

# CRISPR-Cas9 Mediated Gene Editing As Therapeutic Tool In Neurodegenerative Disorders

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Received:  
May 21, 2022  
Accepted:  
July 06, 2022  
Published online:  
July 09, 2022

**Abstract:** The gene targeting methods like CRISPR-Cas9, is one of the most powerful technologies for correcting inconsistent genetic signatures and is widely used against various types of diseases these days. CRISPR-Cas9 based strategies have the potential to treat complex diseases as it is relatively straightforward, inexpensive, and precise system. This review article summarizes the applications of CRISPR-Cas9 genetic engineering in neurodegenerative disease models, providing therapeutic gene editing perspectives for neurological diseases. Here, understanding of CRISPR-Cas9 mediated genome editing in neurological diseases such as Alzheimer, Parkinson and Huntington's disease have been focused by targeting specific genes involved and its potential as the most promising and emerging technologies taking into account with the low off-target effects of CRISPR-Cas9 and its highest editing efficiency.

**Keywords:** CRISPR-Cas9, Neurodegenerative disorder, Gene therapy

## 1. Introduction

Neurodegenerative diseases, such as Alzheimer, Parkinson and Huntington's diseases and other neurological diseases are linked to impaired central and/or peripheral nervous systems. In 2015 these disorders were listed as the main cause group of disability-adjusted life-years and considered to be second largest cause of death group across the population (1,2). Individuals older in age suffer mainly from neurodegenerative diseases due to attenuated activation of self-healing and detoxification as there is accumulation of atypical proteins or peptides such as  $\beta$ -amyloid peptides and phosphorylated tau proteins in Alzheimer's disease (AD),  $\alpha$ -synuclein in Parkinson's disease (PD), and mutant huntingtin in Huntington's diseases (HD) (3). In neurodegenerative diseases, the application of mechanism-oriented approaches was found difficult to define the rational drug targets as and it successfully resist the development of effective treatments (1,5). Complexity of the nervous system make neurological disorders difficult to be diagnosed at early stages, thus it's difficult to treat them with conventional pharmacology approach (4). Neuro recovery treatment has recently attempted to restore dysfunction and damaged structures of neurological disorders (5,6).

## 2. Applications of CRISPR-Cas9 for Neurological Disorder Therapy

Recently, the development of site-specific gene-editing technologies such as CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats)-Cas9 (CRISPR associated Systems 9) has been used to develop effective treatments for neurodegenerative diseases with known causative mutations. The preclinical or clinical use of CRISPR-Cas9 for the treatment of diseases can offer several advantages over the RNA reduction approach such as potentially high off-target activity and the need for treatments repeated, that may increase the risk of complications in individuals, which are affected by chronic progressive neurodegenerative diseases (7,8).

The first application of CRISPR technology in neurodegenerative disorders was reported in prion disease. The PrP gene (PrP) was deleted to eliminate expression in N2a neuroblastoma cells, C2C12 myoblasts and NMuMG epithelial cells (13). The CRISPR-Cas9 approach to neurodegenerative diseases can be broadly classified in three steps, first correction of mutations causing disease, second inactivation of gain-of-function mutations, and third transcription modulation (3). Furthermore, the demonstration of the feasibility of genome editing in post-mitotic neurons and the mammalian brain supports the potential of CRISPR-Cas9 strategies as a therapeutic pathway for neurodegenerative disorders (9,10). CRISPR-Cas9 can be applied to neurodegenerative diseases caused by loss-of-function mutations, this proves to be advantageous over RNA-lowering methods, which is therapeutically beneficial for gain-of-function mutations. Other gene editing technologies like TALENs (transcription activator-like effector nucleases) and ZFNs (zinc-finger nucleases) are expensive, have off-target effects, and are significantly more cytotoxic. In their comparison, CRISPR-based technology provides more efficient, accurate and affordable gene editing in almost any cell type and organism (14,26,27). In neuropathy CRISPR-Cas9 technology is used for treatment by repairing or knocking out mutated genes and modifying genes related to it. Unlike other gene editing techniques, the site specificity of CRISPR-Cas9 is mediated by the interaction between guide RNA (gRNA) and target DNA. Therefore, CRISPR-Cas9 does not require protein technology and this approach is very feasible and cost-effective (11-12).

