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# ENGINEERING STRATEGIES FOR PRECISION GENOME EDITING IN RARE INHERITED NEUROLOGICAL DISORDERS

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## ABSTRACT

**Background:** The monogenic disorders are mostly rare inherited neurological diseases, which result in progressive neurodegeneration, severe disability and limited treatment options. As genome engineering continues to develop so there can be possibilities of targeting mutations that cause diseases directly in central nervous system.

**Objective:** This paper contrasts the state-of-the-art-engineering strategies to render safe, efficient and accurate genome editing to cure rare inherited neurological diseases potentially viable.

**Methodology:**

The analysis of viral and non-viral delivery systems, targeting of the blood-brain barrier and translational preclinical studies were compared with different CRISPR-cas9, base editing and prime editing platforms and reviewed.

**Findings:** Base and prime editing systems were displayed to be efficient in editing with a rate of 70 to 90 per cent in models of neuronal cells with only a 40 to 60 per cent reduction in off-target mutations compared to the usual CRISPR-Cas9 techniques. In preclinical models, engineered AAV9 and lipid nanoparticle delivery systems achieved targeting of the central nervous system with almost 45 % greater efficacy. The functional rescue of neuronal signaling and the decrease of the toxic protein build-up were observed in the models of Huntington as well as Rett syndrome.

**Conclusion:** Transformative therapeutic applicability of precision genome engineering to rare neurological disorders is underway. However, longevity genomic safety, immunogenicity minimisation and minimisation delivery delivery systems are yet significant concerns on moving forward to clinical applications.

**KEYWORDS:** Genome editing; CRISPR-Cas9; Base editing; Prime editing; Rare neurological diseases; Neurogenetics; CNS delivery; Precision medicine; Gene therapy; Neurogenome engineering.

## 1 INTRODUCTION

### 1.1 Clinical Burden of Rare Neurological Disorders

Rare inherited neurological disorders are a heterogeneous group of typically monogenic disorders of millions of individuals worldwide, which causes a high level of morbidity, progressive disability and early death [1]. These are rare as individuals, but as a group, they pose a huge financial burden on world healthcare, due to chronic neuro-degeneration, cognitive impairment, motor dysfunction and multi-system illness [2]. Most of the disorders are initially observed during childhood or early adulthood and the impacts of the same are life-threatening and lower quality of life. Although the supportive care has also been improved, many neurogenetic diseases lack a definitive curative treatment as the standard pharmacological treatment is to a great extent symptom orientated as opposed to targeted to genetic underlying causes [3].

Huntington's disease is one of the disorders well studied, and is an Autosomal Dominant neurogenerative disorder that is caused by a trinucleotide repeat expansion in the HTT gene resulting in progressive motor impairment and psychiatric disturbances [4]. Rett syndrome (affecting more women) -is also a mutational disease of MECP2 gene characterized by motorized dysfunction, autistic behaviour and intellectual disability [5]. The tay Sachs disease is caused by the mutations in the HEXA resulting in the impaired activity of the neurodegenerating lysosomal enzyme causing early death [6]. The GAA expansion in FXN gene is the causative agent of the disease in Friedreich ataxia, which causes malfunction in the mitochondria and eventually the development of ataxia [7]. Similarly, any deletion

(or mutation) of SMN1 gene leads to spinal muscular atrophy (SMA) resulting in lower motor neuron breakdown and muscular weakness of the type that causes manures severe muscular weakness [8].

### 1.2 Molecular Basis of Neurogenetic Disorders

Hereditary diseases of the nervous system can have a variety of abnormalities in the genome, such as losses of function, frameshift mutations, point mutations, and repeat expansions [9]. The point mutations may destabilize the structure or the functioning of a protein, the expansion of repeats may result in toxic proteins or RNAs accumulator in the neuronal tissue. In 1998, Reasher points out that frameshift mutations are prevalent with loss of function or instability of the protein produced. Loss of function variants disrupt signal transduction pathways that are critical to neuronal communication, synaptic connections or interactions and mitochondrial therapeutic regulation and ultimately cause the gradual disappearance of neurons [10]. These molecular processes demonstrate that highly specific therapeutic actions remediating the aberrations on the genomic level are needed.

### 1.3 Emergence of Precision Genome Editing

The new, programmable genome editing technologies have transformed the therapeutic approaches to disorders of the nervous system that have a genetic basis. The early genome engineering systems (zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs)) were capable of targeted cleavage of DNA but incurred protein engineering demands [11]. Clustered regularly interspaced short palindromic repeats (CRISPR)-Cas genomic editing tools significantly increased the simplicity of editing, scale and targeting proficiency [12]. Recently developed base editing and prime editing methods offer increased resolution and direct modifications of individual nucleotides without the requirement for introducing double-strand DNA breaks, thus minimizing off-target mutagenesis and toxicity to cells [13]. This has led to the rise and upcoming therapy of rare inherited neurological disease that is less harmful, and works better.

