EDITORIAL

Prime Editing in Neurogenetic Disorders: Molecular Mechanisms, Therapeutic Applications, and Translational Challenges

Hafsa Aslam[1]* | Fatima Ali[2]

[1]Center of Excellence in Molecular Biology, University of the Punjab, Lahore, Pakistan | [2]School of Molecular Pathology, University of Rome, Italy

***Correspondence:** Hafsa Aslam (hafsaaslam35102@gmail.com)

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ABSTRACT

Genome editing has progressed rapidly, and prime editing is among the most impactful advances since the advent of CRISPR-Cas9. Rather than snipping both strands of DNA and repairing them, prime editors insert a new DNA sequence into the genome via a fused Cas9 nickase and reverse transcriptase, directed to the target sequence by an engineered pegRNA that encodes both the target address and the edit. That combination matters most in tissues where mistakes can't be undone, and few tissues fit that description better than the brain. This editorial briefly discusses the molecular basis of prime editing and then examines its current status for a selection of neurogenetic disorders: Tay-Sachs disease, Duchenne muscular dystrophy, Huntington disease, and Rett syndrome. Preclinical studies have demonstrated prime-editing applications in selected genetic disorders, while evidence for other conditions remains based on related genome-editing approaches, particularly base editing. The translational barriers that currently separate cell-based proof-of-concept from clinical application, including vector packaging constraints, blood-brain barrier penetration, and long-term safety in postmitotic neurons, are also discussed.

Keywords: CRISPR-Associated Protein 9; Gene Editing; Genetic Therapy; Heredodegenerative Disorders, Nervous System; Nervous System Diseases.

The first generation of the CRISPR-Cas9 system for editing genes was based on the direct correction of genes using double-strand breaks and homology-directed repair (HDR), which has unpredictable insertions/deletions (indels), low efficiency in postmitotic cells, and potential off-target risk, which poses safety concerns for its use in the central nervous system (CNS) [1]. Prime editing (PE) directly tackles these challenges. By fusing a Cas9 nickase with a reverse transcriptase, PE is capable of targeted "search-and-replace" editing without a double-strand break or donor template and has been shown to exhibit lower off-target editing than Cas9 nuclease at known Cas9 off-target sites [2]. This precision has assured PE a prominent role in the correction of point mutations, small indels, and repeat expansions which underlie some neurogenetic disorders. This editorial reviews the mechanism of prime editing, outlines its uses in representative monogenic neurogenetic diseases, and highlights the challenges for prime editing-based therapy in the clinic.

MECHANISTIC DISCUSSION

PE in its core PE2 form fuses a Streptococcus pyogenes Cas9 H840A nickase to an engineered Moloney murine leukemia virus reverse transcriptase (M-MLV RT), directed by a pegRNA that both specifies the target site and encodes the desired edit within its 3′ extension (primer-binding site and RT template) [3]. Recent cryo-electron microscopy structures of the Cas9–RT–pegRNA–DNA complex have clarified how the RT domain is held in place relative to Cas9, while the nascent, pegRNA-templated DNA heteroduplex grows along the surface of Cas9 during reverse transcription [4]. The nickase cuts the non-target DNA strand, and the exposed 3′ end is then used as the primer for reverse transcription, which is then used for the synthesis of the corrected sequence directly on the genomic DNA with formation of a 3′ flap that competes with the original 5′ flap for incorporation. Cellular flap resolution and DNA repair pathways favour the edited strand, completing the edit. PE3 includes a second strand of a nicking guide RNA to bias repair further towards the edited allele, with a slight decrease in specificity and an increase in efficiency [5].

One drawback of PE is that the heteroduplex intermediate can be recognized by endogenous DNA mismatch repair (MMR) and can be corrected before the final editing, thus reducing the overall editing efficiency. This dominant-negative MLH1 (MLH1dn) system of transient inhibition of MMR significantly boosts editing efficiency [6]. Two recent advancements have shown the continued rapid progress in optimizing this part of the system: (i) the small, AI-designed MLH1 binder from RFdiffusion and AlphaFold 3 increased the efficiency of the PE7-SB2 system by 18.8-fold beyond that of PEmax editing and (ii) small, independent developments of fusion of polymerase substrate-binding proteins to the RT domain resulted in increased intended-edit rate for substitutions, insertions and deletions [7]. The core PE2 mechanism, from pegRNA binding through flap resolution, is shown in **Figure 1** [16].

