USE OF CRISPR BASE EDITING AND PRIME EDITING IN NEUROLOGICAL DISEASES

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Abstract: Next-generation gene editing technologies, particularly base editing and prime editing, have emerged as highly promising approaches for the treatment of neurological diseases driven by pathogenic mutations and genomic instability. Unlike conventional CRISPR-Cas9 methods that introduce double-strand breaks, these systems enable precise nucleotide conversions or guided sequence replacements, thereby reducing off-target events and increasing safety for central nervous system (CNS) applications. Recent studies demonstrate their applicability in cellular and animal models of conditions such as Alternating Hemiplegia of Childhood, Huntington's disease, and repeat-associated ataxias, showing functional restoration, decreased somatic repeat expansion, and improvement of neurological phenotypes. However, the clinical translation of these tools still faces substantial challenges, including limitations in CNS delivery, target-dependent efficiency, and the need for comprehensive biosafety evaluation. Viral and nonviral platforms—such as optimized AAVs, lipid nanoparticles, and virus-like particles—are under active development to overcome these barriers. Additional gaps remain regarding editing durability, immunogenicity, and scalability. This article provides an integrated analysis of the principles, preclinical applications, technical limitations, and future perspectives of base editing and prime editing in neurological diseases, emphasizing their

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transformative potential and the necessity of rigorous, safety-driven research.

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INTRODUCTION

The rapid advancement of genome editing technologies has profoundly transformed the field of biotechnology and biomedical sciences, offering increasingly accurate, efficient, and safe approaches to DNA manipulation (Devinsky et al., 2025). Among these tools, the CRISPR base editing and the Prime Editing emerge as state-of-the-art strategies capable of promoting specific modifications in the genome without generating double-strand breaks, significantly reducing unwanted effects associated with traditional methods. These techniques open up new possibilities for the investigation and correction of pathogenic genetic variants, especially in tissues of high biological complexity, such as the central nervous system (MacLaren et al., 2020).

The importance of the theme stands out in view of the significant global burden of neurological diseases, which affect millions of people and present, in many cases, complex etiology and limited therapeutic response. Diseases such as Alzheimer's, Parkinson's, hereditary ataxias, genetic epilepsies, and various neuropathies associated with point mutations pose significant medical and socioeconomic challenges (Feigin et al., 2020). In this context, base editing and prime editing methodologies offer promising alternatives to correct pathological variants with greater precision, expanding the potential for personalized treatments and early interventions. In addition, the applicability of these technologies in cellular and animal models contributes to a deeper understanding of the molecular mechanisms underlying neurological diseases (Murray, Harrison and Scholefield, 2025).

The guiding question of this work is: How have base editing and prime editing technologies been applied in the study and correction of mutations associated with neurological diseases, and what are their advances, limitations, and therapeutic perspectives? The rationale lies in the growing scientific and clinical interest in approaches that enable safe and highly specific genetic interventions

in neural tissue, where cell regeneration is limited and the effects of mutations can be particularly devastating. Recent literature demonstrates a rapid increase in the number of studies exploring these technologies in the neurological context, reinforcing the need for a comprehensive and critical review on the topic (Paul, Collins, and Lee, 2022).

Therefore, the general objective of this article is to analyze the state of the art of the use of CRISPR base editing and Prime Editing in neurological diseases, highlighting applications, challenges and future perspectives. Specific objectives include: (i) to describe the molecular principles and functional differences between base editing and prime editing; (ii) review experimental and preclinical studies that use these technologies in models of neurological diseases; (iii) discuss the main therapeutic advances reported, as well as technical, ethical, and safety limitations; and (iv) point out gaps in the literature and possible directions for future research aimed at the clinical application of these tools in neurological conditions (Chiba-Falek et al, 2025a).

METHODOLOGY

This study was conducted as a systematic review strictly following the guidelines established by the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) protocol, ensuring transparency, reproducibility and methodological robustness at all stages of the investigation. The elaboration of the initial protocol included a clear definition of the guiding question, the eligibility criteria and the search strategies, ensuring that the process of selecting the studies was objective and consistent with the scope of the research.