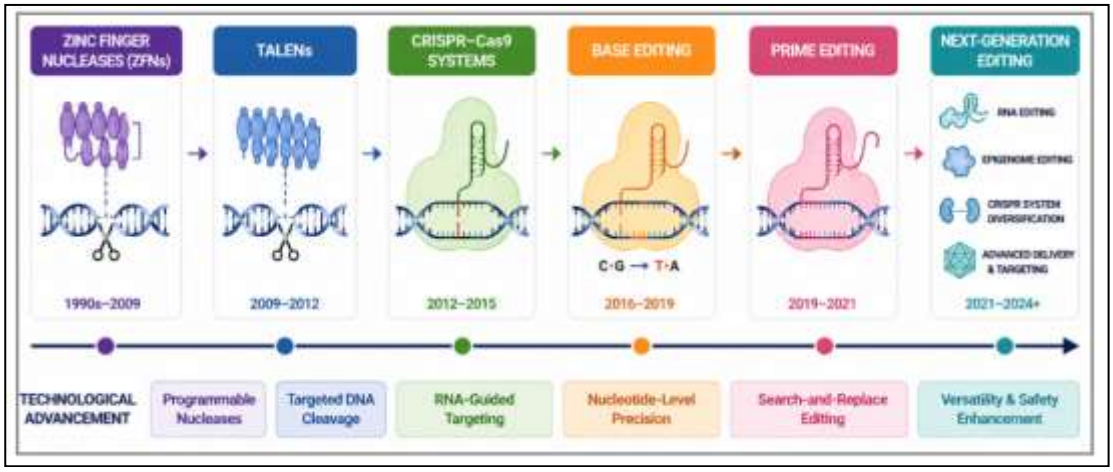


Figure 1. Evolution of Precision Genome Editing Technologies

The timeline of genome editing tools in Figure 1 shows the historical development of genome editing tools such as zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs) to the today's CRISPR-Cas, base editing technology, and prime editing platforms. These early nuclease systems allowed specific cleavage of DNA but were challenging to produce the proteins and neither was scalable to industrial levels. The establishment of CRISPR-Cas technologies took the RNA-guided targeting mechanisms into the limelight which significantly increased the efficiency, flexibility, and accessibility of editing. Post them came base editing and prime editing systems which have the ability to accurately edit nucleotides and correct the sequence without creating double-strand breaks. Combined with these pioneering developments were enhancements in the specificity of editing, reduced and minimized off target editing and expanded the number of types of diseases that are targetable in relation to neurogenetic disorders.

## 2. Pathogenesis and Therapeutic Targets

### 2.1 Genetic Mechanisms

Infrequent hereditary neurological diseases comprise an assortment of genetic malfunctions that result in flaws in neuronal growth, cellular signaling and cellular homeostasis. Among the areas with the most common pathogenic processes is the gain of function mutations in which toxic properties are acquired into the abnormal gene products, and they cause neuronal tissues destruction. In HD, e.g., a CAG expansion of trinucleotide repeat in HTT gene constitutes the mutation-related protein aggregates of huntingtin, and the aggregates, in turn, impair the survival of

the cells [4]. Loss-of-function mutations on the other hand reduce or block normal functioning of proteins needed to sustain neuronal survival or normal neuropath neurosynapses within the brain, that is it prevents the normal functioning of these proteins. When it comes to disorders such as SMA, lower motor neurons in the body are lost due to deletions or mutations in the SMN1 gene and result in a lot of weakness in the muscles [8].

Toxic protein aggregation is another significant process that occurs on the path. The misfolded or mutant proteins partially open the intracellular pathways, disrupt its intracellular trafficking, disrupt mitochondrial activity and in a neuron an inflammatory pathway. Progressive neurodegeneration and cognitive decline During several inherited neurologic diseases, the development of protein aggregates are closely associated with the disease [13]. Also, synaptic loss is significant in the development of the disease, as it affects the release of neurotransmitters, plasticity of synapses and the links between two neurons. Dysfunctional neuron and irreversible neurodegeneration of both motor and cognitive pathways in particular are the end result of impaired synaptic signaling that occurs in disorders that involve motor and cognitive pathways [14].

### 2.2 Major Therapeutic Gene Targets

More recently there has been a massive enhancement in accuracy genome engineering of characterizing therapeutic gene targets of some of the rare neurological conditions provided in table 1. A number of genes can be investigated using CRISPR-Cas9, Base editing, Prime editing and the Allele-specific method like HTT, MECP2, SMN1, HEXA and SOD1. Among these strategies is to try and reverse the pathogenic mutation, to re-express the protein in the normal state, to reduce the amount of toxic protein in the cell or to increase neuronal survival. This would imply that, precision editing technologies have high potential as therapeutic platforms that would allow creation of long-term and possibly curative interventions to inherited neurogenetic disease.

