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Commentary

CRISPR-based prime editing improves therapeutic outcomes for childhood alternating hemiplegia

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Editor's note: A commentary on "*In vivo* prime editing rescues alternating hemiplegia of childhood in mice"

Alternating hemiplegia of childhood (AHC) is an infrequent genetic pathology manifesting primarily as a neurodevelopmental disorder [1]. Affected infants exhibit recurrent paroxysmal manifestations within several months postpartum, including hemiplegia, dystonia, abnormal ocular movements, and seizures [2]. Additionally, patients may present with persistent hypotonia, developmental delay, and cognitive impairments [2]. Approximately 70% of AHC cases are linked to deleterious mutations in the *ATP1A3* gene [3], which encodes the α_3 subunit of the sodium–potassium pump (Na^+/K^+ ATPase), essential for neuronal homeostasis [4]. Dysfunction of ATP1A3 activity can lead to neuronal hyperexcitability or energy metabolism imbalances, resulting in episodic neurological symptoms and developmental anomalies [5]. Furthermore, *ATP1A3* mutations with D801N, E815K, and G947R account for >65% of AHC cases, with incidences of 36%, 22%, and 9%, respectively [1]. Previous studies have revealed a dominant-negative pathogenic mechanism associated with AHC-related *ATP1A3* mutations, wherein the aberrant ATP1A3 protein not only loses its intrinsic functionality but also impedes the functionality of other proteins [4]. This distinct pathological paradigm has posed significant challenges to the advancement of conventional gene therapy approaches. Currently, there are no curative or highly effective treatments available for this disease.

CRISPR-Cas-mediated platforms offer unprecedented tools for precise genomic editing, with the capacity to markedly enhance therapeutic strategies for genetic disorders [6]. Prime editing (PE), leveraging an engineered variant of the CRISPR-Cas9 system, employs a prime editor, which is a fusion of a catalytically inactive Cas9 endonuclease and a reverse transcriptase, along with a prime editing guide RNA (pegRNA) [7, 8]. The pegRNA encompasses sequences for both target-site recognition and the intended nucleotide alteration, which guides the prime editor to the specific location in the genome where the edit is to be made. The prime editor then makes a single-strand break in the DNA, and the reverse transcriptase utilizes the pegRNA as a template to synthesize the desired DNA sequence, effectively replacing the original sequence with intended modification in cells, animals, and human patients [7, 8]. A recent study published in *Cell* reported the use of CRISPR-mediated PE to repair mutations of AHC that rescued clinically significant phenotypes, encompassing the reestablishment of ATPase functionality, with alleviation of paroxysmal episodes, motor impairments, and cognitive deficits, alongside a substantial increase in lifespan of the subjects (Fig. 1) [3], representing a major advance in the design of treatments for numerous neurological disorders that have historically been deemed untreatable.

To enhance PE strategies for maximizing correction efficiency and minimizing indels, Sousa *et al.* optimized engineered pegRNA (epegRNA), an optional nicking guide RNA (ngRNA), reverse transcription template (RTT) lengths across various protospacers with PE6 variants [3]. This optimization achieved correction efficiencies and indel rates for the pathogenic alleles D801N, E815K, L839P, and G947R-C of 43% and 8%, 63% and 5.2%, 70% and 1%, and 74% and 4%, respectively, in induced pluripotent stem cell (iPSC) lines derived from patients with AHC (Fig. 1B). Nonetheless, unintended genetic modifications could occur due to off-target effects on sequences homologous to the target sequence. The likelihood of such inadvertent alterations necessitates rigorous vigilance in any prospective clinical implementation. Ensuring the safety of off-target effects is crucial for the clinical application of PE in treating AHC. Sousa *et al.* utilized CIRCLE-seq to assess off-target editing in AHC patient-derived iPSCs, revealing that PE strategies exhibited fewer genuine off-target loci (8 out of 457 assessed sites) and lower off-target editing levels [3]. These findings from AHC patient-derived iPSCs substantiate the proof-of-concept for efficient PE-mediated correction of mutations responsible for the majority of *ATP1A3*-associated AHC cases.