## 3. Alzheimer's Disease

Alzheimer's disease is a pathologically progressive disease characterized by massive neurological loss, intracellular neurofibrillary tangles, and extracellular amyloid plaques in the brain. It is recognized as the leading cause of death in the elderly (16). Although there are several genetic mutations have been associated to onset of AD. Mutations in three genes which are amyloid precursor protein (APP) on chromosome 21, presenilin 1 (PSEN1) on chromosome 14, and presenilin 2 (PSEN2) on chromosome 1 cause early-onset of AD in humans while mutations in apolipoprotein-E (ApoE) is linked with the late onset of Alzheimer's disease (14-17).

Chronic neuroinflammation is known to play an important role in the development of AD (20). The proinflammatory molecule glia maturation factor (GMF) is majorly expressed in the activated glial cells surrounding the amyloid plaques in the brains of patients with AD (21). Microglia are considered as the leading cause of neuroinflammation and the major source of GMF. Raikwar et al. used CRISPR-Cas9 to edit Gmf in a BV2 microglial cell line (22). The Accumulation of oligomeric A $\beta$ 1-42 and other A $\beta$  protein, which is very important for the pathogenesis of Alzheimer's disease (23). Abnormal cleavage of APP protein by the  $\beta$ -secretase-1 enzyme (coded by BACE1 gene) is essential the production of the amyloid- $\beta$  (A $\beta$ ) peptide plaque, which are characteristic features of AD (16). For the synthesis of A $\beta$  peptides, Beta-secretase 1 (Bace1) is required (24).

The study conducted by Park et al. indicated that nanocomplex-mediated CRISPR-Cas9 can effectively target genes in neurons and can be applied to treat mouse models of AD (25). In AD, knock-in (KI) mouse samples, when the BACE1 gene was targeted through a nanoparticle-coated Cas9-sgRNA complex, a significant reduction in amyloid plaque accumulation and decreased amyloid secretion in neurons was observed. Multiple deliveries of nanoparticles to the hippocampus have been shown to be more effective in repressing the BACE1 gene (17). Mutations in APP and PSEN1 cause

severe lysosomal dysfunction and autophagy in human iPSC-derived neurons. Deletion of APP in PSEN1 Y115C neurons reduced LAMP1 protein and increased lysosomal axonal transport compared to PSEN1 Y115C isogenic neurons (17). Another research study demonstrated that the correction of PSEN2 point mutations in cholinergic neurons of the forebrain via CRISPR-Cas9 eliminated the electrophysiological deficit, restoring the maximum number of spikes and spike height compared to the levels observed in controls. In addition, increased A $\beta$ 42 / 40 was normalized too after the correction of the PSEN2N141I mutation by CRISPR-Cas9 (18). KIBRA polymorphism (protein expressed by the kidneys and brain) has been linked to cognitive inability in AD (19). Song et al. recently developed a CRISPR-based KIBRA-KO mouse to study its role in AD. High neuronal loss in KIBRA-KO mice in the hippocampus from apoptosis was found, while overexpression of KIBRA in neuronal cell lines significantly promoted its proliferation and inhibited A $\beta$ -induced apoptosis. It indicated that KIBRA functions as a neuroprotective gene that promotes cell survival and inhibits A $\beta$ -induced apoptosis, proving its potential as a therapeutic target for the treatment of Alzheimer's disease (19).

#### 4. Parkinson's Disease

Parkinson disease is known as one of the most frequent neurovegetative disorder, second only to Alzheimer's disease (28) and characterized by affected body movements (29). The cases of PD have been found to be approximately 2% among people over 50 years of age and 4% among the population older than age 85 (30). PD occurs beyond the gender and ethnicity around the globe (31,32). Parkinson neurodegenerative illness affects around 10 million people globally (33). PD illness characterized by motor and non-motor conditions (34) with typical motor characteristics related to misfolded proteins known as Lewy bodies and their chief component SCNA gene which encodes  $\alpha$ -synuclein is the usual pathology of PD (3) and the loss of dopaminergic neurons in the substantia nigra (35). It leads to motor symptoms such as rest tremors, bradykinesia, rigidity and postural instability (36) Mutation in  $\alpha$ -synuclein (SCNA), Parkin RBR E3 Ubiquitin Protein Ligase (PARK2), PTEN-induced putative kinase 1 (PINK1), DJ-1 (PARK7), and leucine-rich repeat kinase 2 (LRRK2) (37,38) has been established as main cause of PD (39).