Search strategy

The research question was structured based on the PICO model adapted for biotechnology reviews: What are the applications, advances, and limitations of CRISPR base editing and prime

editing technologies in neurological diseases? Thus, the following criteria were defined: Population (P) – studies involving neurological diseases of genetic origin; Intervention (I) – use of CRISPR base editing or prime editing; Comparison (C) – not applicable due to heterogeneity of studies; Outcome (O) – genetic correction, phenotypic modulation, safety and efficacy assessment. Based on these elements, inclusion and exclusion criteria were outlined.

Inclusion and exclusion criteria

The inclusion criteria involved: (i) original articles published between 2015 and 2025, a period corresponding to the development and consolidation of base editing and prime editing technologies; (ii) studies conducted in cell models, organoids, animal models or human samples; (iii) research that directly applied one of the aforementioned CRISPR technologies to investigate or correct mutations related to neurological diseases; and (iv) texts published in English or Portuguese and available in full. The exclusion criteria were: (i) narrative reviews, editorials, letters, and opinion reports; (ii) studies that used only conventional CRISPR/Cas9 methods without base editing or prime editing; (iii) studies that addressed diseases unrelated to the nervous system; and (iv) studies without adequate methodological description.

Systematic search

It was performed in the PubMed, Scopus and Web of Science databases, using combinations of controlled descriptors (MeSH) and free keywords, such as: “CRISPR base editing”, “prime editing”, “neurological diseases”, “neurogenetic disorders”, “genome editing therapy”. Boolean operators AND and OR were employed to broaden and refine the results. All searches were carried out between August and September 2025. The identified records were imported into the Rayyan software for duplicate removal and initial screening.

Selection of studies

It took place in two stages: (i) screening by title and abstract, carried out by two independent reviewers; and (ii) complete reading of the eligible texts. Divergences were resolved by consensus or by a third reviewer. The complete process of identification, screening, eligibility and final inclusion was documented in a PRISMA flowchart, ensuring the traceability of decisions. In the end, the included studies had their data systematically extracted in a matrix containing information on the experimental model, target mutation, type of editing, results obtained, limitations reported, and contributions to the field. The methodological quality assessment was conducted using criteria adapted from the NIH Quality Assessment Tool, allowing the studies to be classified for risk of bias

LITERATURE REVIEW

Technologies, principles and evolution (base editing vs. prime editing).

Base editing (BE) and prime editing (PE) have emerged as genome editing platforms designed to increase the accuracy of modifications without relying on double-strand breaks (DSBs) and repair by homologous recombination with a donor template. The first functional BE described was the Cytosine Base Editor (CBE), which combines a cytosine-deaminase to the knocked out Cas9 variant (nickase or dCas9) to promote C→T (or G→A conversion on the complementary strand) in a constrained window around the gRNA binding site—allowing for targeted point changes without extensive DNA fragmentation. This approach was published by Komor et al. in 2016 and paved the way for point edits with fewer by-products than classic CRISPR/Cas9 (Komor et al., 2016)which can frequently generate random insertion or deletion of bases (indels.

The extension of the BE repertoire for A→G conversions (ABE — Adenine Base Editors) was achieved by Gaudelli et al. (2017), through the directed evolution of a tRNA-derived adenosine-

deaminase adapted to act on DNA when fused to modified Cas9. ABEs and CBEs have shown, over time, optimized versions (BE2→BE3→BE4/BE4max; variants of ABE with improved activity and edit window), along with engineering Cas (high-fidelity variants) to reduce off-targets and Bystander edits (Gaudelli et al., 2017).

