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Development and Therapeutic Applications of Precise Gene Editing Technology*

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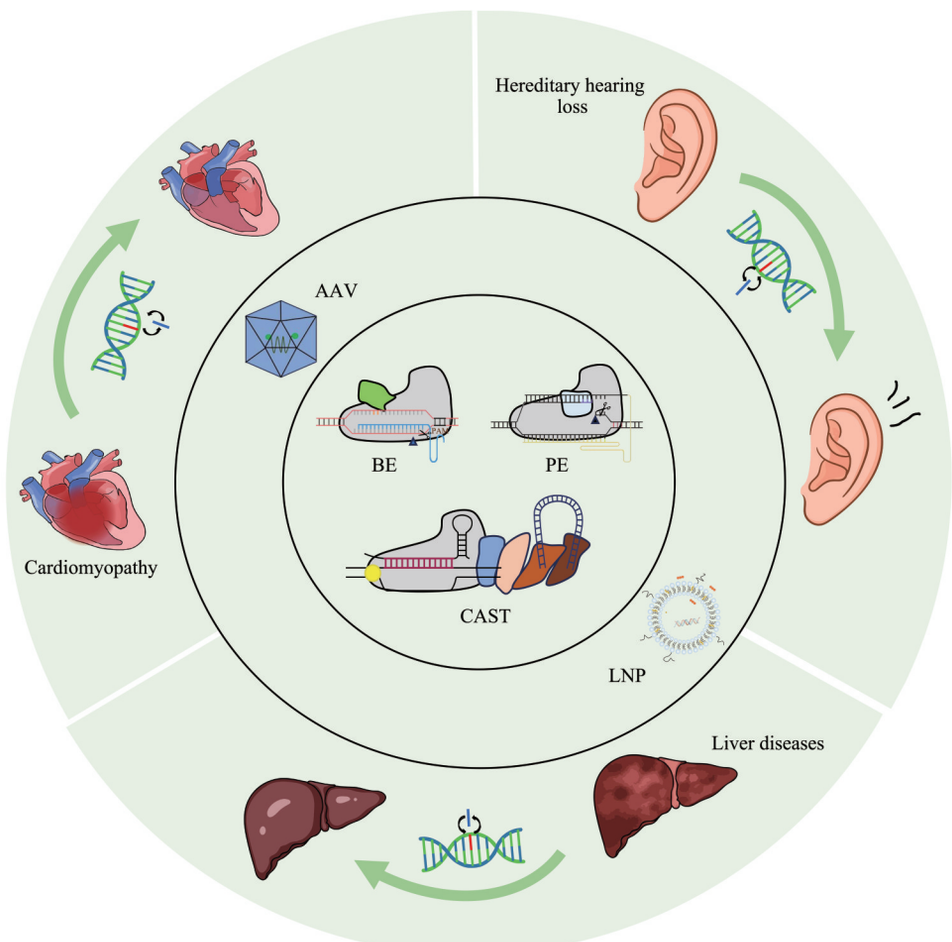
Graphical abstract