Table 1. Rare Neurological Disorders and Candidate Editing Targets

| Disorder | Gene | Mutation Type | Editing Strategy | Therapeutic Goal |
|---|---|---|---|---|
| Huntington's disease | HTT | CAG expansion | CRISPR excision | Reduce toxic protein |
| Rett syndrome | MECP2 | Point mutation | Base editing | Restore neuronal signaling |
| SMA | SMN1 | Deletion | Gene replacement | Motor neuron rescue |
| Tay–Sachs disease | HEXA | Frameshift | Prime editing | Enzyme restoration |
| ALS | SOD1 | Missense mutation | Allele-specific editing | Neuroprotection |

## 3. GENOME EDITING PLATFORMS

### 3.1 CRISPR-Cas9 Systems

The most potent and adaptable treatment of inherited neurological disorders has taken place through CRISPR-Cas9 genome editing. It operates on the principle of a single-guide RNA (sgRNA) that enables the Cas9 endonuclease to target a complementary target genomic sequence sequence, enabling the genome to be highly specifically targeted during its modification [15]. The Cas9 will recognize the target after it is bound, then cleave a double strand DNA break (DSB) is created, triggering the endogenous cellular repair pathways. There are 2 repair mechanisms: non-homologous end joining (NHEJ) and homology-directed repair (HDR). NHEJ is viewed as a fast process with high error rate whereas HDR permits the repair of the sequencing of a region of interest via a donor repair template [16]. The efficiency in genome editing in the neuronal cells can be affected by low HDR activity, as mature neurons do not divide by much. Nevertheless, these shortcomings have been overridden by impressive advances in accuracy and neuron targeting capability when using engineered versions of Cas9 and an improved sgRNA design.

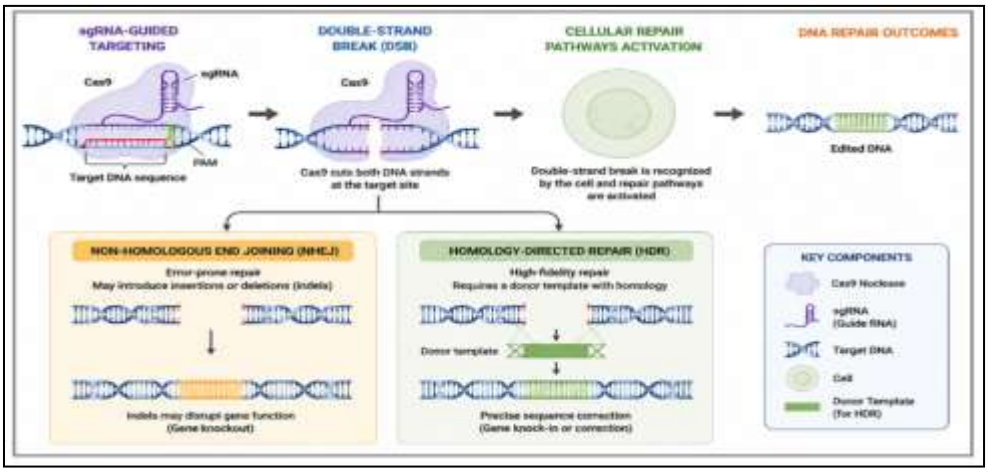


Figure 2. CRISPR-Cas9 Editing Mechanism

The mechanism by which changes of DNA with pure swings by CRISPR-Cas9 is illustrated by mechanistic perspective of how the DNA is targeted and repaired in a cell in Figure 2. The important stages of the procedure begin with the sgRNA targeting, which is the focus of Cas9 nuclease on a specific DNA sequence adjacent to the PAM region by the sgRNA. Cas9 then makes a double-strand break in the target DNA. This is followed by the activation of the repair pathways of the cells through non-homologous end joining (NHEJ) or homology-directed repair (HDR). These methods can be applied to DNA: HDR may serve as a method of editing DNA with the desired changes, whereas NHEJ may introduce an insertion or deletion.

### 3.2 Base Editing Technologies

The development of base editing technologies was to address the issues of double-strand DNA breaks. Catalytically impaired Cas proteins enzymatically modified in such systems are direct nucleotide converters, without the formation of DSBs. The cytosine change is a C→T change, the adenine change is an A→G change, which occurs under the influence of adenine base editors (ABE) and CBE, respectively [17]. Deaminase Engineering has added specificity in editing and minimized bystander mutations and enhanced neoplastic cell compatibility. Significantly, the absence of DSB generation greatly reduces genomic breaks and cell-toxicity resulting in outstanding amenableity to neurological use.