Prime editing, described by Anzalone et al. in 2019, it broadened the scope by allowing any→any replacements, small insertions, and deletions without DSBs and without separate exogenous template. The PE system combines a Cas9 nickase with a reverse transcriptase (RT) and uses a pegRNA (prime editing guide RNA) that contains the sequence to be written and a tail that serves as a template for the RT, pegRNA guides the nickase to the target site and provides the information to be incorporated, which is then synthesized by the RT directly at the site. PE has enormous versatility, but on the other hand it is a larger protein and, initially, less efficient in some sites than BEs, which led to successive optimizations (PE2/PE3, improvements in pegRNA design, RT engineering and Cas variants).

From a mechanistic point of view, the crucial differences that guide the choice between BE and PE are: (i) type of change desired (BE limited to specific transitions — C→T/T→C or A→G/G→A — while PE allows transitions, transversions, insertions, and deletions); (ii) edit window and context (BE has edit window determined by deaminase/Cas and often manages Bystander edits on adjacent bases, while PE offers greater positional accuracy); and (iii) publisher size and delivery requirements (PE is structurally larger, complicating delivery by limited-capacity vectors). Recent studies continue to refine specificity, reduce genomic errors, and characterize cellular repairs that modulate outcomes (e.g., uracil repair pathways and BE mismatch) (Gu et al., 2025).

Preclinical studies and application models in neurological diseases.

The application of BE and PE in neurological diseases focuses primarily on monogenic diseases caused by point mutations (or small insertions/deletions) and on strategies to mitigate

pathological repeats. Examples and relevant preclinical evidence:

Proof-of-concept in patient-derived cell cultures (iPSC-neurons) and in animal models have demonstrated that BE can reverse loss-of-function mutations by restoring protein expression and cellular phenotypes. Gene-driven projects such as MECP2 (Rett syndrome), lysosomal disease genes such as Tay-Sachs, and other nonsense mutations have shown in vitro edits with sufficient efficiency to consider subsequent in vivo studies (Chang et al., 2021).

Prime editing for complex corrections and nonsense mutations, PE has been applied to repair premature stop codons and reinstall correct coding sequence in cellular models of neurogenetic diseases; its ability to generate alterations that are not accessible to BE, such as multinucleotide transversions or insertions, is especially useful for certain mutations found in rare CNS diseases. Reports from 2024–2025 describe molecular and phenotypic rescues in animal models for specific neurological conditions (Sousa et al., 2025).

Repeat expansion diseases, base editing approaches have been explored to “stop” pathogenic repeats (e.g. CAG in Huntington, introducing substitutions that reduce repeat somatic instability, with preclinical reports of phenotype mitigation in murine models. These interventions aim both to reduce the production of toxic protein and to stabilize the genome in the face of expansion (Chiba-Falek, 2025b).

In vivo evidence and transactional relevance, recent advances show that both BE and PE can be delivered to neural tissue with partial restoration of function and behavioral improvement in animal models, as the 2024–2025 studies report recovery of enzyme activity, motor improvement, and reduction of paroxysmal episodes in specific models. These studies mark important preclinical milestones, but often rely on local vaccination (intracerbral injections) or wide-expression vectors to demonstrate efficacy (Caso and Davies, 2022)achieving targeted genomic change at unprecedented efficiencies with considerable application in laboratory animal research. Despite its ease of use and wide application, there remain concerns about the precision of this technology and a number of unpredictable consequences have been reported, mostly resulting from the DNA double-strand break

(DSB.

Delivery to the SNC, safety and technical limitations

Delivery to the central nervous system (CNS) is the main practical limitation for BE/PE therapies. The blood-brain barrier (BBB), post size (especially for PE), the need to target specific cell types, and the persistence/timing of expression are critical obstacles. Delivery strategies investigated:

AAVs (adeno-associated viruses), widely used in gene therapy for CNS (good neuronal tropism profiles for certain serotypes), but have limited capacity (~4.7 kb): PE often exceeds this capacity, requiring strategies such as split-intein systems (splitting the editor into two particles), code compression, or the use of compact promoters. AAVs have low insertional risk compared to integrative vectors, but they can induce immune responses and have dose/scale implications. 2024–2025 reviews discuss AAV optimizations and limitations to deliver bulky editors to the brain (Davis et al., 2024) prime editing guide RNA stability and modulation of DNA repair. The resulting dual-AAV systems, v1em and v3em PE-AAV, enable therapeutically relevant prime editing in mouse brain (up to 42% efficiency in cortex.

