**Utilising CRISPR/Cas 9 for Gene Editing to Address Hereditary Movement Disorder**

Isha Upadhyay

Department of Zoology,

University of Lucknow

Email: [ishaupadhyay17@gmail.com](mailto:ishaupadhyay17@gmail.com)

Ankita Singh

Department of Zoology,

University of Lucknow

Email: [kmrvishwajit9@gmail.com](mailto:kmrvishwajit9@gmail.com)

**Abstract**

Gene therapy offers a promising approach for addressing hereditary movement disorders like Ataxia, Dystonia, Huntington's disease, and Parkinson's disease. A method known as genome editing, which involves the modification of DNA by inserting, deleting, or replacing specific sequences, has gained much attention in the past few years. A prominent tool in this field is the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and CRISPR-associated protein 9 (CRISPR/Cas9) system. Cas9 is obtained from the bacteria, *Campylobacter jejuni*. CRISPR-Cas was originally a part of the *Streptococcus pyogenes* adaptive immune system, but CRISPR/Cas9 now serves as a valuable tool for genome editing, causing breaks in the DNA strands under the guidance of RNA (gRNA). In contrast to alternative genome editing techniques like zinc-finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs), CRISPR/Cas9 stands out for its potential clinical applicability due to its convenient in vivo delivery. It is highly specific and cuts only a limited number of sites in the genome. This article explores and assesses the viability of CRISPR/Cas9 in the preclinical research and its potential use in gene therapy for hereditary movement disorders.

Keywords- CRISPR-Cas9, gene therapy, gene editing, hereditary movement disorders

1. **Introduction**

The Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-Cas (CRISPR-associated proteins) technology has emerged as a groundbreaking gene-editing tool over the past decade, enabling scientists to precisely and efficiently alter the DNA sequences. This article delves into the multifaceted aspects of CRISPR-Cas9 technique that have revolutionised the fields of genetics, biotechnology, and medicine.

CRISPR-Cas9 was initially identified as a peculiar DNA sequence in bacteria, but, when paired with CRISPR-Cas proteins, this system forms a natural defence mechanism used by eubacteria and archaea for protection against invading viruses and plasmid. The Cas9 protein, in particular, acts as molecular "scissors," which is an RNA-guided DNA endonuclease enzyme and is now being utilised as a genome editing tool to induce double-strand breaks (DSB) in DNA. This system is crucial for acquired immunity necessary to defend against invading viruses and plasmids (P Horvath 2010). In 1987, researchers discovered CRISPR in the genome of *Escherichia coli*. They identified it as a sequence of 29 nucleotides that repeated several times, interspaced with variable sequence fragments of 32 (Y Ishino et al. 1987).

Genome editing, a form of genetic engineering, involves the precise modification of DNA through the insertion, deletion or replacement in the genome using nucleases which enable precise modification of genes by introducing DSBs at the target location in the genome. Nucleases that can be used in genomic editing include zinc-finger nuclease (ZFNs) and transcription activator-like effector nuclease (TALENs) which create site-specific DSBs at the target locations. One particularly significant tool in genome editing for this purpose is the CRISPR-Cas9, which is an RNA-guided engineered nuclease (RGEN) system, which has synthetic guide RNA (gRNA) that introduces a DSB at a specific location in the target genome (Barrangou et al. 2007; Garneau 2011). CRISPR-Cas is associated with the adaptive immune system of *Streptococcus pyogenes*. Notably, it offers distinct advantages in clinical applicability compared to other editing technologies.

In medicine, CRISPR-Cas9 holds immense promise for treating genetic disorders. By editing disease-causing mutations, researchers aim to correct the genetic defects at the root level. This technology has the potential to pave the way for ground-breaking therapies, offering hope to patients suffering from previously incurable genetic conditions.

     II.          **Mechanism of action of CRISPR-Cas9 system in gene editing**

The mechanism of action of the CRISPR-Cas9 system, which is classified as RGEN and recognises a specific target sequence of 23 base pairs which is distinct from that of ZFNs and TALENs (T. Gaj et al. 2013; E. Deltcheva et al. 2011). Cas9 is used as a nuclease by CRISPR-Cas9, which recognises genomic DNA using gRNA. (PJ Mali et al. 2013; MJ Moscou et al. 2009). The gRNA recognizes approximately 20 base pair nucleotide and requires protospacer adjacent motif (PAM), which recruits Cas9, where Cas9 is guided by a specific sequence of gRNA that is related to trans-activating crRNA (tracrRNA) and forms the complementary DNA (cDNA) target sequence, resulting in site-specific DSB (JE Garneau 2010; M Jinek 2012).