To further investigate whether PE gene editing can alleviate the pathology of AHC *in vivo*, Sousa *et al.* used a v1em dual adeno-associated virus (AAV) system, used in U.S. Food and Drug Administration-approved drugs to treat spinal muscular atrophy (SMA), to deliver PE for treating two AHC mouse models, which carry the *Atp1a3* D801N and E815K mutation found in human patients (Fig. 1C). On the day of the mice's birth, the

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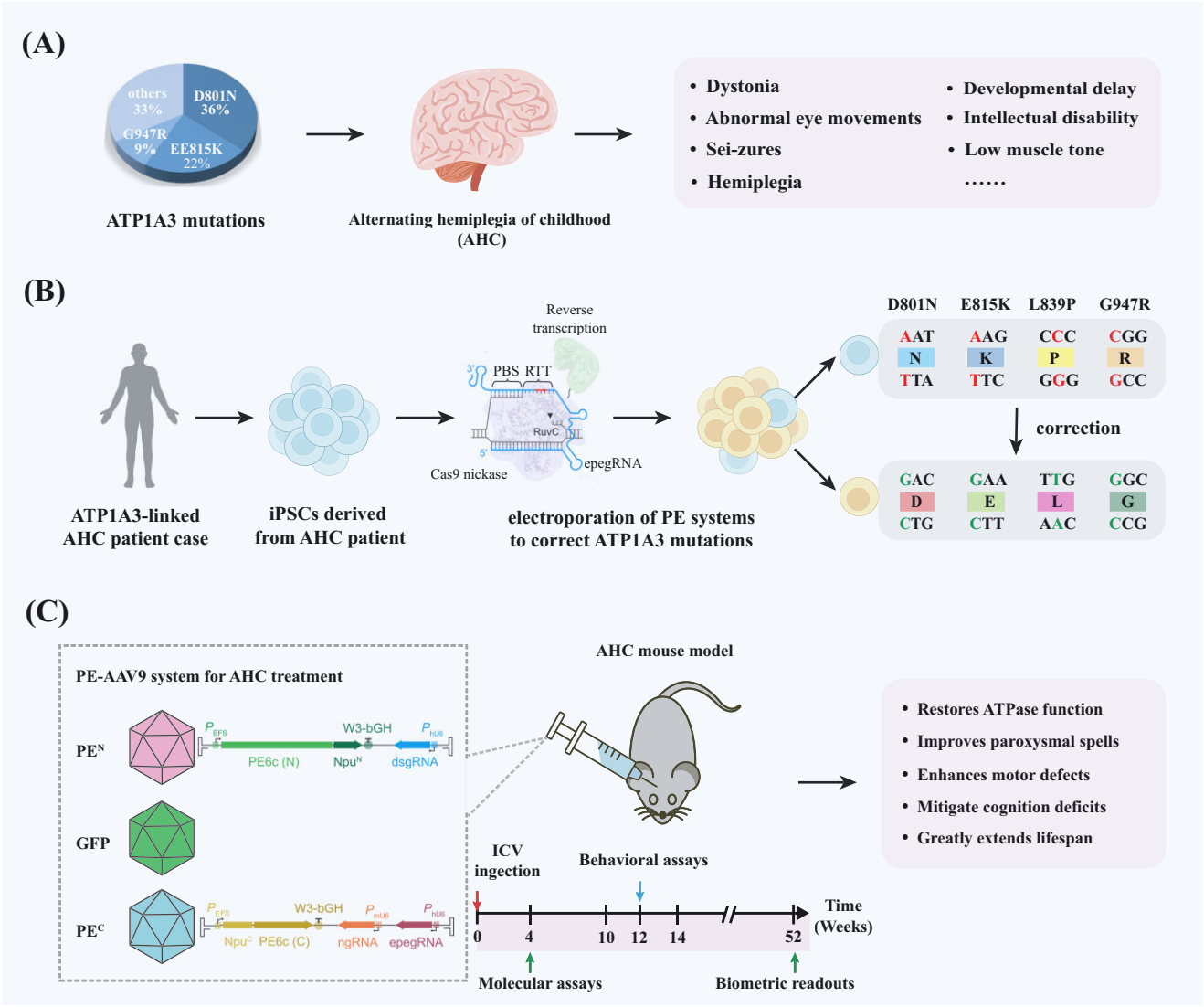

Figure 1. Programmable PE rescues AHC. (**A**) Base mutation in *ATP1A3* gene results in impaired wild-type ATP1A3 activity, which causes AHC. (**B**) PE corrects human *ATP1A3* mutation in induced pluripotent stem cells (iPSCs) derived from AHC patients. Using the electroporation-delivered prime editor, engineered pegRNA and ngRNA target *ATP1A3* mutations, resulting in gene editing of the *ATP1A3* loci, subsequently rescuing ATP1A3 activity. (**C**) PE-AAV9 treatment in an AHC mouse model with *ATP1A3* D801N and E815K mutation. Sousa *et al.* [3] used the adeno-associated virus (AAV) vector for co-packaged PE systems to correct mutation in the central nervous system, resulting in restoration of ATPase activity, amelioration of paroxysmal spells, motor defects, and cognition deficits, and a dramatic extension of animal lifespan. RTT, Reverse transcription template. PBS, primer binding site. GFP, Green fluorescent protein. Adapted from Sousa et al., Cell, 2025 [3].