THERAPEUTIC APPLICATIONS

In monogenic diseases, there is a clear point mutation, small deletion, or frameshift, which has been furthest advanced towards disease-relevant validation for prime editing. In Tay-Sachs disease, the original prime-editing study demonstrated correction of the pathogenic four-base-pair HEXA deletion in human cells and also evaluated editing in primary postmitotic mouse cortical neurons, providing early evidence of prime-editing feasibility in neuronal models [1]. A selectable all-in-one prime-editing system using a piggyBac transposon was developed, demonstrating an approach for efficient prime-editor delivery and selection in human cell lines [8]. However, this study does not establish therapeutic correction of SOD1-associated ALS.

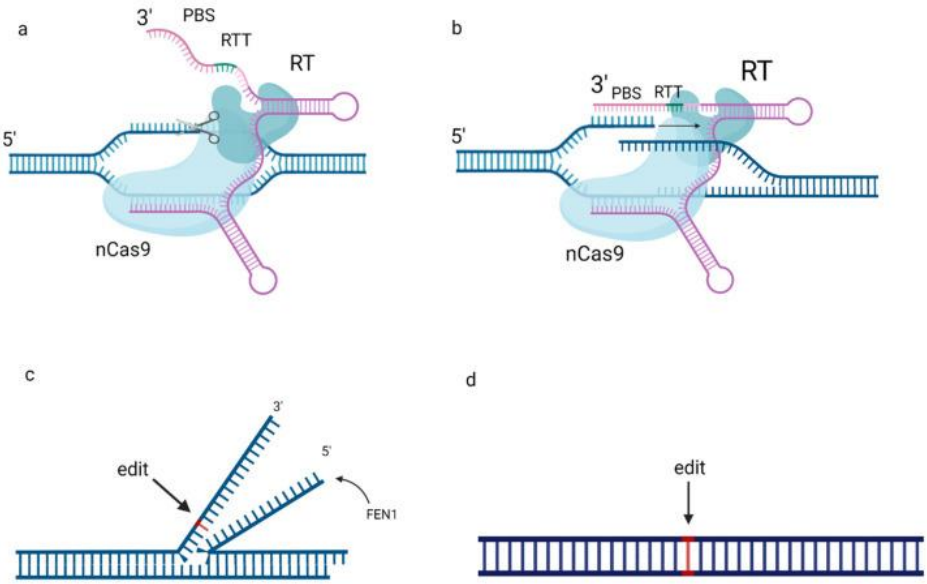


***Figure 1**: **Molecular mechanism of prime editing.** (a) The prime editor complex consists of Cas9 nickase (nCas9) fused to reverse transcriptase (RT), together with prime editing guide RNA (pegRNA), which directs target recognition and exposes the DNA strand for nicking. The nicked DNA end binds to the primer-binding site (PBS) of pegRNA. (b) The nicked DNA strand serves as a primer for RT, which synthesizes the edited DNA sequence using the reverse transcription template (RTT) of pegRNA. (c) The resulting heteroduplex is resolved, and the unedited 5′ DNA flap is cleaved by flap endonuclease 1 (FEN1). (d) DNA repair incorporates the intended genetic modification into the genome.*

In Duchenne muscular dystrophy (DMD), high-capacity adenovector delivery of advanced prime editing systems resulted in reading-frame correction in patient-derived myoblasts and mesenchymal stem cells, restoring dystrophin synthesis and dystrophin–β-dystroglycan linkages, and the same platform corrected DMD iPSC-derived cardiomyocytes and supported dual-PE-mediated exon 51 deletion [9]. This work has highlighted that PE efficiency is greater in cycling cells compared to postmitotic cells, directly applicable to any CNS application.

Direct prime editing evidence in disease-relevant models remains limited in the case of Huntington disease and Rett syndrome. The most recent examples were base editing, where AAV9-mediated delivery of base editors to disrupt the pathogenic HTT CAG repeat with stabilizing interruptions led to reduced somatic repeat expansion in the CNS of Huntington disease mice [10]. An overview of representative prime-editing and related genome-editing studies, their experimental models, and current evidence status is provided in **Table I**.

Table I: Representative prime-editing and related genome-editing studies relevant to genetic disorders.