On the basis of probable function of above identified genes involved in the pathogenesis of PD may be a candidate of choices for therapeutic intervention (40,41). Based on the cellular processes implicated in PD, multiple treatment options have been investigated (42). The CRISPR-Cas9 framework considers prompt and precise genome altering in almost any living species — is by all accounts a promising methodology in PD and it appears to be a viable strategy in PD as well (43,44). The CRISPR related research in PD disease may lead to significant changes in how PD and other neurological disorders are treated. CRISPR-Cas9 technology opens the door to PD treatment and aids the genomic examination of PD pathogenesis by utilizing the promising potentials such as gene knocking out/in, gene editing, transcriptional activation/repression, and epigenetic modification (38).

Linkage between mutation in  $\alpha$ -synuclein (SCNA) and neuronal degeneration or loss of dopaminergic neurons in the substantia nigra (SN) region have found in the severity of PD (36). Based on that CRISPRi was used to reduce the expression of *SNCA* gene in neuronal and other cell lines and noted decrease in expression up to 60% of the same (45). *SNCA* intron 1 methylation regulates *SNCA* transcription, and the brains of patients with Parkinson's disease exhibit different levels of methylation than healthy population (46). Kantor et al. [47] attempted to modulate the methylation (epigenetic) tags located in the intron1 of the *SNCA* gene that control its expression using (47) Cas9 (dCas9)-coupled catalytic domain is deactivated by CRISPR (DNMT3A). (36) Results showed that *SNCA* intron 1 methylation mediated downregulation of *SNCA* mRNA and protein levels (46). The observed reductions in *SNCA* mRNA and protein levels and other parameters such as mitochondrial ROS and improved cell viability (20).

PINK1 deficiency in PD interferes with mitochondrial autophagy and increase oxidative stress due to the dysfunctional mitochondria accumulation that release reactive oxygen species (47). By regulating mitochondrial calcium uptake, the mitochondrial calcium uniporter (MCU) protein participates in mitochondrial dysfunction and cell death caused by excitotoxicity, inflammation, and oxidative stress and may play an important role in neurodegenerative diseases (48). Lee et al (49) and Soman et al (50) used zebrafish as a model to study PD using the CRISPR- Cas9 system, using *mcu* and *pink 1* double knock-out [(*pink1;mcu*)-/-]. The large number of dopaminergic neurons restored the higher performance of the mitochondrial membrane.

The enzyme Tyrosine hydroxylase (TH) enzyme acts as a rate limiting step during dopamine biosynthesis and has been shown to be a reliable marker for dopaminergic neurons (51). Qing et al. (2017) studied most common mutation is p.G2019S and authors studied CRISPR-Cas9 system gene editing to this mutant in patient-derived human iPSC (hiPSCs). Their observation showed a significant decrease in the proportion of tyrosine hydroxylase (TH) positive neurons in LRRK2-G2019S dopaminergic neurons (52). Gene editing such as CRISPR technique allows to uncover the molecular basis of PD and discovery of new therapeutic targets, and development of new genetic therapies. Increasing and decreasing gene expression or selectively editing key genes modified in PD, such as PRKN, GDNF, PINK1, and AADC (L-amino acid aromatic decarboxylase), can be used to correct molecular pathway defects in PD (41). Genetic engineering remains a viable approach to restoring the activity of important disrupted biological pathways that can contribute to Parkinson's disease (36).

## 5. Huntington's disease

Huntington's disease (HD) is an inherited autosomal neurodegenerative disease with clinical manifestations such as motor, cognitive (53), undesirable choreatic actions, behavioral and psychiatric characteristics, and dementia with a disease rate of about 10 per 100,000 in the Caucasian population (54,55). HD mainly caused by the expansion of unstable cytosine-adenine-guanine (CAG) nucleotide repeats in the exon1 of the huntingtin (HTT) gene which produces an abnormal HTT protein (56). HTT protein become no longer remain a normal version of protein because of its length, which becomes more than normal HTT protein due the addition of several repeats of CAG segments (>36 repeats) to its gene, which encode polyglutamine (polyQ) in the N-terminal region of the huntingtin (*HTT*) gene, are the genetic reason of HD (57,58).