Due to CRISPR-Cas9 ability to simultaneously disrupt several genes, it can be used to research genetic interaction and create models for multigenic disorders. By regulating the short specific part of gRNA, the Cas protein can target particular DNA sequences. One significant issue with Cas is the occurrence of off-target effects, which involve the non-specific recognition and digestion of non-targeted DNA sequences. Cas protein can be dual-guide RNAs or single-guide RNAs. For CRISPR-Cas9 applications to human disease to be successful, the technique for preventing off-target effects needs to be further researched.

   III.          **CRISPR-Cas9 as a therapeutic intervention for hereditary movement disorder**

The pathophysiology of hereditary movement disorders has been associated with a multitude of genes, rendering them viable candidates for the utilisation of the CRISPR-Cas9 system in the development of therapeutic strategies aimed at modifying the course of the diseases (Wooseok et al. 2016). Hereditary movement disorders encompass conditions such as ataxia, dystonia, chorea, spastic paraparesis, Huntington's disease, and Parkinson's disease, among others, all of which manifest due to heightened occurrences of genetic mutations.

Trinucleotide repeat disorders, exemplified by Huntington's disease, are characterised by an elongated polyglutamine region that surpasses a defined threshold. This expansion triggers protein misfolding and aggregate formation, ultimately exerting profound impacts on neuronal cellular and molecular processes [A Riner et al. 2011]. The prospect of rectifying the anomalous CAG repeat sequence within normal cells or amending mutations within patient-derived cells holds promise through tailored nucleases, as demonstrated in modified nuclease studies (PQ Tan et al. 2005; K Freude et al. 2014). Conversely, in the context of Parkinson's disease (PD), the pathological underpinning frequently involves the presence of misfolded protein entities termed Lewy bodies. The central constituent of these aggregates, α-synuclein, significantly contributes to PD pathology. A principal genetic contributor to PD is the Synuclein Alpha (SCNA) gene, responsible for encoding α-synuclein. A prevalent genetic anomaly in PD is a mutation within the leucine-rich repeat kinase 2 (LRRK2) gene, culminating in dopaminergic neuron toxicity. By leveraging the precision of CRISPR/Cas9 technology, it becomes conceivable to rectify the LRRK2 mutation, potentially mitigating the incidence of PD within afflicted families. Likewise, spinocerebellar ataxia types 1, 2, 3, 6, 7, and 17, characterised by autosomal dominant inheritance, manifest as trinucleotide repeat disorders in which an abnormal protein with an extended polyglutamine tract accumulates, precipitating cerebellar ataxia. This shared pathogenic mechanism underscores the role of aberrant protein accumulation in neurodegenerative processes. It is noteworthy that while multifactorial etiological factors are implicated in Parkinson's disease, Alzheimer's disease, and amyotrophic lateral sclerosis, an analogous manifestation of abnormal misfolded proteins is evident.

CRISPR-Cas9 can be used to modify abnormal protein production, and accumulation appears to be effective in their diseases. Some hereditary movement disorder occurs in autosomal recessive patterns caused by the loss-of-function mutation of a particular gene. Genes responsible for hereditary movement disorder can be knocked in a specific transgene by CRISPR-Cas9. Some hereditary movement disorder occurs in autosomal recessive patterns caused by the loss-of-function mutation of a particular gene. Genes responsible for hereditary movement disorder can be knocked in a specific transgene by CRISPR-Cas9.

   IV.          **Futuristic trends of CRISPR-Cas9**

The emergence of CRISPR-Cas-mediated genome editing technologies has ushered in a transformative and versatile avenue for the manipulation, control, and observation of genetic material. These technologies are regarded as a significant landmark in 21st-century molecular biology. Thus far, CRISPR-Cas systems have found wide-ranging utility in dissecting gene functions, advancing human genetic therapies, precision-oriented drug creation, establishing animal models, and enhancing livestock breeding. These applications substantiate their immense potential for further advancement.

The evolution of the CRISPR system into a gene-editing tool has sparked a revolutionary shift in the life sciences. The advent of next-generation gene editing technologies has further enhanced the versatility of the CRISPR system, offering researchers potent and innovative tools to explore biological systems and study human diseases. CRISPR technologies hold significant promise as potential treatments for genetic diseases and genetic disorders in humans. Base editing screening has emerged as a valuable method to investigate the connections between gene mutations and their effects. Its remarkable ability to knock out specific genes without causing extensive chromosomal rearrangements by introducing a premature stop codon or disrupting the splice site. CRISPR-Cas9 holds the potential for targeted manipulation of aberrant protein synthesis and accumulation, demonstrating efficacy in mitigating the underlying pathology of associated disorders. Certain hereditary movement disorders manifest through autosomal recessive inheritance patterns, attributed to loss-of-function mutations within specific genes. The genetic loci accountable for these hereditary movement disorders can be precisely integrated into a designated transgene via CRISPR-Cas9 technology, offering a mechanism for functional restoration.