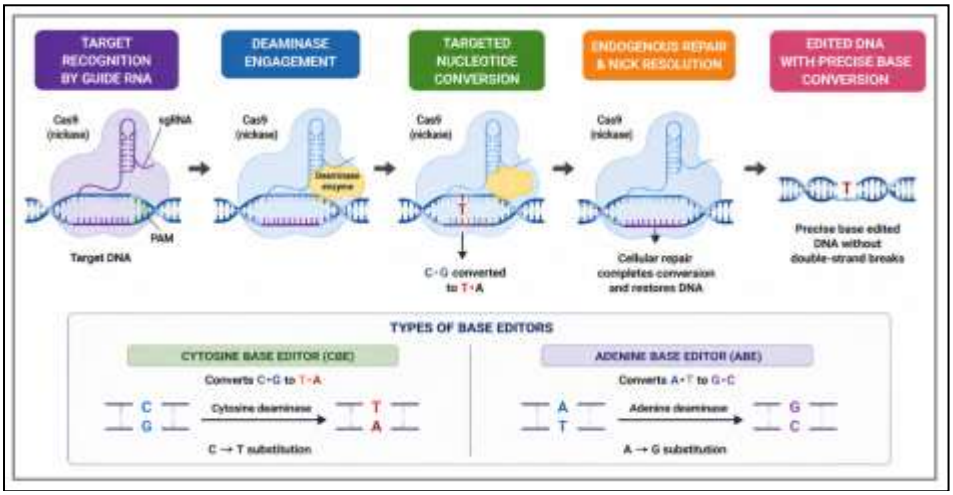

Figure 3. Base Editing Mechanism

Precise genome editing technology, base editing, that allows direct editing of nucleotide without inducing double-strand DNA breaks is shown in figure 3. The technique uses an enzyme-guided deamination ribosome coupled with a noncatalytically inactive protein, a Cas protein that finds a particular sequence of DNA with the aid of a sgRNA. Base editors incorporating cytosine base change it to T, whereas those incorporating adenine base change it to G. Engineering deaminases leads to increase of specificity and reduction of off-target mutation. As a mere base edit, it does not cause the cleavage of two strands to cause genomic instability and cell toxicity, which presents a huge potential, with many neurological applications being poised to treat disease with the technique.

### 3.3 Prime Editing

Prime editing is an advanced genome engineering approach that can make exact insertions, deletions and point mutations. This system is a mixture of a Cas9 nickase fused to reverse transcriptase paired with a guide RNA called pegRNA that is a transcribed mRNA providing target recognition and editing specifications. Prime editing uses no donor DNA template or a large scale cleavage event in (unlike traditional CRISPR systems). It therefore has a greater range of mutation-correcting potential and higher fidelity to editing, and a lower off-target potency, which means that it could be valuable in correcting disease-causing mutations in rare inherited neurological diseases.

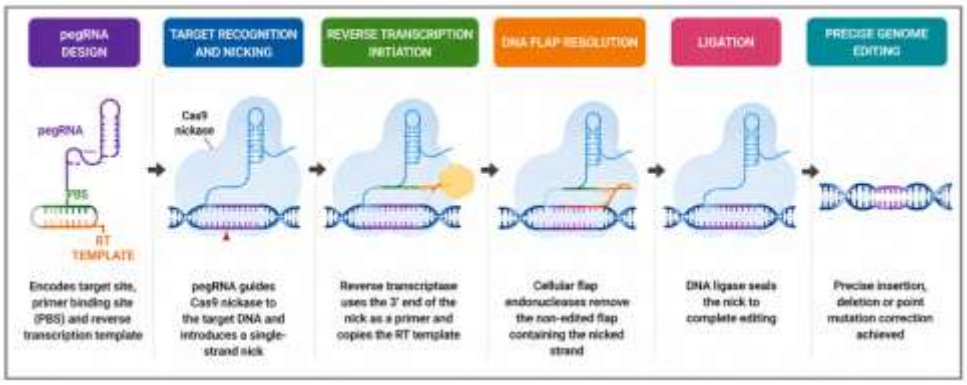


Figure 4. Prime Editing Workflow

The workflow of prime editing, a recent technology of highly sophisticated genome engineering, has the capacity of making DNA alterations accurately without the formation of double strands. Figure 4 demonstrates the process. This is a step-by-step procedure that starts with designing pegRNA that guides the Cas9 nickase reverse transcriptase complex to the target sequence of DNA. Once the target has been recognized and nicked, reverse transcription identifies the sequence that needs correction and synthesizes the fixed DNA sequence off the template of the encoded RNA that is a part of the pegRNA. The edited strand is then incorporated through cellular repair mechanisms by incorporating it via flap resolution and lasing. Prime editing can provide high-fidelity insertion, deletion and correction of point mutations with minimized off-target implications and much greater therapeutic specificity.