Lipid nanoparticle (LNP) and non-viral vectors, LNPs, which have proven clinical success in mRNA vaccines, have been adapted for delivery to the CNS by surface modification or via direct administrations; LNPs can carry editor-coding RNAs (mRNA) or RNPs, avoiding sustained expression that can increase immune risk. Recent reviews point to advances in the functionalization of LNPs to cross BBB and improve tropism, but neuronal efficiency and persistence are still variable (Vargas et al., 2024).

Second Guo et al., (2023), predominant concerns include off-target (unwanted genomic targets), Bystander edits (for BE, editing of adjacent bases within the window), mosaicism (partial editing between cells of the same tissue), and immune effects against viral components or exogenous proteins (Cas9, RT). Studies show that BE has a distinct profile of off-targets, including accidental

changes in DNA and, in some cases, deaminase-mediated edits in RNA. PE tends to produce fewer structural undesirables, but can still generate unwanted insertions/deletions and relies heavily on pegRNA design and host repair response. Modern protocols employ high-fidelity Cas variants, optimized gRNA/pegRNA designs, and deep sequencing pipelines to map off-targets (DNA-seq, RNA-seq, GUIDE-seq, Digenome-seq, etc.)

Additional technical limitations, variable efficiency per locus/genome, chromatin context dependence, need to optimize conditions for specific cell types (postmitotic neurons versus dividing cells), and regulatory challenges for permanent CNS interventions. Even with robust proofs of concept, the safety margin required for human interventions is high, requiring extensive genotoxicity, immunogenicity, and biodistribution evaluation panels (Schep et al., 2024).

Research perspectives, gaps and priorities.

Based on recent literature, the most relevant priorities and gaps to move BE/PE to the clinic in neurological diseases are: Robust and scalable delivery platforms for the CNS, developing optimized AAVs, functionalized LNPs, or novel nanostructures capable of crossing BBB with cellular selectivity (neuronal vs. glial), high efficiency, and lower immunogenicity. Engineering of functional and compact split-protein systems for PE is critical (An et al., 2024).

Better understanding of the cellular determinants of the editing result, investigate how DNA repair pathways, chromatin status, and cellular metabolism of neurons influence BE and PE results, to reduce variability and mosaicism; studies from 2024–2025 show that repair pathways strongly shape CBE outcome (Gu et al., 2025).

Strict off-target reduction and mapping, standardize sensitive experimental panels (genomics and transcriptomics) for off-target detection, and Bystander edits and to develop even more specific enzyme variants (desaminases/RT and high-fidelity Cas). Recent work proposes prime editors with minimized genomic errors and new tools to track off-targets (Chauhan, Sharp and Langer, 2025).

More predictive translational models, use of human brain organoids, multicellular co-cultures, animal models with human-like phenotype, and robust dose/time studies to evaluate long-term efficacy and safety. Inter-species and inter-model heterogeneity still hinders clinical extrapolations (Schene et al., 2020).

Ethical, regulatory and access aspects, permanent interventions in the brain raise questions about consent, reversibility, unknown long-term risks and equity in access. Policies and regulatory frameworks will need to evolve in parallel with scientific evidence to ensure that clinical trials are conducted in a safe and socially responsible manner (Wiley et al., 2025).

RESULTS

Table 1: Most relevant publications on the use of base editing and prime editing in neurological diseases.