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Abstract The advent of gene editing represents one of the most transformative breakthroughs in life science, making genome manipulation more accessible than ever before. While traditional CRISPR/Cas-based gene editing, which involves double-strand DNA breaks (DSBs), excels at gene disruption, it is less effective for accurate gene modification. The limitation arises because DSBs are primarily repaired *via* non-homologous end joining (NHEJ), which tends to introduce indels at the break site. While homology-directed repair (HDR) can achieve precise editing when a donor DNA template is provided, the reliance on DSBs often results in unintended genome damage. HDR is restricted to specific cell cycle phases, limiting its application. Currently, gene editing has evolved to unprecedented levels of precision without relying on DSB and HDR. The development of innovative systems, such as base editing, prime editing, and CRISPR-associated transposases (CASTs), now allow for precise editing ranging from single nucleotides to large DNA fragments. Base editors (BEs) enable the direct conversion of one nucleotide to another, and prime editors (PEs) further expand gene editing capabilities by allowing for the insertion, deletion, or alteration of small DNA fragments. The CAST system, a recent innovation, allows for the precise insertion of large DNA fragments at specific genomic locations. In recent years, the optimization of these precise gene editing tools has led to significant improvements in editing efficiency, specificity, and versatility, with advancements such as the creation of base editors for nucleotide transversions, enhanced prime editing systems for more efficient and precise modifications, and refined CAST systems for targeted large DNA insertions, expanding the range of applications for these tools. Concurrently, these advances are complemented by significant improvements in *in vivo* delivery methods, which have paved the way for therapeutic application of precise gene editing tools. Effective delivery systems are critical for the success of gene therapies, and recent developments in both viral and non-viral vectors have improved the efficiency and safety of gene editing. For instance, adeno-associated viruses (AAVs) are widely used due to their high transfection efficiency and low immunogenicity, though challenges such as limited cargo capacity and potential for immune responses remain. Non-viral delivery systems, including lipid nanoparticles (LNPs), offer an alternative with lower immunogenicity and higher payload capacity, although their transfection efficiency can be lower. The therapeutic potential of these precise gene editing technologies is vast, particularly in treating genetic disorders. Preclinical studies have demonstrated the effectiveness of base editing in correcting genetic mutations responsible for diseases such as cardiomyopathy, liver disease, and hereditary hearing loss. These technologies promise to treat symptoms and potentially cure the underlying genetic causes of these conditions. Meanwhile, challenges remain, such as optimizing the safety and specificity of gene editing tools, improving delivery systems, and overcoming off-target effects, all of which are critical for their successful application in clinical settings. In summary, the continuous evolution of precise gene editing technologies, combined with advancements in delivery systems, is driving the field toward new therapeutic applications that can potentially transform the treatment of genetic disorders by targeting their root causes.

Key words precise gene editing, CRISPR/Cas system, base editing, prime editing, gene therapy
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Creating animal models with genetic mutations has long been the gold standard for studying many human diseases[1]. These models were originally generated by gene targeting technology, which relies on homologous recombination and culturable embryonic stem cells[1]. The concept of gene editing is established when programmable nucleases, including zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and clustered regularly interspaced short palindromic repeats (CRISPR) -associated nucleases (Cas), are used to recognize specific genomic sequences and generate double-strand breaks (DSBs) [2-3]. DSBs dramatically increase the frequency of homologous recombination, enabling the construction of animal models independent of embryonic stem cells[4]. The past decade has undoubtedly been the era of CRISPR/Cas-based gene editing, which shows excellent advantages over other nucleases due to its easy programming and broad accessibility.

Traditional CRISPR/Cas-based gene editing excels at gene disruption, as DSBs are repaired *via* non-homologous end joining (NHEJ), resulting in high frequencies of insertions or deletions[5-6]. Though precise gene editing can be achieved by homology-directed repair (HDR) when donor DNA templates exist, the presence of DSBs often causes undesired damage to the genome[7]. Additionally, HDR is largely restricted to the G2 and S phases of cell cycle, and does not play a role in post-mitotic cells[8]. Therefore, developing precise gene editing technology independent of DSB and HDR is essential. The latest

precise gene editing tools use nuclease-deficient Cas proteins, Cas nickase (nCas) or dead Cas (dCas), which retain their genome-targeting ability but fail to generate DSBs[9]. Base editors (BEs) and prime editors (PEs) have been developed and continuously optimized by fusing nCas with deaminases and reverse transcriptases, respectively[10-12]. They can produce desired genomic modifications without needing DSBs and HDR and can be applied to dividing and nondividing cells[10-12]. CRISPR-associated transposases (CAST) is a recently developed technology that can catalyze the integration of large DNA fragments into the genome, further enriching the precision gene editing toolbox[13].