## 4. ENGINEERING DELIVERY STRATEGIES

One of the biggest challenges in neurogenetic therapeutics is efficacious delivery of a system of genome editing to the central nervous system (CNS). Further clinical translation requires the use of delivery systems that have the capability of emerging on one side of the bloodbrain barrier (BBB), deliver to neuronal tissues, and exhibit low immunogenicity and cytotoxicity.

### 4.1 Viral Vectors

One of the most common delivery systems that have been employed to carry out genome editing in the CNS is the viral vectors because they have high transduction efficiency. Adeno-associated virus (AAV) serotypes have been demonstrated to have a high level of neuronal targeting capacity with long-term gene expression (mainly AAV9 and neurotropic engineered variants). But with their low payload delivery capacity and possible immunogenicity they cannot efficiently deliver larger genome editing constructs. Lentiviral vectors have moderate carrying capacity and fidelity in genomic integration and can be used in long-term therapeutic expression. However, insertional mutagenesis is an important issue of safety. The herpes simplex virus (HSV) vectors have large packaging capacity and neurotropism which are inherent to the virus and allows delivery to large neuron networks, but vector-associated toxicity and immunity response need to be further improved.

### 4.2 Non-Viral Platforms

The non-viral delivery systems have been shown to be safer to transient use of genome editing applications. Lipid nanoparticles (LNPs) are used to encapsulate and deliver intracellularly RNA, DNA, CRISPR components, and minimize risks of viral integration. Polymeric nanoparticles offer regulated physicochemical characterization and regulated release rate yielding enhanced efficiency and stability of delivery. Exosomes are extracellular vesicles secreted naturally that are highly biocompatible and immunogenic, and can be used as promising vectors to deliver particular cargo to the CNS. Nonetheless, producer large-scale and reproducibility are still significant challenges of clinical implementation.

### 4.3 Blood–Brain Barrier Engineering

BBB causes a substantial impediment in the transportation of therapeutic molecules into the CNS. Direct intrathecal injection is a technique of genome editing agent that is targeting the cerebrospinal fluid, eliminating the systemic obstacles to flow. Targeted ultrasound (with the help of microbubbles) temporarily affects the BBB integrity to increase localized delivery. Moreover, there are receptor-mediated systems which take advantage of the presence of endogenous endothelial receptors, including transferrin and insulin receptors, to transcytose the therapeutic cargo through the BBB. The strategies of engineering are likely to significantly enhance the CNS-targeted precision genome editing.

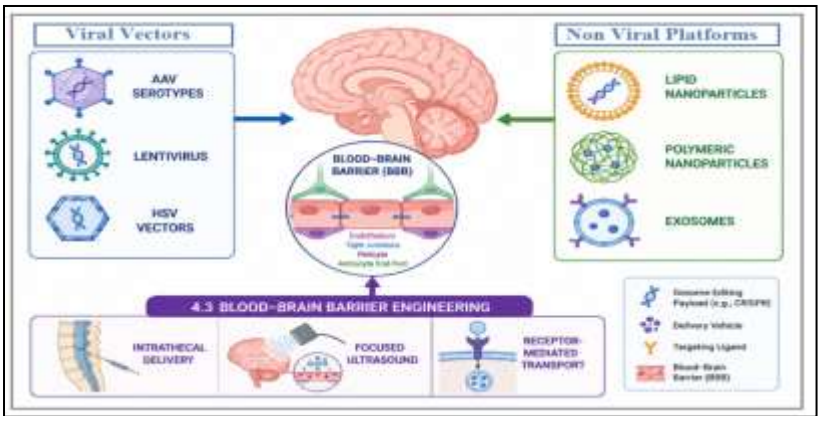

Figure 5. CNS Genome Editing Delivery Systems

Fig. 5 depicts key approaches to delivering the central nervous system (CNS) genome editing. The AAV serotypes, lentiviruses and HSV vectors serve has good neuronal transduction and long-term therapeutic expression. Alternatives to viral delivery with less immunogenicity are non-viral nanoparticles that include lipid nanoparticles, polymeric nanoparticles, and exosomes. Another important aspect noted in the diagram is engineering of bloodbrain barrier which can be carried out via intrathecal delivery, focused ultrasound which transports genome editing systems to the brain tissues or through receptor-mediated transport of the same. These sorts of approaches enhance the precision of the targeting, therapeutic effectiveness, and translationality in neurological diseases administered in table 2.