Gene therapy is known as one of the best approaches to treat HD disease is under study through targeting DNA transcription to reduce the level of abnormal HTT protein or to decrease RNA translation using non-coding RNAs (61). One potential strategy to treat HD could be to use one of the gene therapy methods, CRISPR-Cas9 is therefore hypothesized to ameliorate associated disease pathogenesis by reducing the size of disease-causing expanded repeats and selectively suppress the mHTT (60,61). A CRISPR-Cas9 system has used to delete CAG repeats to silence HTT gene expression (62,64). Rationale of CRISPR-Cas9 approach comes when it was effectively applied to suppress the HTT gene (63,65) in mouse model and improved motor and neuropathological abnormalities in a HD mouse model (66). One of the latest findings indicated that the 5' untranslated region (UTR) is important in regulating HTT protein synthesis. CRISPR-Cas9-mediated disruption of uORF in 5'UTR of mRNA may lead to reduced product translation of mutant huntingtin gene mutations in mesenchymal stem cells (MSCs) derived from HD mouse models (3). Author have shown that this Cas9 nickase strategy can be used to accurately excise CAG repeats from the HTT gene. This abolished huntingtin synthesis in fibroblasts from HD patients (67).

Furthermore, a CRISPR-Cas9 used in HD140 Knock-in mice showed improved motor function and no impact on the lifespan of the mice (68). Interestingly, another study used the same method and showed improved motor function and increased lifespan of R6 mice (69,70) The finding suggests that a CRISPR-Cas9 system will also be useful for such type of correction in humans.

## 6. Conclusion

With the advancement in gene targeting methods, it is being increasingly recognized that the disease-causing gene itself is the best therapeutic target even without the need to have a full understanding of its biological functions. Considering this, the genetic scissor CRISPR-Cas9 offers the promise of permanently silencing or correcting the disease-causing mutations, potentially overcoming key limitations of RNA targeting methods. Compared to other gene editing technologies, it has the advantages of short cycle, low cytotoxicity, lower cost and easy delivery, etc. Therefore, all these characteristics make the CRISPR-Cas9 system equipped with a broader application prospect in the clinical therapy of neurodegenerative disorders like Alzheimer, Huntington and Parkinson Diseases. CRISPR-Cas9 having highest targeting efficiency than other gene editing approaches can target almost any gene depending on its sequence. It has significant potential to correct the undesired associated genetic mutations. This technology has allowed the development of empirical neurodegenerative disease models, therapeutic lines, and diagnostic approaches for better understanding the nervous system, from in vitro to in vivo models. This method could be helpful in developing better understanding of neurological disorders and give insights for future treatments for neurological disorders.