Author	Year	Title of the work	Key findings
Sousa et al.	2025	In vivo prime editing rescues alternating hemiplegia of childhood in mice	saram prime editing and base editing to correct mutations in the ATP1A3 gene (which cause AHC) in human cell models and two murine models of HCA. The editing was efficient (up to ~48% in DNA, ~73% in mRNA), restored ATPase activity, improved motor and cognitive symptoms, reduced paroxysmal episodes, and significantly extended the lives of mice.
Matuszek et al.	2025	Base editing of trinucleotide repeats that cause Huntington's disease and Friedreich's ataxia reduces somatic repeat expansions in patient cells and in mice	They developed base editors (CBE and ABE) to introduce disruptions to the repeated trinucleotides (CAG in HTT, GAA in FXN) — simulating more stable alleles. In patient cells and in mice, this approach reduced the somatic expansion of repeats in the central nervous system.
BenDavid et al.	2024	Emerging Perspectives on Prime Editor Delivery to the Brain	Review article that discusses the challenges and strategies for delivering prime editors to the brain, especially considering barriers such as the blood-brain barrier. It points to nanomedicines and delivery systems (viral and non-viral) as promising avenues for neurological gene therapy.

Source: Authors, 2025.

DISCUSSION

The experimental studies by Sousa et al. (2025) and de Matuszek et al. (2025) cited in the Table, represent important preclinical milestones. Sousa et al. demonstrate in vivo correction of variants that cause Alternating Hemiplegia of Childhood (ATP1A3) using prime editing and base editing with relevant efficiencies and functional recovery in murine models. Matuszek et al. apply base editors to interrupt trinucleotide repeats (CAG/GAA), reducing somatic expansion in patient cells and in animal models of Huntington and Friedreich. The review by Bem-David et al. (2024) complements these results by discussing the practical challenges of delivering prime editors to the brain. These studies, taken together, show two complementary trajectories, such as the directional therapeutic application for severe point variants (PE/BE for ATP1A3) and the strategic modification of repetitive elements to attenuate progressive disease processes (BE for repeats).

The efficiency levels reported by Sousa et al., according to values in the range of tens of percent in DNA and even higher in RNA/protein, with phenotypic rescues, are consistent with the incremental progress observed since the original descriptions of BE and PE.

Komor et al. (2016) and Gaudelli et al. (2017) showed that base editors can generate high local efficiencies without DSBs, and Anzalone et al. (2019) demonstrated the versatility of Prime Editing for search-and-replace corrections. Recent in vivo results extend these principles where these authors have established in vitro capabilities and limitations, and Sousa and Matuszek validate therapeutic applications in nervous tissue, which confirms technological maturation, but with locus and vector-dependent efficiencies that vary greatly between studies. In other words, the 2025 findings follow the trajectory observed since 2016–2019, but now with in vivo proofs of concept that were previously mostly theoretical.

The main practical difference between the classic benchtop studies and those reported in

the table is the delivery solution. Komor et al. (2016), Gaudelli et al. (2017) and Anzalone et al. (2019) established the editors, however, in vivo translation requires delivery to the CNS and here the recent publications, including the studies in Table 1, which explore varied strategies such as neonatal intracerebral injection, split-intein AAVs, optimized LNPs or VLPs (virus-like particles).

Reviews and technical reports such as that of BenDavid (2024), containing work on split-AAV and VLPs, emphasize that the choice of delivery system determines effective efficiency, cell distribution, and immune safety, which explains why high in vitro efficiencies can fall in vivo or vary between brain regions. Thus, the positive findings of Sousa et al. (2025) and Matuszek et al. (2025) demonstrate that therapeutic edits in the CNS are feasible, but depend on delivery solutions that still require optimization for clinical scalability.

Pioneering work has warned of off-targets, bystander edits (especially for BE), and possible desaminase-mediated RNA edits. Later studies, as well as by the BE/PE authors themselves, invested in higher-fidelity variants and gRNA/pegRNA designs to mitigate these effects. The 2025 publications brought here continue this line as they report deep sequencing monitoring and phenotypic toxicity assessments, but also acknowledge limitations such as mosaicism, possible unintended edits, and immune response to Cas/vehicle remain real concerns.