D801N-PE-AAV9 and E815K-PE-AAV9 systems were administered via intracerebroventricular (ICV) injection into D801N and E815K mice, respectively (Fig. 1C). Sousa *et al.* achieved a correction rate of up to 48% and 27% at the DNA level and 73% and 68% at the mRNA level in the central nervous system (CNS) of D801N and E815K mice [3]. This restoration of ATP1A3 Na^+/K^+ ATPase activity in the hippocampus improved seizure episodes, motor deficits, and cognitive impairments, significantly extending the lifespan of *ATP1A3* mutant mice (Fig. 1C). Together, PE-AAV9 treatment attenuates typical symptoms of AHC disease *in vivo*, which may rescue neurological disease in animals.

PE could have greater advantages over current conventional pharmacological therapies in directly tackling the underlying etiological factors of diseases. Nevertheless, determining the optimal deployment of PE through adeno-associated viral vectors or alternative delivery mechanisms remains a critical question. Which specific tissues and organs are amenable to targeted intervention? Sousa *et al.* employed AAV9 due to its neurotropic transduction characteristics to administer PE systems to the neurons and glial cells in ATP1A3 mutant mice [3]. The application of AAV9 in gene therapy marks notable progress in the management of neurological diseases, especially AHC and SMA. Its proficiency in efficiently transducing neurons and traversing the blood–brain barrier has established it as a formidable asset in expanding the domain of gene therapy for CNS disorders. The achievements observed in AHC not only underscore the therapeutic promise of PE-AAV9 but also set the groundwork for forthcoming treatments aimed at other neurodegenerative diseases.

To what extent can genetic defects be rectified by PE-AAV9 or other CRISPR-mediated gene editing technologies? Achieving high editing efficiency may be paramount, especially in endeavors to treat genetic disorders. For example, contrary to the

advantages observed with PE, the administration of the intact *ATP1A3* gene through AAV9 delivery fails to ameliorate phenotypic manifestations pertinent to AHC pathology [9]. It is plausible that *ATP1A3* transgene expression was inadequate to counteract the hypothesized dominant-negative impact of the pathogenic ATP1A3 variant, thereby lacking therapeutic efficacy. Sousa *et al.* documented a 48% editing efficiency by PE-AAV9, which resulted in a 6-fold increase in healthy *ATP1A3* expression compared to the pathogenic *ATP1A3* expression in *ATP1A3*-treated D801N mice. This finding suggests that absolute correction is not a requisite for therapeutic advantage, but underscores the critical necessity for highly effective treatment methodologies, thereby opening the possibility of revisiting this strategy for other pathologies as well.

A critical inquiry pertains to the administration routes for gene editing therapies and how to optimize these strategies. Currently, delivery modalities encompass intravenous injection, localized injection, oral administration, inhalation, and biological vectors such as AAVs, among others. Despite Sousa *et al.*'s utilization of ICV injection for AAV9-mediated AHC PE therapy, alternative methodologies like intrathecal injection of AAV9 or intravenous administration of blood–brain barrier-penetrating AAV capsids can also achieve safe and effective gene transduction within the CNS [10]. Each delivery route presents unique benefits and limitations. We need to select the most appropriate method based on specific contexts, considering factors such as therapeutic efficacy, safety profiles, and patient compliance.

As with all gene therapies, determining the optimal age for intervention and assessing the adequacy of long-term administration of gene editors remains paramount. The clinical diagnosis of AHC typically spans several months to years, depending on the manifestation and frequency of symptoms, as well as the observations and clinical judgments of the physician. Therefore, for mice, the treatment age of newborns is much lower than the diagnostic age for numerous prevalent human diseases. This necessitates further research into later therapeutic windows to ascertain the effective time threshold for AHC gene editing and age-dependent phenotypic reversibility. Moreover, in animal experiments, it is challenging to benchmark the age of a given mouse against the corresponding human age. Finally, to utilize base editing in AHC, the safety of such interventions and the effective temporal window of genomic correction must be ensured. If this method effectively rectifies the pathogenic mutations in critical tissues associated with AHC, it holds immense promise for enhancing health, extending lifespan, and improving the quality of life for individuals harboring this mutation.

Taken together, the preliminary findings by Sousa *et al.* indicate that the administration of PE-AAV9 system therapy is sufficient to rectify *ATP1A3*-associated AHC phenotypes and significantly prolong lifespan in a murine model. These results imply a promising avenue for future gene editing therapies targeting human AHC and other genetic pathologies. Beyond AHC, PE holds broad significance for the field of gene therapy. With its ability to precisely correct pathogenic variants while minimizing indel formation, PE provides a versatile platform that could complement or even surpass the conventional approaches of CRISPR-Cas9 and base editing. Continual optimization of PE could therefore expand the therapeutic landscape for a wide range of monogenic and complex genetic diseases, underscoring its transformative potential in precision medicine.

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Conflict of interest

The authors declare no conflict of interest.

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