Optimizing delivery vehicles is crucial for improving the efficiency and safety of gene editing. Commonly used delivery vehicles include viral vectors (such as adeno-associated virus (AAV)) and non-viral vectors (such as lipid nanoparticles (LNPs)). Viral vectors are widely used due to their high transfection efficiency, but they may have immunogenicity and potential carcinogenic risks[14]. Non-viral vectors typically have lower immunogenicity but their transfection efficiency may be lower[15]. With advancements in technology, more efficient and safe delivery vehicles have been developed and optimized, driving the development and application of gene editing technology *in vivo*.

Gene editing ignites hope for patients with genetic disorders, providing them with a path to a cure for their diseases. On December 8, 2023, the US Food and Drug Administration (FDA) approved the first CRISPR-based gene editing therapy, Casgevy, for the treatment of sickle cell diseases. Casgevy uses CRISPR/Cas9 to edit the enhancer region and suppress the expression of BCL11A, thereby increasing fetal hemoglobin expression levels in $CD34^+$ hematopoietic stem cells of the patients[16-17]. In addition to Casgevy, many other gene editing therapies are in development or in clinical stages[18]. The advent and continuous improvement of precise gene editing suggest that the goal of curing the root causes of disease is being achieved at an accelerated pace.

This review highlights the recent advances in precise gene editing technologies and delivery systems, and summarizes the applications of these precise tools in treating genetic disorders.

1 Precision gene editing tools

Nuclease-mediated gene editing can achieve precise genomic modification by relying on DSB and HDR, which have played pivotal roles across various research fields. However, DSB represents a double-edged sword for cells, which can greatly improve the efficiency of homologous recombination on the one hand and cause uncontrollable damage to the genome on the other[7]. The dependence on HDR also renders this precision editing ineffective in terminally differentiated cells[8]. These shortcomings limit the clinical application of this gene editing technology and also promote the development of a new generation of precision gene editing tools that do not rely on DSB and HDR.

BEs are a class of CRISPR-based precise gene editing tools that can mediate the conversion of one nucleotide to another at a target genomic locus. BEs recognize and target specific genomic sequences *via* nCas, generating a single-strand break. The conversion of nucleotides is then realized by a deaminase and other regulatory elements fused to nCas[19] (Figure 1a). The initial two kinds of BEs are cytosine base editors (CBEs) and adenine base editors (ABEs), which can achieve the transition of C-to-T and A-to-G, respectively[10-11]. Since their emergence, CBEs and ABEs have been continuously optimized. To comprehensively address concerns regarding off-target editing of BEs, a transformer base editor (tBE) system has been recently developed by introducing a cleavable deoxycytidine deaminase inhibitor (dCDI) [20]. The tBE system is inactive at off-target sites, but it is activated only at specific target sites by clearing off the dCDI domain. Through structure-guided design, an ABE9 variant has been developed by introducing N108Q and L145T mutations into ABE8e, which refines the editing window to the A5 position of the protospacer sequence and reduces both adenine and cytosine bystander editing[21]. To reduce the Cas9-dependent or Cas9-independent DNA/RNA off-target editing of CBE, ABE has also been repurposed for cytosine editing, resulting in the adenine deaminase TadA-8e-derived cytosine base editor (Td-CBE) [22]. Nowadays, BEs can mediate not only nucleotide transitions but also certain kinds of nucleotides. C-to-G base editor (CGBE) replaces the uracil-DNA glycosylase inhibitor (UGI) of CBE with

a uracil-DNA glycosylase (UNG), resulting in the C-to-G transversion[22]. A programmable A-to-Y base editor (AYBE) is developed by fusing an ABE with hypoxanthine excision protein N-methylpurine DNA glycosylase (MPG), which can excise hypoxanthine and catalyze A-to-C and A-to-T transversions[23]. By fusing an ABE8e with mouse alkyladenine DNA glycosylase (mAAG), an A-to-C base editor (ACBE) is created and can precisely install A-to-C transversions[24]. In addition, by fusing two deaminases simultaneously with nCas9, a dual BE is created, allowing for C-to-T and A-to-G conversions at the same genomic target site and significantly expanding the practicality and adaptability of BEs[25].