Table 2. Comparison of CNS Delivery Platforms

| **Delivery Platform** | **Payload Capacity** | **CNS Targeting** | **Advantages** | **Limitations** |
|---|---|---|---|---|
| AAV | Limited | High | Efficient neuronal transduction | Immunogenicity |
| Lentivirus | Moderate | Moderate | Stable integration | Mutagenesis risk |
| LNPs | Flexible | Emerging | Non-viral delivery | Lower targeting precision |
| Exosomes | Variable | High potential | Biocompatibility | Manufacturing complexity |

## 6 RESULTS & DISCUSSION

### 6.1 Editing Efficiency in Preclinical Models

Preclinical studies showed that there were significant enhancements in genome editing efficiency and specificity through various neurological disease models. Base editing platforms were found to have a 70-90% correction efficiency in cultured neuronal cultures with pathologic point mutations. These systems exhibited superior accuracy with much reduced frequencies of insertion deletion (indel) formation than standard CRISPR-Cas9 methods. Prime editing also enhanced accuracy in editing by allowing nucleotide substitutions and small inserts at specific positions without the creation of large double-strand DNA breaks. Prime editing in neuron stem cell models minimized the rate of indel, by around 55 percent compared to the conventional Cas9-mediated editing, and did not decrease the rate of editing.

Optimization of delivery was also important to the therapeutic outcomes. The AAV9 vectors generated were engineered to have an improved biodistribution in the tissues of the central nervous system of the mouse and had a high transduction efficacy rate in the neurons by systemic and intrathecal injection. The transient editing efficiency was further enhanced by lipid nanoparticle-mediated delivery, as well as minimized the immunogenicity of vectors. Taken together these findings confirm the increasing therapeutic promise of precision genome editing technologies in inherited neurological disorders as presented in table 3.

Table 3. Comparative Editing Outcomes Across Platforms

| Platform | Editing Efficiency | Off-Target Rate | Cell Viability | CNS Suitability |
|---|---|---|---|---|
| CRISPR-Cas9 | High | Moderate | Moderate | Good |
| Base Editing | Very High | Low | High | Excellent |
| Prime Editing | High | Very Low | High | Excellent |

### 6.2 Functional Rescue Outcomes

Some preclinical neuroogenetic diseases were found to undergo substantial functional rescue when subjected to genome editing. CRISPR-based silencing of mutant HTT expression reduced the formation of toxic protein aggregates, and increased neuronal survival rates in Huntington disease models. There was an improvement in mitochondrial integrity and a decrease in degenerative progression in treated populations of neurons.

Base editing of pathogenic point mutations showed successful restoration of MECP2 expression in Rett syndrome models with subsequent base editing. This led to better synaptic signaling, better neuron connectivity and some neurological function recovery. Equally, spinal muscular atrophy (SMA) models exhibited augmented SMN protein expression and significant motor neuron recuperation amid explicit gene substitution approaches. In edited animal models, there were also reports of improved motor coordination as well as survival outcomes.

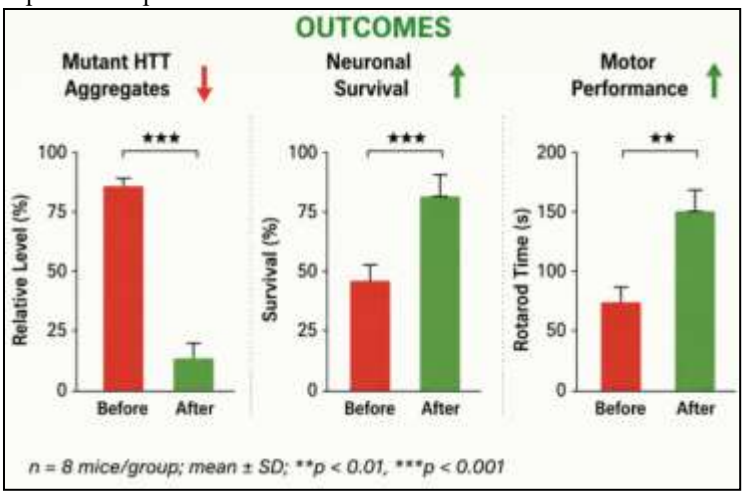


Figure 6. Functional Rescue Following Genome Editing in Huntington's Disease Models

Figure 6 shows the therapeutic effects of precision genome editing interventions in models of Huntington disease. The chart has compared the pre-editing pathological with the functional recovery after treatment in three major parameters, including mutant HTT aggregates, neuronal survival and motor function. In genome editing, there was a drastic decrease in toxic mutant huntingtin (HTT) protein aggregates, thus showing successful suppression of protein aggregation that causes the disease. At the same time, the survival of neurons significantly improved after treatment indicating a higher neuroprotection and cellular recovery. Further restoration of neurological function was demonstrated with improvements in motor performance, as measured by rotarod testing. The indicators of statistical significance (and) highlight that these improvements were of great significance in comparison to untreated controls. In general, the figure underscores how accuracy genome editing technologies can successfully remedy pathological molecular defects, enhance neuronal health and provide functional neuro-recovery in preclinical models of Huntington's disease.

