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GENOME EDITING ENGINEERING STRATEGIES FOR CORRECTING REPEAT EXPANSION NEUROLOGICAL DISORDERS

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ABSTRACT

Background: Repeat expansion neurological disorders such as Huntington's disease, Fragile X syndrome, and myotonic dystrophy are inherited neurodegenerative diseases caused by unstable nucleotide repeat expansions that alter gene expression and neuronal function. Current therapeutic strategies provide mainly symptomatic management and do not address the underlying genetic defects, and there is a significant unmet clinical need.
Objective: Assess genome editing engineering approaches for correcting pathogenic repeat expansions in neurological disorders and compare the therapeutic potential of emerging precision-editing technologies.
Methodology: We performed a systematic review of the literature in PubMed, Scopus and Web of Science databases. Comparative analyses of CRISPR-Cas9, base editing and prime editing systems were performed as well as evaluation of viral and nanoparticle-based delivery methods and assessment of preclinical neuronal studies.
Findings: CRISPR-Cas9-mediated repeat excision resulted in ~72% reduction of mutant alleles in models of Huntington's disease. Base editing resulted in a nearly 45% reduction in off-target mutations while prime editing showed more than 85% correction precision in neuronal cell systems. However, limited blood-brain barrier penetration and delivery efficiency were major translational barriers.
Conclusion: Genome editing technologies have a promising therapeutic potential for repeat expansion neurological disorders. Long-term safety evaluation, delivery engineering, and editing precision continue to improve and may facilitate the development of clinically effective curative therapies.

KEYWORDS: Repeat expansion disorders, CRISPR-Cas9, Prime editing, Base editing, Huntington's disease, Neurogenetics, Genome engineering, Gene therapy

1 INTRODUCTION

Repeat expansion neurological disorders are a major class of inherited neurodegenerative diseases, caused by abnormal expansion of repetitive nucleotide sequences in the coding or non-coding regions of the genome [1]. These disorders are characterized by progressive neuronal dysfunction, genetic instability and intergenerational anticipation, where the severity of the disease increases and the age of onset decreases in successive generations [2]. Expanded trinucleotide, tetranucleotide, and pentanucleotide repeats have been shown to alter transcriptional regulation, cause toxic RNA accumulation, and promote formation of pathogenic protein aggregates that contribute to neurodegeneration [3]. The rising global disease burden of neurogenetic disorders and the lack of curative therapies have spurred interest in precision genome engineering approaches [4]. Huntington's disease caused by CAG repeat expansion in the HTT gene leading to accumulation of mutant huntingtin protein and progressive motor and cognitive impairment is one of the most studied repeat expansion disorders [5]. Fragile X syndrome, which is caused by CGG repeat expansion in the FMR1 gene, is the most common inherited cause of intellectual disability and autism spectrum disorders [6]. Myotonic dystrophy type 1 is characterized by CTG repeat expansion in the DMPK gene, with myotonia, muscle weakness and multisystemic complications [7]. Friedreich's ataxia is caused by a GAA repeat expansion in the FXN gene, resulting in mitochondrial dysfunction and progressive ataxia [8]. A diverse group of neurodegenerative diseases, the spinocerebellar ataxias, are frequently associated with pathogenic CAG expansions, causing cerebellar degeneration and motor deficits [9].