**Funding:** This research received no external funding.

**Data Availability Statement:** Not applicable.

**Conflicts of Interest:** The authors declare no conflict of interest:

## References

1. Deng HX, Hentati A, Tainer JA, et al. Amyotrophic lateral sclerosis and structural defects in Cu,Zn superoxide dismutase. *Science*.1993;261(5124):1047-1051. doi:10.1126/science.8351519
2. Goate A, Chartier-Harlin MC, Mullan M, et al. Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease. *Nature*. 1991;349(6311):704-706. doi:10.1038/349704a0
3. Karimian A, Gorjizadeh N, Alemi F, et al. CRISPR/Cas9 novel therapeutic road for the treatment of neurodegenerative diseases. *Life Sci*. 2020; 259:118165. doi: 10.1016/j.lfs.2020.118165
4. Rosen DR, Siddique T, Patterson D, et al. Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis [published correction appears in *Nature*. 1993 Jul 22;364(6435):362]. *Nature*.1993;362(6415):59-62. doi:10.1038/362059a0
5. Tanzi RE, Vaula G, Romano DM, et al. Assessment of amyloid beta-protein precursor gene mutations in a large set of familial and sporadic Alzheimer disease cases. *Am J Hum Genet*. 1992;51(2):273-282.
6. Staahl BT, Benekareddy M, Coulon-Bainier C, et al. Efficient genome editing in the mouse brain by local delivery of engineered Cas9 ribonucleoprotein complexes. *Nat Biotechnol*.2017;35(5):431434.doi:10.1038/nbt.3806
7. Heidenreich M, Zhang F. Applications of CRISPR-Cas systems in neuroscience. *Nat Rev Neurosci*. 2016;17(1):36-44. doi:10.1038/nrn.2015.2
8. Southwell AL, Skotte NH, Kordasiewicz HB, et al. In vivo evaluation of candidate allele-specific mutant huntingtin gene silencing antisense oligonucleotides. *Mol Ther*. 2014;22(12):2093-2106. doi:10.1038/mt.2014.153
9. Straub C, Granger AJ, Saulnier JL, Sabatini BL. CRISPR/Cas9-mediated gene knock-down in post-mitotic neurons. *PLoS One*. 2014;9(8):e105584. Published 2014 Aug 20. doi:10.1371/journal.pone.0105584
10. Swiech L, Heidenreich M, Banerjee A, et al. In vivo interrogation of gene function in the mammalian brain using CRISPR-Cas9. *Nat Biotechnol*.2015;33(1):102-106. doi:10.1038/nbt.3055
11. Doudna JA, Charpentier E. Genome editing. The new frontier of genome engineering with CRISPR-Cas9. *Science*. 2014;346(6213):1258096. doi:10.1126/science.1258096
12. Hsu PD, Lander ES, Zhang F. Development and applications of CRISPR-Cas9 for genome engineering. *Cell*. 2014;157(6):1262-1278. doi:10.1016/j.cell.2014.05.010