Compared to methodological reviews and articles on off-target mapping that use tools such as GUIDE-seq, Digenome-seq, and RNA-seq, recent studies tend to present more complete safety panels, but still do not provide long-term follow-up time in humans. In short: there is clear progress in detecting and reducing adverse effects, but the evidence for long-term safety remains incomplete.

Results such as those of Sousa et al. (2025) on ATP1A3 correction with phenotypic improvement and Matuszek et al. (2025) on reducing somatic expansion represent crucial translational steps that show that gene edits can produce significant functional effects on the CNS. However, when contrasting with the literature that evaluates clinical applicability, and with the limits described by Anzalone (2019) and reviews on delivery, some gaps need to be filled before large clinical trials.

Cell distribution and heterogeneity of many diseases require correction in multiple cell types

such as neurons, astrocytes, microglia, and microglia, and preclinical studies often focus on specific regions or populations, since extrapolating to the human brain requires demonstrations of wide and controlled distribution (O'Carroll, Cook and Young, 2020). Therapeutic scale and window requiring neonatal corrections or early injections in murine models may not reflect efficacy in adult patients or in diseases with a narrow therapeutic window. This is remembered in reviews on translationality (Bunuales et al., 2024). Long-term follow-up data on persistence of editing, phenotypic stability, and late risk of tumorigenesis and cellular dysfunction need long-term studies in large models before human trials.

As the data in Table 1 fit the general picture and methodological recommendations, they show positive convergence with the findings that confirm that BE/PE are no longer only in vitro tools, but also work in vivo in the CNS with measurable therapeutic effects, endorsing the predictions made by Komor et al. (2025), Gaudelli et al. (2025), and Anzalone et al. (2025). This strengthens the case for regulatory advances and robust preclinical phase designs.

Regarding the need for an experimental standard, it is recommended that future studies publish standardized sets of safety data, such as databases of off-targets detected by multiple techniques, biodistribution by qPCR/sequencing and immune response and make direct comparisons between vectors and protocols, such as AAV split vs LNP Vs VLP), which is also suggested in the reviews of BenDavid (2024) and Kalter et al. (2025).

Research priorities are optimization of delivery to the CNS, engineering editors with less RNA activity, systematic study of mosaicism, and investigation in non-rodent models, such as non-human primates (when warranted) before clinical transition. These priorities already appear in both reviews and experimental articles.

CONCLUSION

The consolidation of base editing and prime editing technologies represents a significant

advance in the field of gene therapy applied to neurological diseases, offering tools capable of correcting mutations with high precision and minimal generation of double-strand breaks. The analyzed studies demonstrate that these platforms have already gone beyond the conceptual stage, achieving robust results in cellular and animal models for pathologies such as Alternating Hemiplegia of Childhood, Huntington's disease, and hereditary ataxias. The ability to modulate trinucleotide repeats, correct pathogenic variants, and restore complex cellular functions in the central nervous system indicates that next-generation editing systems have real potential for therapeutic interventions in conditions previously without effective treatment options. These advances reinforce the translational relevance of CRISPR-based tools and demonstrate that their continuous optimization can redefine the landscape of precision neurological medicine.

However, despite the promising progress, the transition of these technologies to clinical applications requires caution and systematic investigation of critical aspects still pending. Challenges include efficient and safe delivery to the CNS, mitigating risks associated with off-target edits and mosaicism, and the need for long-term safety evidence in more complex preclinical models. In addition, improvements in vectors, non-viral strategies, and higher-fidelity editor engineering are essential to expand clinical applicability. Thus, although base editors and prime editors have already demonstrated therapeutic feasibility, progress towards clinical practice depends on coordinated efforts that integrate technological innovation, rigorous functional validation, and biosafety assessment. As a result, these platforms emerge as central pillars for the future of neurological gene therapies, as long as they are supported by a solid, continuous and multidisciplinary research agenda.

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