Study	Target gene/application	Editing strategy	Experimental model	Evidence status
Anzalone et al., 2019 [1]	HEXA – Tay-Sachs disease	Correction of the pathogenic four-base-pair deletion using prime editing	Human cells and postmitotic mouse cortical neurons	Direct prime-editing evidence
Eggenschwiler et al., 2021 [8]	Prime-editing platform development	Selectable all-in-one prime-editing system using a piggyBac transposon	Human cell lines	Platform development; disease-specific SOD1 correction not established by the cited reference
Wang et al., 2024 [9]	DMD – Duchenne muscular dystrophy	Reading-frame correction and exon-directed repair using advanced prime editors	Patient-derived myoblasts, mesenchymal stem cells, and iPSC-derived cardiomyocytes	Direct prime-editing evidence
Matuszek et al., 2025 [10]	HTT – Huntington disease	Base editing to introduce stabilizing interruptions in pathogenic trinucleotide repeats	Patient-derived cells and mouse models	Related genome-editing evidence; not prime editing
Kanno et al., 2025 [15]	Disease-associated genetic variants	Prime-editing-based introduction of disease-relevant single-nucleotide variants	Human induced pluripotent stem cells	Disease-model generation; not therapeutic correction evidence

***Abbreviations**: Duchenne muscular dystrophy; HEXA, hexosaminidase A; HTT, huntingtin; iPSC, induced pluripotent stem cell.*

TRANSLATIONAL CHALLENGES

The assembled PE2/PE3 cassette is ~6.3 kb, which is much longer than what a single adeno-associated virus (AAV) genome can accommodate (approximately 4.7kb; and AAV is the most well-studied CNS-tropism). The workaround is splitting the editor across dual AAV vectors, using a truncated reverse transcriptase and split-intein reconstitution to rejoin the halves once inside the cell, validated by a study, though so far mainly in mouse liver rather than at scale in the CNS [11]. The problem is only its ability to enter the brain. Capsid variants of AAV specifically engineered to transduce CNS tissue have been generated with significantly improved transduction efficiency of neurons compared to AAV9, and systemically injected AAV.PHP.eB is capable of achieving long-term, cell-type-specific transgene expression throughout the brain and spinal cord with neuron- or glia-specific promoters [12, 13]. Meanwhile, there have been some genuine advances on the receptor side, too: Several engineered capsids were found to interact with the same receptors, namely LY6C1 and carbonic anhydrase IV, which the BBB actually uses; a

finding that will help facilitate more rational, structure-driven capsid design instead of trial-and-error work using directed evolution [14]. None of this, though, has yet been shown with a full dual-AAV prime editing payload delivered systemically to the CNS.

There's a subtler issue underneath the delivery problem, and it's biological rather than engineering. Most PE efficiency numbers come from dividing cell lines or patient-derived iPSCs, the protocol described for building isogenic disease-variant lines, where cells that failed to edit correctly can simply be diluted out over passages, or selected against [15]. Neurons don't offer that luxury. There's no in vivo mechanism to enrich for successfully edited cells in a static, non-dividing population, so the efficiency needed for real therapeutic benefit in brain tissue is probably higher than what dividing-cell data would suggest. Add to that the unresolved questions around long-term safety in non-dividing neurons, immune responses to repeated or high-dose viral dosing, and the lack of any standardized off-target testing protocol built specifically for CNS-delivered PE, and it's clear why this remains further from the clinic than the mechanism section might imply.

FUTURE PROSPECTS

The near-term trajectory probably runs through continued AI-assisted optimization of the editor itself; the MLH1-binding domain work and RT-fusion strategies, combined with continued directed evolution of CNS-tropic, liver-detargeted AAV capsids and neuron-specific promoter systems. Personalized delivery strategies matched to a disorder's neuroanatomical distribution, and standardized in vivo off-target assessment protocols specific to postmitotic tissue, will be necessary before prime editing can move from cell-based proof-of-concept to CNS clinical trials

CONCLUSION

Prime editing represents a promising genome-editing approach for correcting pathogenic genetic variants associated with neurogenetic and neuromuscular disorders. Preclinical studies have demonstrated its potential in selected disease-relevant cellular models, including Tay-Sachs disease and Duchenne muscular dystrophy, although the evidence varies across experimental systems. In contrast, findings in Huntington disease and Rett syndrome remain limited and should be distinguished from direct prime-editing validation. Overcoming AAV packaging limitations, improving blood–brain barrier penetration, and establishing long-term safety in postmitotic neurons will be essential for translating these experimental advances into potential clinical applications.

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