CRISPR-derived PEs are another class of precise gene editing tools independent of DSB and HDR. PE is composed of nCas-conjugated engineered reverse transcriptase and prime editing guide RNA (pegRNA)[12, 26] (Figure 1b). The pegRNA is based on the sgRNA and has two additional RNA sequences: a prime binding site (PBS) and a reverse transcription template (RTT) storing the editing information of targeting site. nCas9 binds to DNA under the guidance of the pegRNA and cleaves a single strand of DNA. The PBS hybridizes to the 3' single-stranded DNA, allowing the reverse transcriptase to synthesize the edited strand with RTT as a template. PE can achieve all possible single base conversions and allows the precise introduction of <40 bp insertions or <80 bp deletions[12]. The PE system is optimized by engineering the PE protein and pegRNA architecture, generating PEmax and epegRNA that significantly increase PE editing efficiency[27-28]. Additional strategies to improve PE outcomes have also been conducted by fusing an engineered DNA mismatch repair-inhibiting protein. To inhibit the cyclization of classical pegRNA, which may hinder editing, a 20-nt Csy4 RNase recognition site is fused to the 3' end of the pegRNA to construct an extended pegRNA, which is then fused with a nick-sgRNA. The PE protein is fused with a Csy4 RNase that can cleave at the Csy4 recognition site to separate the extended pegRNA and nick-sgRNA. They constitute the enhanced prime editing system (ePE), markedly boosting the PE editing efficiency[29]. Using a phage-assisted evolution selection for PE with smaller reverse transcriptases, several compact PE tools have been developed that exceed PEmax in efficiency and are more compatible with AAVs[30]. Various PE variants with PAM flexibility have also been generated to expand the range of genomic targets of Pes[31]. When conjugated to serine recombinases, PEs are able to integrate large DNA sequences into the target genomic sites. These approaches include prime-editing-assisted site-specific integrase gene editing (PASSIGE) [32] and programmable addition *via* site-specific targeting elements (PASTE) [33]. In a recent article, phage-assisted evolution has been used to improve Bxb1 recombinase activity, thus significantly increasing the targeted integration efficiencies of PASSIGE[34]. Of note, a novel PE-based tool, named amplification editing, enables programmable DNA duplication up to 100 Mb, expanding the scope of precise gene editing to chromosome scale[35].

The CAST system is a recently developed CRISPR-Cas system associated with the Tn7 transposon found in cyanobacteria[36]. This system combines the functions of CRISPR-Cas and transposons, allowing large DNA fragments to be inserted into specific target sites through an RNA-guided process. Currently, Type I-F and Type V-K CAST systems have been successfully adopted to insert donor DNA into specific genomic loci in mammalian cells[37-38]. The two CAST systems have distinct characteristics. Type I-F CAST exhibits higher on-target specificity and product purity than type V-K CAST, whereas type V-K CAST has the advantage of its compact size. To improve integration specificity and product purity of type V-K CAST, a Homing Endonuclease-assisted Large-sequence Integrating CAST-compleX (HELIX) system has been developed by fusing a nicking homing endonuclease to TnsB, enabling targeted large DNA insertions in human cells with integration efficiencies of less than 0.05%[37]. As for type I-F CAST, two systems from *V. cholerae* and *Pseudoalteromonas* have been optimized through protein and gRNA design to achieve large genomic insertions in mammalian cells with single-digit efficiency[38]. Despite their low efficiencies, the application of CAST systems in eukaryotic gene editing offers infinite possibilities for future gene therapy.