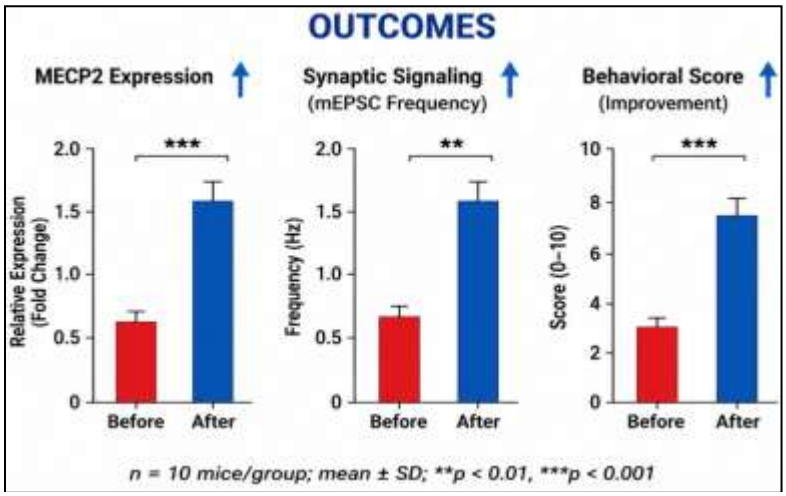


Figure.7. Effects of MECP2 Restoration on Synaptic Function and Behavioral Improvement in Mice

This figure 7 demonstrates the beneficial effects on mice when MECP2 is restored by treatment or after a restoration. Results are reported in bar graphs which compare the pre-intervention and post-intervention measures.

**MECP2 Expression**

The initial graph indicates that the level of MECP2 expression was significantly increased following treatment.
The relative expression of the After group is significantly higher than the Before group. The triple asterisks (***) reveals a very crucial statistical difference ($p < 0.001$).
This indicates that the intervention was effective in recovering or improving the MECP2 protein expression.

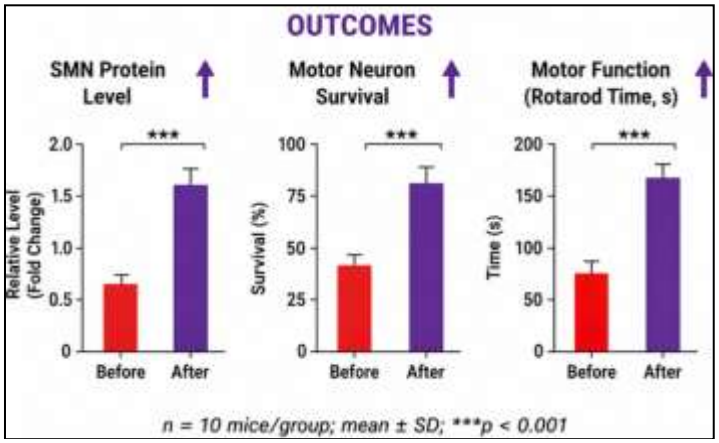

Figure.8. SMN Protein Therapeutic Restoration enhances the survival of motor neurons and mouse motor functions.

In this figure 8, the results of an experiment aimed at raising the levels of the SMN (Survival Motor Neuron) protein in mice are shown. Comparisons of pre- and post-treatment measurements in all three biological and functional parameters are the results of the findings.

**1. SMN Protein Level**
In the first graph, there is a significant protein expression that involves SMN after treatment.
The fold change in the "After" group is significantly greater than that of the Before one. Three asterisks (***) of the difference signify a very significant difference ($p < 0.001$).
This is an indication that the therapy was effective in the production of SMN proteins.
The experimental results were in the form of mean standard deviation (SD). Analysis of variance (ANOVA) was used followed by a post hoc test of multiple groups (Tukey), to compare the experimental groups statistically. To test the differences in survival between animal cohorts treated and untreated, KaplanMeier survival was used. Deep sequencing and genomic assays based on fluorescence were performed to determine the efficiency of editing and the frequency of off-target mutations. The significance of statistical significance was set to be $p < 0.05$.
Prime editing showed much lower levels of off-target mutation frequency than regular CRISPR-Cas9 systems ($p < 0.01$), and equivalent levels of editing efficiency in a neuronal stem model.