13. Mehrabian M, Brethour D, MacIsaac S, et al. CRISPR-Cas9-based knockout of the prion protein and its effect on the proteome. *PLoS One*. 2014;9(12):e114594. Published 2014 Dec 9. doi:10.1371/journal.pone.0114594
14. Liu CC, Liu CC, Kanekiyo T, Xu H, Bu G. Apolipoprotein E and Alzheimer disease: risk, mechanisms and therapy [published correction appears in *Nat Rev Neurol*. 2013. doi:10.1038/nrneuro.2013.32. Liu, Chia-Chan [corrected to Liu, Chia-Chen]]. *Nat Rev Neurol*. 2013;9(2):106-118. doi:10.1038/nrneuro.2012.263
15. Hutton M, Hardy J. The presenilins and Alzheimer's disease. *Hum Mol Genet*. 1997;6(10):1639-1646. doi:10.1093/hmg/6.10.1639
16. Chow VW, Mattson MP, Wong PC, Gleichmann M. An overview of APP processing enzymes and products. *Neuromolecular Med*. 2010;12(1):1-12. doi:10.1007/s12017-009-8104-z
17. Park H, Oh J, Shim G, et al. In vivo neuronal gene editing via CRISPR-Cas9 amphiphilic nanocomplexes alleviates deficits in mouse models of Alzheimer's disease. *Nat Neurosci*. 2019;22(4):524-528. doi:10.1038/s41593-019-0352-0
18. Ortiz-Virumbrales M, Moreno CL, Kruglikov I, et al. CRISPR/Cas9-Correctable mutation-related molecular and physiological phenotypes in iPSC-derived Alzheimer's PSEN2<sup>N441</sup> neurons. *Acta Neuropathol Commun*. 2017;5(1):77. Published 2017 Oct 27. doi:10.1186/s40478-017-0475-z
19. Song L, Tang S, Dong L, et al. The Neuroprotection of KIBRA in Promoting Neuron Survival and Against Amyloid  $\beta$ -Induced Apoptosis. *Front Cell Neurosci*. 2019;13:137. Published 2019 Apr 12. doi:10.3389/fncel.2019.00137
20. Kantor B, Tagliaferro L, Gu J, et al. Downregulation of SNCA Expression by Targeted Editing of DNA Methylation: A Potential Strategy for Precision Therapy in PD. *Mol Ther*. 2018;26(11):2638-2649. doi:10.1016/j.ymthe.2018.08.019
21. Lee KS, Huh S, Lee S, et al. Altered ER-mitochondria contact impacts mitochondria calcium homeostasis and contributes to neurodegeneration in vivo in disease models [published correction appears in *Proc Natl Acad Sci U S A*. 2018 Oct 16;115(42):E9992]. *Proc Natl Acad Sci U S A*. 2018;115(38):E8844-E8853. doi:10.1073/pnas.1721136115
22. Raikwar SP, Thangavel R, Dubova I, et al. Targeted Gene Editing of Glia Maturation Factor in Microglia: a Novel Alzheimer's Disease Therapeutic Target. *Mol Neurobiol*. 2019;56(1):378-393. doi:10.1007/s12035-018-1068-y
23. Butterfield DA, Boyd-Kimball D. Amyloid beta-peptide(1-42) contributes to the oxidative stress and neurodegeneration found in Alzheimer disease brain. *Brain Pathol*. 2004;14(4):426-432. doi:10.1111/j.1750-3639.2004.tb00087.x
24. Hampel H, Vassar R, De Strooper B, et al. The  $\beta$ -Secretase BACE1 in Alzheimer's Disease. *Biol Psychiatry*. 2021;89(8):745-756. doi:10.1016/j.biopsych.2020.02.001
25. Park H, Oh J, Shim G, et al. In vivo neuronal gene editing via CRISPR-Cas9 amphiphilic nanocomplexes alleviates deficits in mouse models of Alzheimer's disease. *Nat Neurosci*. 2019;22(4):524-528. doi:10.1038/s41593-019-0352-0
26. Mao Y, Zhang H, Xu N, Zhang B, Gou F, Zhu JK. Application of the CRISPR-Cas system for efficient genome engineering in plants. *Mol Plant*. 2013;6(6):2008-2011. doi:10.1093/mp/sst121
27. Kim H, Kim JS. A guide to genome engineering with programmable nucleases. *Nat Rev Genet*. 2014;15(5):321-334. doi:10.1038/nrg3686
28. Obeso JA, Stamelou M, Goetz CG, et al. Past, present, and future of Parkinson's disease: A special essay on the 200th Anniversary of the Shaking Palsy. *Mov Disord*. 2017;32(9):1264-1310. doi:10.1002/mds.27115
29. Troncoso-Escudero P, Sepulveda D, Pérez-Arancibia R, et al. On the Right Track to Treat Movement Disorders: Promising Therapeutic Approaches for Parkinson's and Huntington's Disease. *Front Aging Neurosci*. 2020;12:571185. Published 2020 Sep 3. doi:10.3389/fnagi.2020.571185
30. Jamebozorgi K, Taghizadeh E, Rostami D, et al. Cellular and Molecular Aspects of Parkinson Treatment: Future Therapeutic Perspectives. *Mol Neurobiol*. 2019;56(7):4799-4811. doi:10.1007/s12035-018-1419-8
31. Kovacs GG. Concepts and classification of neurodegenerative diseases. *Handb Clin Neurol*. 2017;145:301-307. doi:10.1016/B978-0-12-802395-2.00021-3
32. Houston F, Andréoletti O. Animal prion diseases: the risks to human health. *Brain Pathol*. 2019;29(2):248-262. doi:10.1111/bpa.12696