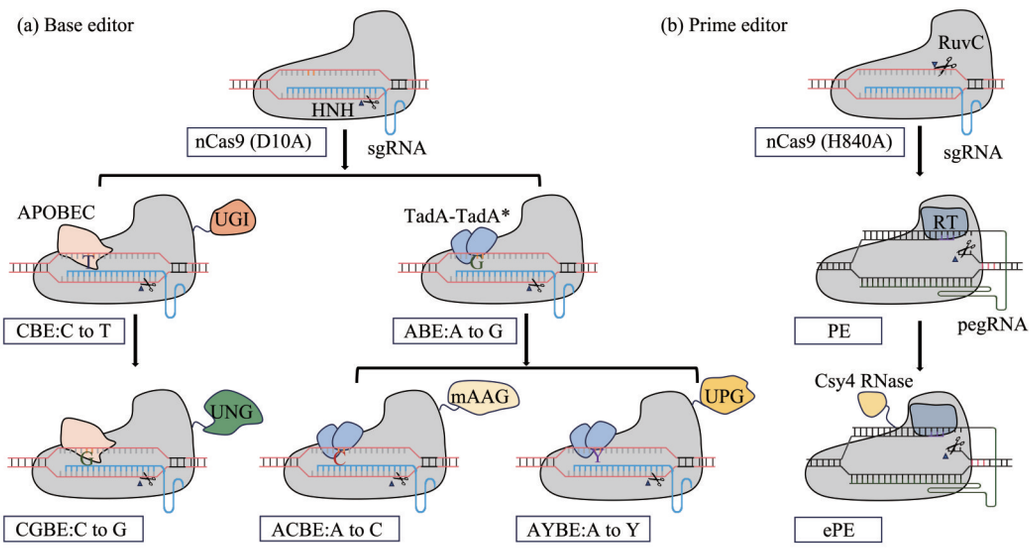

Fig. 1 Schematic of CRISPR−based base editors (a) and prime editors (b)

BE, base editor; HNH, His-Asn-His endonuclease; APOBEC, apolipoprotein B mRNA editing catalytic polypeptide-like; UGI, uracil-DNA glycosylase inhibitor; UNG, uracil-DNA glycosylase; TadA, deoxyadenosine deaminases; mAAG, mouse alkyladenine DNA glycosylase; MPG, N-methylpurine DNA glycosylase; PE, prime editor; RuvC, homology domain of UV-sensitive gene product C activity for resolving Holliday junction; RT, reverse transcriptase.

2 Delivery systems

Safe and efficient delivery of gene editors to target tissues and cells is one of the key and challenging factors for their therapeutic applications. A suitable delivery system should strike a balance between maximizing editing efficiency and minimizing off-target effects. A good delivery method can bring great advantages to gene editing. In recent years, various viral and non-viral vectors have been continuously developed and improved.

AAVs are small viruses with a diameter of approximately 26 nm and are favored in the field of gene therapy because of their low immunogenicity, low gene-integration capacity, long-time expression for carried exogenous genes, and cell-type-specific infection[14]. Several AAV-based gene therapy drugs have been approved by the FDA, and others have entered the clinical and preclinical stages. The tropism of an AAV to specific tissues or cells depends on its serotype[39]. Several novel AAV vectors have been developed in recent years. AAV-X1 variants identified by directed evolution and semi-rational engineering can specifically and efficiently transduce brain endothelial cells following systemic administration to mice[40]. AAV-MaCPNS1 and AAV-MaCPNS2 variants evolved from AAV9 efficiently transduce the peripheral nervous systems in rodents and both the central and peripheral nervous systems in non-human primates (NHPs) after intravenous administration[41]. By screening about 100 cell-penetrating peptides (CPPs) and inserting them into various AAVs, a recombinant AAV is named AAV. CPP. 16 has been created and shown an enhanced ability to cross the blood-brain barrier[42]. By using structure-guided evolution, an AAV variant termed Ark313 is obtained, which exhibits high transduction efficiency in mouse T cells and opens avenues in T cell gene editing[43].