The meaning of gene therapy has been vastly changed by the Gene editing technology.  However, there is still lots of fundamental and translational work to be done to understand the full potential of CRISPR-Cas9, particularly in the area of genetic diseases and genetic disorders. In recent years, it has been seen that an increasing number of CRISPR related clinical trials and promises are being proposed worldwide. CRISPR-Cas9 has been widely used for creating related cellular or animal models of human diseases; developing the CRISPR-Cas9 technique as a therapeutic tool for gene editing can pave the way for the treatment of human disorders. It can be postulated that the CRISPR-Cas9 system could correct or repair mutations caused by both monogenic recessive and dominant-negative disorders to reach therapeutic advantages.

Recent findings indicate that the CRISPR-Cas system exhibits a higher efficiency in-vitro compared to in vivo. This intriguing disparity suggests a promising avenue for expediting medical research through the strategic utilisation of genetically modified cell models. Such an approach has the potential to significantly reduce the time required for research endeavours. To date, researchers have effectively harnessed CRISPR-Cas systems to carry out precise genetic modifications across diverse cell lines. These cell types encompass a range from tumour cells to mature adult cells, as well as versatile stem cells. This versatile application allows for the emulation of a diverse array of human diseases, thereby facilitating a deeper understanding of their underlying mechanisms. Furthermore, this technique paves the way for the exploration of innovative therapeutic approaches. Nonetheless, practical utilisation of CRISPR-Cas systems presents extant challenges that necessitate resolution. Vigorous endeavours are imperative to ascertain enduring safety and efficacy, mandating meticulous evaluation and ongoing study.