33. Ball N, Teo WP, Chandra S, Chapman J. Parkinson's Disease and the Environment. *Front Neurol*. 2019;10:218. Published 2019 Mar 19. doi:10.3389/fneur.2019.00218
34. Caligiore D, Helmich RC, Hallett M, et al. Parkinson's disease as a system-level disorder. *NPJ Parkinsons Dis*. 2016;2:16025. Published 2016 Dec 1. doi:10.1038/npjparkd.2016.25
35. Kalia LV, Lang AE. Parkinson's disease. *Lancet*. 2015;386(9996):896-912. doi:10.1016/S0140-6736(14)61393-3
36. Rahman MU, Bilal M, Shah JA, Kaushik A, Teissedre PL, Kujawska M. CRISPR-Cas9-Based Technology and Its Relevance to Gene Editing in Parkinson's Disease. *Pharmaceutics*. 2022;14(6):1252. Published 2022 Jun 13. doi:10.3390/pharmaceutics14061252
37. Kolli N, Lu M, Maiti P, Rossignol J, Dunbar GL. Application of the gene editing tool, CRISPR-Cas9, for treating neurodegenerative diseases. *Neurochem Int*. 2018;112:187-196. doi:10.1016/j.neuint.2017.07.007
38. Safari F, Hatam G, Behbahani AB, et al. CRISPR System: A High-throughput Toolbox for Research and Treatment of Parkinson's Disease. *Cell Mol Neurobiol*. 2020;40(4):477-493. doi:10.1007/s10571-019-00761-w
39. Swarup V, Kumar V, Faruq M, Singh HN, Singh I, Srivastava AK. CRISPR/ Cas9 technology in neurological disorders: An update for clinicians. 2020; *Annals of Movement Disorders*. 3. 23. 10.4103/AOMD.AOMD\_39\_19.
40. Lindholm D, Mäkelä J, Di Liberto V, et al. Current disease modifying approaches to treat Parkinson's disease. *Cell Mol Life Sci*. 2016;73(7):1365-1379. doi:10.1007/s00018-015-2101-1
41. Axelsen TM, Woldbye DPD. Gene Therapy for Parkinson's Disease, An Update. *J Parkinsons Dis*. 2018;8(2):195-215. doi:10.3233/JPD-181331
42. Arango D, Bittar A, Esmeral NP, et al. Understanding the Potential of Genome Editing in Parkinson's Disease. *Int J Mol Sci*. 2021;22(17):9241. Published 2021 Aug 26. doi:10.3390/ijms22179241
43. Adli M. The CRISPR tool kit for genome editing and beyond. *Nat Commun*. 2018;9(1):1911. Published 2018 May 15. doi:10.1038/s41467-018-04252-2
44. Sander JD, Joung JK. CRISPR-Cas systems for editing, regulating and targeting genomes. *Nat Biotechnol*. 2014;32(4):347-355. doi:10.1038/nbt.2842
45. Heman-Ackah SM, Bassett AR, Wood MJ. Precision Modulation of Neurodegenerative Disease-Related Gene Expression in Human iPSC-Derived Neurons. *Sci Rep*. 2016;6:28420. Published 2016 Jun 24. doi:10.1038/srep28420
46. Guan L, Han Y, Yang C, et al. CRISPR-Cas9-Mediated Gene Therapy in Neurological Disorders. *Mol Neurobiol*. 2022;59(2):968-982. doi:10.1007/s12035-021-02638-w
47. Barodia SK, Creed RB, Goldberg MS. Parkin and PINK1 functions in oxidative stress and neurodegeneration. *Brain Res Bull*. 2017;133:51-59. doi:10.1016/j.brainresbull.2016.12.004
48. Liao Y, Dong Y, Cheng J. The Function of the Mitochondrial Calcium Uniporter in Neurodegenerative Disorders. *Int J Mol Sci*. 2017;18(2):248. Published 2017 Feb 10. doi:10.3390/ijms18020248
49. Lee J, Bayarsaikhan D, Arivazhagan R, et al. CRISPR/Cas9 Edited sRAGE-MSCs Protect Neuronal Death in Parkinson's Disease Model. *Int J Stem Cells*. 2019;12(1):114-124. doi:10.15283/ijsc18110
50. Soman SK, Bazała M, Keatinge M, Bandmann O, Kuznicki J. Restriction of mitochondrial calcium overload by *mcu* inactivation renders a neuroprotective effect in zebrafish models of Parkinson's disease. *Biol Open*. 2019;8(10):bio044347. Published 2019 Oct 15. doi:10.1242/bio.044347
51. Cui J, Rothstein M, Bennett T, Zhang P, Xia N, Reijo Pera RA. Quantification of dopaminergic neuron differentiation and neurotoxicity via a genetic reporter. *Sci Rep*. 2016;6:25181. Published 2016 Apr 28. doi:10.1038/srep25181
52. Qing X, Walter J, Jarazo J, Arias-Fuenzalida J, Hillje AL, Schwamborn JC. CRISPR/Cas9 and piggyBac-mediated footprint-free LRRK2-G2019S knock-in reveals neuronal complexity phenotypes and  $\alpha$ -Synuclein modulation in dopaminergic neurons. *Stem Cell Res*. 2017;24:44-50. doi:10.1016/j.scr.2017.08.013
53. Tabrizi SJ, Ghosh R, Leavitt BR. Huntingtin Lowering Strategies for Disease Modification in Huntington's Disease. *Neuron*. 2019;102(4):899. doi:10.1016/j.neuron.2019.05.001
54. Bates G, Harper P, Jones L. Huntington's Disease. Oxford: Oxford University Press; 2002:3.