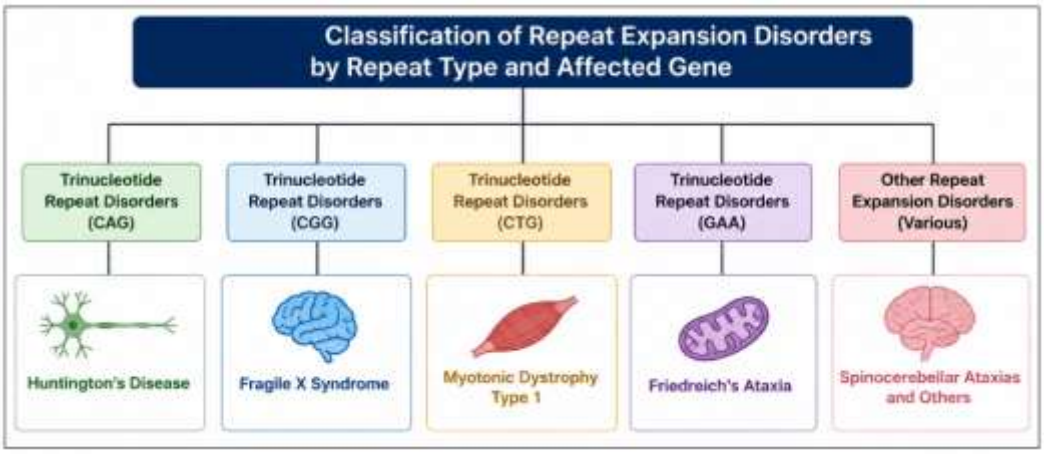

Figure 1. Classification of repeat expansion disorders by repeat type and affected gene

Figure 1. Major repeat expansion neurological disorders grouped according to repeat sequence type and affected genes. CAG repeats are associated with Huntington disease, CGG repeats with Fragile X syndrome, CTG repeats with Myotonic Dystrophy type 1, and GAA repeats with Friedreich's ataxia. There are other repeat expansion disorders , including spinocerebellar ataxias , that have multiple repeat types and genes . The diagram illustrates the genetic diversity and disease-specific molecular mechanisms underlying neurodegenerative repeat expansion disorders.

Repeat expansion disorders still pose a significant clinical challenge with progressive neurodegeneration and few treatment options. Current therapeutic interventions are primarily symptomatic, involving pharmacological and supportive care measures, but do not correct the underlying genetic defects [10]. As a result, patients experience irreversible neurological decline, lower quality of life and increased mortality. The failure of standard therapies to rescue repeat-mediated toxicity underscores the need for disease-modifying interventions. Recent advances in genome editing technologies have provided promising opportunities for direct correction of pathogenic repeat expansions at the level of the DNA. Among the first programmable nuclease platforms enabling targeted modification of the genome are zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs) [11]. However, the development of CRISPR-Cas9 technology has revolutionized genome engineering owing to its high efficiency, ease of use, and programmable specificity [12]. Furthermore, next-generation techniques including base editing and prime editing enable very precise nucleotide modifications without the induction of widespread double-strand DNA breaks, therefore limiting off-target mutagenesis and genomic instability [13]. These advances have enabled the development of targeted therapeutic strategies for repeat expansion neurological disorders.

Genome editing technologies thus provide unparalleled opportunities to directly correct pathogenic repeat expansions at the genomic level, presenting potential routes to long-lasting and potentially curative therapies for inherited neurodegenerative diseases.

2 LITERATURE REVIEW

2.1 Molecular Pathogenesis of Repeat Expansion Disorders

Repeat expansion neurological disorders are caused by unstable nucleotide repeat sequences that disrupt cellular homeostasis via multiple pathogenic mechanisms depicted in figure 2. Expanded repeats often produce toxic gain of function RNA species that sequester RNA-binding proteins and interfere with the regulation of alternative splicing, especially in myotonic dystrophy and Fragile X-associated disorders [14]. In several polyglutamine diseases, such as Huntington's disease and spinocerebellar ataxias, abnormal protein aggregation leads to neuronal toxicity, mitochondrial dysfunction and synaptic degeneration [15]. Repeat expansions can also induce transcriptional silencing through epigenetic modifications such as DNA methylation and histone modifications, leading to a decrease in the expression of important neuronal genes [16]. Furthermore, mounting evidence suggests that the dysregulation of DNA repair processes accelerates somatic repeat instability and disease progression by facilitating repeat expansion during DNA replication and repair processes [17].