**Reference**

1. Horvath, P., & Barrangou, R. (2010). CRISPR/Cas, the immune system of bacteria and archaea. *Science (New York, N.Y.)*, *327*(5962), 167–170. https://doi.org/10.1126/science.1179555
2. Ishino, Y., Shinagawa, H., Makino, K., Amemura, M., & Nakata, A. (1987). Nucleotide sequence of the iap gene, responsible for alkaline phosphatase isozyme conversion in Escherichia coli, and identification of the gene product. *Journal of bacteriology*, *169*(12), 5429–5433.<https://doi.org/10.1128/jb.169.12.5429-5433.1987>
3. Im, W., Moon, J., & Kim, M. (2016). Applications of CRISPR/Cas9 for Gene Editing in Hereditary Movement Disorders. *Journal of movement disorders*, *9*(3), 136–143.<https://doi.org/10.14802/jmd.16029>
4. Rodríguez-Rodríguez, D. R., Ramírez-Solís, R., Garza-Elizondo, M. A., Garza-Rodríguez, M. L., & Barrera-Saldaña, H. A. (2019). Genome editing: A perspective on the application of CRISPR/Cas9 to study human diseases (Review). *International journal of molecular medicine*, *43*(4), 1559–1574. https://doi.org/10.3892/ijmm.2019.4112
5. 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.
6. 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.
7. Gaj, T., Gersbach, C. A., & Barbas, C. F., 3rd (2013). ZFN, TALEN, and CRISPR/Cas-based methods for genome engineering. *Trends in biotechnology*, *31*(7), 397–405.<https://doi.org/10.1016/j.tibtech.2013.04.004>
8. Deltcheva, E., Chylinski, K., Sharma, C. M., Gonzales, K., Chao, Y., Pirzada, Z. A., Eckert, M. R., Vogel, J., & Charpentier, E. (2011). CRISPR RNA maturation by trans-encoded small RNA and host factor RNase III. *Nature*, *471*(7340), 602–607.<https://doi.org/10.1038/nature09886>
9. Mali, P., Yang, L., Esvelt, K. M., Aach, J., Guell, M., DiCarlo, J. E., Norville, J. E., & Church, G. M. (2013). RNA-guided human genome engineering via Cas9. *Science (New York, N.Y.)*, *339*(6121), 823–826.<https://doi.org/10.1126/science.1232033>
10. Moscou, M. J., & Bogdanove, A. J. (2009). A simple cipher governs DNA recognition by TAL effectors. *Science (New York, N.Y.)*, *326*(5959), 1501. https://doi.org/10.1126/science.1178817
11. He, X., Tan, C., Wang, F., Wang, Y., Zhou, R., Cui, D., You, W., Zhao, H., Ren, J., & Feng, B. (2016). Knock-in of large reporter genes in human cells via CRISPR/Cas9-induced homology-dependent and independent DNA repair. *Nucleic acids research*, *44*(9), e85.<https://doi.org/10.1093/nar/gkw064>
12. Shao M, Xu T, Chen C. The big bang of genome editing technology: development and application of the CRISPR/CAS9 system in disease animal models. *Sci Press Zool Res*. 2016;37(2):1–11.
13. Ceasar SA, Rajan V, Prykhozhij SV, Berman JN, Ignacimuthu S. Insert, remove or replace: a highly advanced genome editing system using CRISPR/Cas9. *Biochim Biophys Acta Mol Cell*. 2016;1863(9):2333–2344. doi:10.1016/j.bbamcr.2016.06.009
14. Mei Y, Wang Y, Chen H, Sun ZS, Da JX. Recent progress in CRISPR/Cas9 technology. *J Genet Genomics*. 2016;43(2):63–75. doi:10.1016/j.jgg.2016.01.001
15. Jiang F, Doudna JA. CRISPR-Cas9 structures and mechanisms. *Annu Rev Biophys*. 2017;46(1):505–529. doi:10.1146/annurev-biophys-062215-010822
16. Misganaw Asmamaw & Belay Zawdie (2021) Mechanism and Applications of CRISPR/Cas-9-Mediated Genome Editing, Biologics: Targets and Therapy, 15:, 353-361, DOI: [10.2147/BTT.S326422](https://doi.org/10.2147/BTT.S326422)
17. Pandey, V. K., Tripathi, A., Bhushan, R., Ali, A., & Dubey, P. K. (2017). Application of CRISPR/Cas9 Genome Editing in Genetic Disorders: A Systematic Review Up to Date. In Journal of Genetic Syndromes &amp; Gene Therapy (Vol. 08, Issue 02). OMICS Publishing Group. https://doi.org/10.4172/2157-7412.1000321
18. Karimian, A., Gorjizadeh, N., Alemi, F., Asemi, Z., Azizian, K., Soleimanpour, J., Malakouti, F., Targhazeh, N., Majidinia, M., & Yousefi, B. (2020). CRISPR/Cas9 novel therapeutic road for the treatment of neurodegenerative diseases. In Life Sciences (Vol. 259, p. 118165). Elsevier BV. https://doi.org/10.1016/j.lfs.2020.118165
19. Mani, I. (2021). CRISPR-Cas9 for treating hereditary diseases. In Progress in Molecular Biology and Translational Science (pp. 165–183). Elsevier. https://doi.org/10.1016/bs.pmbts.2021.01.01
20. Liu P-Q, Tan S, Mendel MC, Murrills RJ, Bhat BM, et al. (2005) Isogenic human cell lines for drug discovery: Regulation of target gene expression by engineered zincänger protein transcription factors. J Biomol Screen 10: 304-313.
21. Freude K, Pires C, Hyttel P, Hall V (2014) Induced pluripotent stem cells derived from Alzheimer’s disease patients: Нe promise, the hope and the path ahead. J Clin Med 3: 1402-1436.
22. Xiong, X., Chen, M., Lim, W. A., Zhao, D., & Qi, L. S. (2016). CRISPR/Cas9 for Human Genome Engineering and Disease Research. *Annual review of genomics and human genetics*, *17*, 131–154. <https://doi.org/10.1146/annurev-genom-083115-022258>
23. Zarei, A., Razban, V., Hosseini, S. E., & Tabei, S. M. B. (2019). Creating cell and animal models of human disease by genome editing using CRISPR/Cas9. *The journal of gene medicine*, *21*(4), e3082. <https://doi.org/10.1002/jgm.3082>
24. Ding, Q., Regan, S. N., Xia, Y., Oostrom, L. A., Cowan, C. A., & Musunuru, K. (2013). Enhanced efficiency of human pluripotent stem cell genome editing through replacing TALENs with CRISPRs. *Cell stem cell*, *12*(4), 393–394. <https://doi.org/10.1016/j.stem.2013.03.006>
25. Xu, Y., & Li, Z. (2020). CRISPR-Cas systems: Overview, innovations and applications in human disease research and gene therapy. *Computational and structural biotechnology journal*, *18*, 2401–2415.