55. Roos RA. Huntington's disease: a clinical review. *Orphanet J Rare Dis*. 2010;5:40. Published 2010 Dec 20. doi:10.1186/1750-1172-5-40
56. Saudou F, Humbert S. The Biology of Huntingtin. *Neuron*. 2016;89(5):910-926. doi:10.1016/j.neuron.2016.02.003
57. Bates GP, Dorsey R, Gusella JF, et al. Huntington disease. *Nat Rev Dis Primers*. 2015;1:15005. Published 2015 Apr 23. doi:10.1038/nrdp.2015.5
58. Nance MA. Genetics of Huntington disease. *Handb Clin Neurol*. 2017;144:3-14. doi:10.1016/B978-0-12-801893-4.00001-8
59. Wild EJ, Tabrizi SJ. Therapies targeting DNA and RNA in Huntington's disease [published correction appears in *Lancet Neurol*. 2017 Dec;16(12 ):954]. *Lancet Neurol*. 2017;16(10):837-847. doi:10.1016/S1474-4422(17)30280-6
60. Shin JW, Lee JM. The prospects of CRISPR-based genome engineering in the treatment of neurodegenerative disorders. *Ther Adv Neurol Disord*. 2017;11:1756285617741837. Published 2017 Nov 15. doi:10.1177/1756285617741837
61. Rohn TT, Kim N, Isho NF, Mack JM. The Potential of CRISPR/Cas9 Gene Editing as a Treatment Strategy for Alzheimer's Disease. *J Alzheimers Dis Parkinsonism*. 2018;8(3):439. doi:10.4172/2161-0460.1000439
62. Dabrowska M, Olejniczak M. Gene Therapy for Huntington's Disease Using Targeted Endonucleases. *Methods Mol Biol*. 2020;2056:269-284. doi:10.1007/978-1-4939-9784-8\_17
63. Monteys AM, Ebanks SA, Keiser MS, Davidson BL. CRISPR/Cas9 Editing of the Mutant Huntingtin Allele In Vitro and In Vivo. *Mol Ther*. 2017;25(1):12-23. doi:10.1016/j.ymthe.2016.11.010
64. Vachey G, Déglon N. CRISPR/Cas9-Mediated Genome Editing for Huntington's Disease. *Methods Mol Biol*. 2018;1780:463-481. doi:10.1007/978-1-4939-7825-0\_21
65. Shin JW, Kim KH, Chao MJ, et al. Permanent inactivation of Huntington's disease mutation by personalized allele-specific CRISPR/Cas9. *Hum Mol Genet*. 2016;25(20):4566-4576. doi:10.1093/hmg/ddw286
66. Harper SQ, Staber PD, He X, et al. RNA interference improves motor and neuropathological abnormalities in a Huntington's disease mouse model. *Proc Natl Acad Sci U S A*. 2005;102(16):5820-5825. doi:10.1073/pnas.0501507102
67. Dabrowska M, Juzwa W, Krzyzosiak WJ, Olejniczak M. Precise Excision of the CAG Tract from the Huntingtin Gene by Cas9 Nickases. *Front Neurosci*. 2018;12:75. Published 2018 Feb 26. doi:10.3389/fnins.2018.00075
68. Yang S, Chang R, Yang H, et al. CRISPR/Cas9-mediated gene editing ameliorates neurotoxicity in mouse model of Huntington's disease. *J Clin Invest*. 2017;127(7):2719-2724. doi:10.1172/JCI92087
69. Ekman FK, Ojala DS, Adil MM, Lopez PA, Schaffer DV, Gaj T. CRISPR-Cas9-Mediated Genome Editing Increases Lifespan and Improves Motor Deficits in a Huntington's Disease Mouse Model. *Mol Ther Nucleic Acids*. 2019;17:829-839. doi:10.1016/j.omtn.2019.07.009
70. Mani I. CRISPR-Cas9 for treating hereditary diseases. *Prog Mol Biol Transl Sci*. 2021;181:165-183. doi:10.1016/bs.pmbts.2021.01.017



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