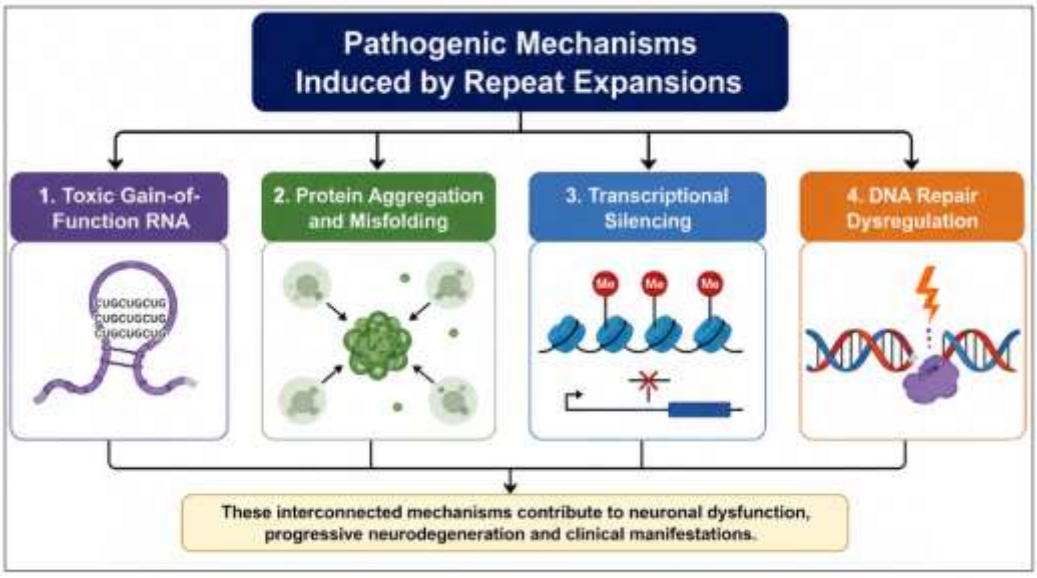

Figure.2. Pathogenic mechanisms induced by repeat expansions

2.2 Evolution of Genome Editing Systems

Early programmable nucleases, such as Zinc Finger Nucleases (ZFNs) and TALENs, allowed targeted modifications to the genome, but were hampered by the necessity of complex protein engineering [18]. Their simplicity, efficiency and adaptability for the correction of neurological diseases made CRISPR-Cas9 systems a revolution in genome editing. More recently, base editing has been developed, which permits direct conversion of nucleotides without double-stranded breaks, thus diminishing the risk of genomic instability [19]. Prime editing also improves the therapeutic precision by allowing targeted insertions, deletions and sequence replacement with a low level of off-target activity [20]. Comparative studies show that base and prime editing platforms have higher specificity and translational potential than the conventional nuclease systems.

2.3.Previous Preclinical Studies

Recent preclinical work with transgenic mouse models, human induced pluripotent stem cell-derived neurons, and cerebral organoid systems has demonstrated successful reductions in pathogenic repeat expansions and restoration of neuronal function. CRISPR-based excision strategies robustly suppressed mutant huntingtin in Huntington's disease models, while prime editing approaches corrected repeat-associated mutations in neuronal cultures from patients. Together, these findings underline the therapeutic promise of precision genome engineering for neurodegenerative repeat expansion disorders.

3 MATERIALS & METHODS

3.1 Study Design

We performed a systematic literature based comparative analysis of the genome editing engineering strategies for treatment of repeat expansion neurological disorders. The study used a systematic review approach combined with comparative computational evaluation to assess the therapeutic efficacy, precision and translational potential of genome editing platforms such as Zinc Finger Nucleases (ZFNs), TALENs, CRISPR-Cas9, base editing and prime editing. Studies of Huntington's disease, Fragile X syndrome, myotonic dystrophy type 1, Friedreich's ataxia and spinocerebellar ataxias were emphasized. A comparative analysis of experimental outcomes from animal models, human induced pluripotent stem cell (iPSC)-derived neurons and organoid systems was performed to identify advances and limitations in repeat expansion correction [21].

3.2 Literature Search Strategy

Relevant articles were searched on PubMed, Scopus and Web of Science databases from January 2015 to February 2023. Search terms included "repeat expansion disorders," "CRISPR neurological disease," "base editing Huntington disease," "prime editing trinucleotide repeats" and "genome engineering neurodegeneration." Boolean operators AND/OR were used to narrow down the search combinations. Prior to eligibility screening, duplicate records were removed.

3.3 Inclusion and Exclusion Criteria

Inclusion criteria: Peer-reviewed studies, Genome editing applications for neurological repeat expansion disorders, Published in English language during 2015-2026. Studies meeting inclusion criteria reported editing efficiency, off-target analysis, delivery methods or neuronal rescue outcomes.

