding and misfolding. Nature 2003;426:884-890.
49. Soto C. Unfolding the role of protein misfolding in neurodegenerative diseases. Nat Rev Neurosci 2003;4:49-60.
50. Hartl FU, Hayer-Hartl M. Converging concepts of protein folding in vitro and in vivo. Nat Struct Mol Biol 2009;16:574-581.
51. Blackburn JS, Mink JW, Augustine EF. Pediatric movement disorders: five new things. Neurol Clin Pract 2012;2:311-318.
52. Anheim M, Tranchant C, Koenig M. The autosomal recessive cerebellar ataxias. N Engl J Med 2012;366:636-646.
53. He X, Tan C, Wang F, Wang Y, Zhou R, Cui D, et al. Knock-in of large reporter genes in human cells via CRISPR/Cas9-induced homology-dependent and independent DNA repair. Nucleic Acids Res 2016;44:e85.
54. Straub C, Granger AJ, Saulnier JL, Sabatini BL. CRISPR/Cas9-mediated gene knock-down in post-mitotic neurons. PLoS One 2014;9:e105584.
55. Incontro S, Asensio CS, Edwards RH, Nicoll RA. Efficient, complete deletion of synaptic proteins using CRISPR. Neuron 2014;83:1051-1057.
56. Shen Z, Zhang X, Chai Y, Zhu Z, Yi P, Feng G, et al. Conditional knockouts generated by engineered CRISPR-Cas9 endonuclease reveal the roles of coronin in C. elegans neural development. Dev Cell 2014;30:625-636.
57. Jao LE, Wente SR, Chen W. Efficient multiplex biallelic zebrafish genome editing using a CRISPR nuclease system. Proc Natl Acad Sci U S A 2013;110:13904-13909.
58. Swiech L, Heidenreich M, Banerjee A, Habib N, Li Y, Trombetta J, et al. In vivo interrogation of gene function in the mammalian brain using CRISPR-Cas9. Nat Biotechnol 2015;33:102-106.
59. Hendriks WT, Warren CR, Cowan CA. Genome editing in human pluripotent stem cells: approaches, pitfalls, and solutions. Cell Stem Cell 2016;18:53-65.
60. Vannocci T, Faggianelli N, Zaccagnino S, della Rosa I, Adinolfi S, Pastore A. A new cellular model to follow Friedreich's ataxia development in a time-resolved way. Dis Model Mech 2015;8:711-719.
61. Coppola A, Moshé SL. Animal models. Handb Clin Neurol 2012;107:63-98.
62. Tu Z, Yang W, Yan S, Guo X, Li XJ. CRISPR/Cas9: a powerful genetic engineering tool for establishing large animal models of neurodegenerative diseases. Mol Neurodegener 2015;10:35.
63. Wang X, Cao C, Huang J, Yao J, Hai T, Zheng Q, et al. One-step generation of triple gene-targeted pigs using CRISPR/Cas9 system. Sci Rep 2016;6:20620.
64. Niu Y, Shen B, Cui Y, Chen Y, Wang J, Wang L, et al. Generation of gene-modified cynomolgus monkey via Cas9/RNA-mediated gene targeting in one-cell embryos. Cell 2014;156:836-843.
65. Holm IE, Alstrup AK, Luo Y. Genetically modified pig models for neurodegenerative disorders. J Pathol 2016;238:267-287.
66. Yen ST, Zhang M, Deng JM, Usman SJ, Smith CN, Parker-Thornburg J, et al. Somatic mosaicism and allele complexity induced by CRISPR/Cas9 RNA injections in mouse zygotes. Dev Biol 2014;393:3-9.
67. Takahashi M, Suzuki M, Fukuoka M, Fujikake N, Watanabe S, Murata M, et al. Normalization of overexpressed α-synuclein causing Parkinson's disease by a moderate gene silencing with RNA interference. Mol Ther Nucleic Acids 2015;4:e241.
68. Aronin N, DiFiglia M. Huntingtin-lowering strategies in Huntington's disease: antisense oligonucleotides, small RNAs, and gene editing. Mov Disord 2014;29:1455-1461.
69. LeWitt PA, Rezai AR, Leehey MA, Ojemann SG, Flaherty AW, Eskandar EN, et al. AAV2-GAD gene therapy for advanced Parkinson's disease: a double-blind, sham-surgery controlled, randomised trial. Lancet Neurol 2011;10:309-

319.
70. Bartus RT, Brown L, Wilson A, Kruegel B, Siffert J, Johnson EM Jr, et al. Properly scaled and targeted AAV2-NRTN (neurturin) to the substantia nigra is safe, effective and causes no weight loss: support for nigral targeting in Parkinson's disease. Neurobiol Dis 2011;44:38-52.
71. Perdomini M, Belbellaa B, Monassier L, Reutenauer L, Messaddeq N, Cartier N, et al. Prevention and reversal of severe mitochondrial cardiomyopathy by gene therapy in a mouse model of Friedreich's ataxia. Nat Med 2014;20:542-547.
72. Kaplitt MG, During MJ. GAD gene therapy for Parkinson's disease. In: Kaplitt MG, During MJ, editors. Translational neuroscience. New York: Springer, 2016;89-98.
73. Palfi S, Gurruchaga JM, Ralph GS, Lepetit H, Lavisse S, Buttery PC, et al. Long-term safety and tolerability of ProSavin, a lentiviral vector-based gene therapy for Parkinson's disease: a dose escalation, open-label, phase 1/2 trial. Lancet 2014;383:1138-1146.
74. Talan J. News from the Society for Neuroscience Annual Meeting: gene editing techniques show promise in silencing or inhibiting the mutant Huntington's disease gene. Neurol Today 2015;15:14-16.
75. Chen Y, Xiong M, Dong Y, Haberman A, Cao J, Liu H, et al. Chemical control of grafted human PSC-derived neurons in a mouse model of Parkinson's disease. Cell Stem Cell 2016;18:817-826.
76. Reardon S. First CRISPR clinical trial gets green light from US panel. Nature News. 2016 Jun 22.