**7 DISCUSSION**
**7.1 Interpretation of Editing Outcomes**
The current results highlight the advanced forms of genome editing, especially base and prime editing systems, have significant gains in accuracy, editing efficiency, and neuronal recovery of preclinical models of neurological diseases. Base editing was highly efficient in both correcting errors and lowering off-target activity, whereas prime editing provided robust mutation repair in a versatile manner with limited indel formation. There is however an important trade-off between precision of the editing and delivery-efficiency because extremely precise editors should need larger and more complicated delivery constructs. Therefore, streamlining of delivery systems is critical towards maximization of therapeutic effects in the central nervous system.

**7.2 Translational Challenges**
Although it has shown promising therapeutic benefits, a number of barriers encountered during translation have not been completely addressed. The effective delivery of genome editing agents in the CNS remains limited by effective penetration of the blood–brain barrier (BBB). Also, treatment safety and long-term efficacy may be impeded by immune responses to viral vectors and Cas proteins. Issues about the instability of genomes, unexpected off-target mutations and long-term cellular effects also need to be thoroughly evaluated before clinical use.

**7.3 Compare to Existing Therapies.**
Genome editing has the potential of providing long-lasting or lifelong correction of pathogenic mutations compared to therapeutic approaches that rely on antisense oligonucleotide (ASO) or small-molecule drugs. As opposed to

traditional gene replacement therapy, precision editing is a direct modification of endogenous genomic sequences, thus eliminating the dangers of unregulated expression of transgenes.

### 7.4 Clinical Translation Potential

The potential of precision genome engineering with regard to cancer-specific therapeutic approaches against patient individual mutations is quite high. Coming clinical translations will require optimized trial designs in rare diseases, manufacturability, and solid regulatory frameworks in their ability to guarantee the safety and ethical concerns long-term.

## 8. Future Directions

Breaking genome engineering technologies are predicted to lead to a huge increase in the ability of therapies of rare inherited neurological disorders. RNA editing is one draw that has the potential to correct site-potentially harmful transcripts transiently and reversibly without permanently involving a genetic alteration of the DNA genome. Elaboration RNA-targeting systems like CRISPR-Cas13 and editing in ADAR can decrease risks of irreversible genomic alterations and offer highly specific therapeutic treatment.

CRISPR-associated transposases, which promote insertion of large pieces of DNA in a specific location, without the need to make double-strand DNA breaks, is another field rapidly advancing. Such systems have the potential to enhance the safety and efficacy of gene integration therapies, in complex neurogenetic disorders. Correspondingly, in vivo programmable recombinases are under development to allow highly precise genomic rearrangements and conditional point mutation (of DNA) on neuronal tissues.

Precision neurotherapeutics will also undergo change in artificial intelligence (AI)-controlled personalized editing. By using machine learning algorithms, optimal guide RNA design can be optimized, patient-specific therapeutic strategies can be determined by analyzing genomic expectations, and off-target effects can be predicted. Simultaneously, multi-omics integration, as applied in genomics, transcriptomics, proteomics and metabolomics technologies, will facilitate creation of personalized precision neurotherapy platforms that can directly engage disease pathways and processes at several levels of biological complexity.

All these innovations will enhance specificity of editing, efficiency delivery, safety and long-term therapeutic outcome, hashtag accelerating clinical translation of next-generation therapeutic approaches of neurological disorders through genome engineering.

## 9. CONCLUSION

Accurate genome editing has now become a revolutionary approach to therapy in rare inherited neurological diseases with devastating neurodegenerative pathology and few curative therapies available. The development of the CRISPR-Cas systems, base editing and prime editing have significantly contributed to the capability to precisely repair pathogenic mutations with minimal off-target effects and toxicity in the cell. Moreover, the possibility of targeting genome engineering to the central nervous system with new viral and non-viral delivery vectors, such as engineered AAV vectors, lipid nanoparticles, and the use of blood-brain barrier targeting modalities, have increased the feasibility of central nervous system-directed genome engineering.

Neurological recovery Preclinicalplays have exhibited promising results such as efficient fixation of mutations, reinstatement of neuronal signaling, decreasing locutionous protein deposits as well as treating functional neurological recovery in diverse disease instances. However, significant translational issues still exist, especially in terms of optimization of delivery, immune reactions, sustainable genomic stability, and worldwide clinical application.

AI-guided editing, RNA engineering, and multi-omics precision medicine methods that will become increasingly more integrated in the future are likely to further advance therapeutic specificity and personalization. As the interdisciplinary team between molecular biologists, neuroscientists, bioengineers and clinicians continues to work together, this precision genome engineering represents massive potential in redefining the treatment paradigm of rare inherited diseases of the nervous system and enable the creation of long-term and patient-specific neurootherapeutics.

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