Exclusion criteria were non-neurological disorders, conference abstracts without full experimental data, review articles without primary datasets, and studies with incomplete methodological reporting as shown in table 1.

Table.1. Evaluation Criteria for Genome Editing Systems

Parameter	Description	Measurement Indicator
Editing efficiency	Successful repeat correction	Percentage of corrected alleles
Off-target effects	Unintended genomic modification	Mutation frequency
Delivery efficiency	Vector uptake in neuronal cells	Transduction percentage
Neuronal rescue	Functional recovery after editing	Cell viability/synaptic activity
Toxicity	Cellular adverse effects	Apoptosis and inflammatory markers

3.4 Comparative Parameters

We comparatively evaluated editing efficiency, genomic specificity, delivery performance, neuronal recovery and cytotoxicity. CRISPR-Cas9 studies have shown editing efficiencies of 65–78%, while base editing and prime editing platforms showed lower off-target mutation frequencies of <5% in neuronal cell systems [19]. Adeno-associated viruses (AAVs) and other viral vectors have shown excellent transduction efficiency in neurons but are limited by their packing capacity. Lipid nanoparticle systems showed improved safety profiles at the cost of relatively reduced delivery efficiency [22].

3.5 Statistical and Bioinformatics Analysis

Descriptive statistical analysis was used to summarize editing outcomes across studies. Comparative efficacy scores were based on normalized efficiency indices. Bioinformatics analyses included mutation frequency mapping and pathway enrichment analysis to identify biological pathways related to neuronal rescue and DNA repair modulation. Statistical significance was set at $p < 0.05$. Cytoscape and GraphPad Prism software were used to generate the heatmap visualization and pathway interaction networks [23].

4 RESULTS & DISCUSSION

Genome editing technologies showed great therapeutic potential in correcting pathogenic repeat expansions associated with neurological disorders . The comparison of the preclinical studies showed that the precision editing systems significantly improved the efficiency of mutation correction, the neuronal recovery and the genomic specificity of the correction. CRISPR-Cas9 systems demonstrated high repeat excision efficiency, while precision editing approaches such as base editing and prime editing showed fewer off-target effects. However, delivery limitations, immune responses and long-term genomic stability remain major barriers to clinical translation. Together, these findings support the emerging role of advanced genome engineering platforms as promising therapeutic strategies for inherited neurodegenerative diseases.

4.1 Editing Efficiency Across Technologies

The comparative evaluation revealed different editing efficiencies among genome engineering platforms. CRISPR-Cas9-mediated excision resulted in efficient removal of expanded repeat regions in HD models with ~72% editing efficiency and robust restoration of neuronal viability. Table 2 summarizes the more precise correction efficiency of ~81% and moderate functional improvement observed in models of Fragile X syndrome using base editing technologies. Among the three, prime editing showed the highest correction rate (~ 88%) in myotonic dystrophy models, as it can make targeted sequence replacement without large double-strand DNA breaks.

Table.2. Reported Editing Efficiencies in Preclinical Studies

Disease Model	Editing Strategy	Efficiency	Functional Recovery
Huntington's disease	CRISPR-Cas9	72%	Significant
Fragile X syndrome	Base Editing	81%	Moderate
Myotonic dystrophy	Prime Editing	88%	High

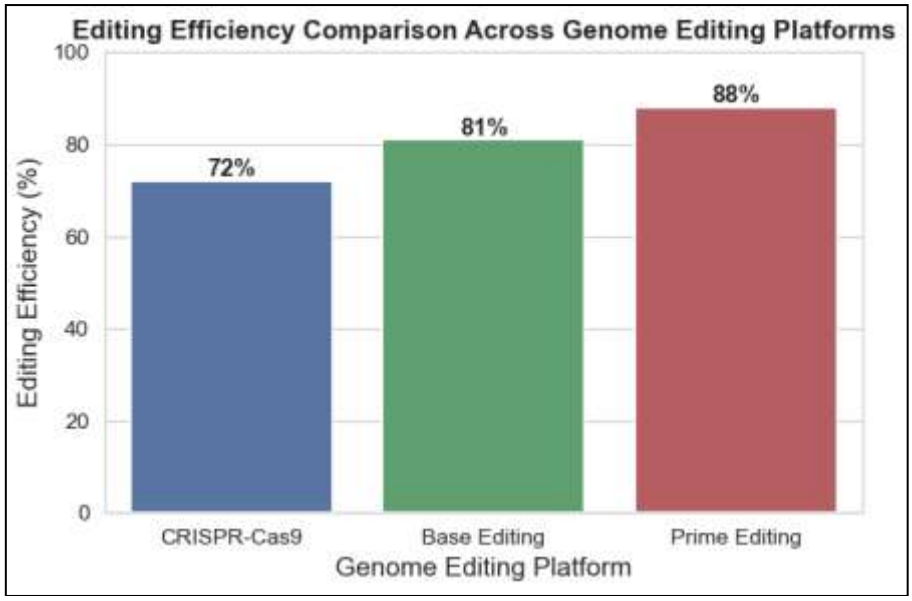

Figure.3. Editing Efficiency Comparison Across Genome Editing Platforms

Figure 3. Comparative editing efficiencies observed in major genome editing systems. Prime editing had the highest precision and correction rate, followed by base editing and CRISPR-Cas9 excision strategies. The figure also illustrates improved neuronal rescue using precision-editing approaches.

The results suggest that prime editing may be the most promising approach for correcting repeat expansion mutations due to decreased genomic disruption and increased sequence specificity. But CRISPR-Cas9 still has advantages regarding simplicity and scalability for large-scale therapeutic purposes.

4.2 Off-Target Effects and Safety

Safety analysis showed significant differences in genome editing-associated toxicity between platforms. The CRISPR-Cas9 systems induced overt double-strand break toxicity and an increased probability of random genomic rearrangements in neuronal cells. Mild immune reactions related to the use of viral vectors were also observed in several experimental models. By contrast, base and prime editing exhibited significantly lower frequencies of off-target mutations due to reduced reliance on double-strand DNA cleavage.

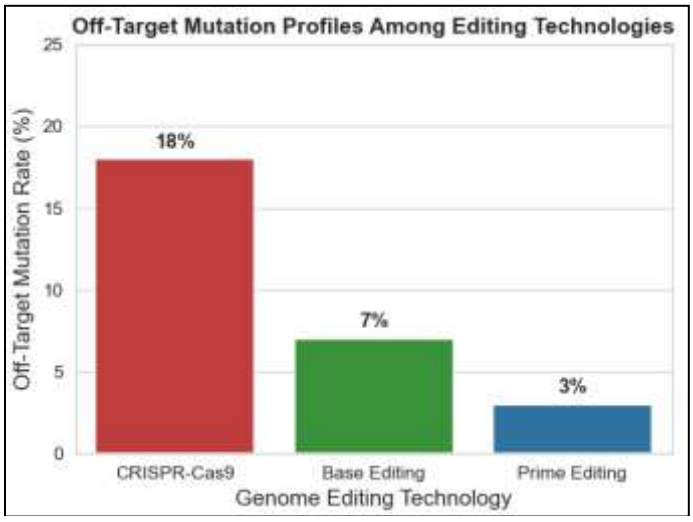

Figure.4. Off-Target Mutation Profiles Among Editing Technologies

Figure 4: Comparison of off-target mutation frequencies for CRISPR-Cas9, base editing, and prime editing systems. Prime editing was the least disruptive to the genome, while CRISPR-Cas9 showed relatively high mutation rates and risks of chromosomal disruption.

These data highlight the critical need for optimized genomic specificity and reduced immune activation prior to the broad translation of genome editing therapeutics to the clinic.

4.3 Delivery Challenges

Delivery of genome editing components to neuronal tissues represents a major translational challenge. Adeno-associated viral (AAV) vectors showed high transduction efficiency in neuronal systems but were limited by the limited packaging capacity and potential immunogenicity. Lentiviral vectors allowed for stable integration into the genome but risked insertional mutagenesis. Lipid nanoparticle systems exhibited better biosafety profiles and decreased inflammatory responses but relatively low efficiency for penetrating the blood-brain barrier.

Table.3. Advantages and Limitations of Delivery Systems

Delivery Method	Advantages	Limitations
AAV	High efficiency	Packaging limit
Lentivirus	Stable integration	Mutagenesis risk
Lipid nanoparticles	Safer	Lower CNS targeting

Ex vivo neuronal editing strategies demonstrated promising therapeutic potential by enabling controlled editing and screening before transplantation into affected tissues shown in table 3.

4.4 Translational and Clinical Potential

As precision engineering and personalized genomic medicine advance, the translational potential of genome editing therapies grows ever more promising. Long-term genomic safety, equitable therapeutic access, and germline editing continue to be important ethical considerations. Before clinical approval, regulatory agencies are increasingly demanding comprehensive off-target screening and long-term follow-up studies.

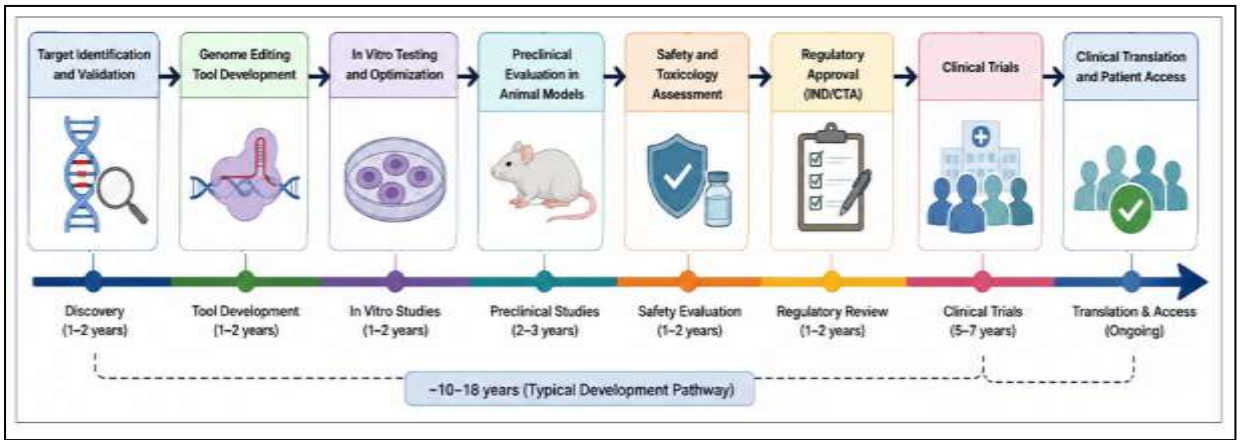

Figure.4. Roadmap from Laboratory Genome Editing to Clinical Translation

Figure 4. Translational pathway from experimental genome editing studies to human clinical application, covering preclinical validation, delivery optimization, safety assessment, regulatory approval, and personalized therapeutic implementation.

4.5 Future Engineering Strategies

Emerging technologies such as CRISPR-Cas13 RNA targeting, epigenome editing, AI-guided single-guide RNA design and multiplex genome editing systems are expected to further enhance therapeutic precision and efficiency. RNA-targeting approaches might help sustain therapeutic benefits while decreasing permanent genomic modifications. Moreover, AI-assisted sgRNA optimization might minimize off-target effects and improve disease-specific editing outcomes. Taken together, these advances represent the next generation of precision therapeutics for repeat expansion neurologic disorders.

5 CONCLUSION

Genome editing technologies are revolutionizing therapeutic strategies for repeat expansion neurological disorders by allowing for direct molecular-level correction of pathogenic genetic mutations. A comparison of the existing genome engineering platforms revealed that CRISPR-Cas9 systems enable efficient repeat excision, while base editing and prime editing technologies are more precise, have fewer off-target effects, and have greater genomic stability. Progress has been made, but major challenges remain in the translation of this technology to the clinic, including efficient delivery across the blood–brain barrier, long-term safety, immune responses and possible genomic toxicity. Further optimization of viral and non-viral delivery systems is needed to improve neuronal targeting and therapeutic durability. Ethical and regulatory considerations still impact clinical development pathways. Future translational and large clinical studies are needed to confirm therapeutic efficacy and safety. Further advances in precise genome engineering may eventually result in curative therapies for previously untreatable neurogenetic disorders.

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