Non-viral vectors are progressively commercialized due to their lower immunogenicity, easier assembly, and higher gene packaging capacity[44]. Lipid nanoparticle systems are one of the most promising systems for realizing the clinical potential of genetic drugs and are widely accepted with their successful application in mRNA vaccines for COVID-19. The inhalable LNP delivery system RCB-4-8 LNP enables the pulmonary delivery of Cas9 mRNA through the trachea, achieving editing

efficiency comparable to that of dual AAV vector delivery[45-46]. Systemic administration of LNPs is naturally enriched in the liver, making non-liver gene editing challenging. The emergence of SORT-LNP has broken the limitations of traditional LNP delivery technology through intravenous injection and achieved specific mRNA delivery to organs such as the lungs and spleen[47]. Currently, SORT-LNP has been gradually applied in the treatment of various mouse disease models, such as B-cell lymphoma and sickle cell disease[47-48].

3 Therapeutic applications of precision editing technology

The introduction of gene editing technology has had a landmark impact on the treatment of human genetic diseases. According to their principles, base editing and prime editing technology can directly correct the expression of disease-causing genes, offering the potential for cures rather than mere symptom alleviation. These technologies have already demonstrated significant progress in treating various diseases in preclinical studies (Table 1).

3.1 Cardiomyopathy

Cardiomyopathies account for about 17 million deaths per year and are estimated to affect about 1−2 per 1 000 people worldwide. Compared to other forms of cardiac gene therapy that are limited by difficult-to-control expression levels and potential compensatory mechanisms, cardiac gene editing is more precise, permanent, and allows for direct editing of disease-causing genes. Recent studies have revealed successful application of cardiac gene editing in several models of cardiomyopathy.

Dilated cardiomyopathy (DCM), which is marked by impaired systolic function, is a major contributor to heart failure[49]. Mutations in the RNA binding motif protein 20 (*RBM20*), a striated muscle-specific splicing factor, are common causes of familial DCM. Researchers have used ABE and PE separately to correct the p. R634Q and p. R636S pathogenic mutations in human iPSC-derived cardiomyocytes. In an *RBM20* R636Q mutant mouse model, delivering ABE components restores cardiac function and corrects the cardiac transcriptional profile, highlighting the therapeutic potential of DCM[50].

Hypertrophic cardiomyopathy (HCM) is often caused by mutations in cardiac sarcomeric proteins, and over one-third of HCM-causing variants occur in the myosin heavy chain 6 (*MYH6*) gene that encodes the β-myosin heavy chain. The heterozygous *MYH6* p. R403Q missense variant is associated with a high incidence of HCM. A previous study has demonstrated that correcting the mutation in zygotes and embryos of p. R403Q HCM mice using the ABEmax-NG system reduces the expression levels of mutant RNA and rescues the HCM phenotype in postnatal mice and their offspring[51]. Sysmetic AAV delivery of ABEmax-VRQR or ABE8e targeting the mutant site has successfully corrected the *MYH6* p. R403Q variant in both human cell models and mouse models, significantly reducing pathological changes and restoring normal cardiac function[52-53].

Base editing can also be effective in treating other cardiomyopathies. Pathogenic variants in *SCN5A* can cause long QT syndrome type 3, a life-threatening genetic disease. Using dual AAV delivery of split ABE into the hearts of mice carrying *Scn5a* p.T1307M mutation corrects the variant *Scn5a* allele, thus ameliorating arrhythmia phenotypes in mice, and offering a strategy for the treatment of hereditary arrhythmias[54]. An ABE-mediated gene-editing strategy has been employed in the mouse germline to ablate the autophosphorylation site of CaMKIIδ, which is protective in afterload-induced heart failure. This suggests that reversing CaMKIIδ overactivation using base-editing therapies would provide a new direction for the treatment of cardiomyopathies[55]. Duchenne muscular dystrophy (DMD) is a disease caused by a deficiency of myotonic dystrophy proteins, characterized by progressive degeneration of the heart and skeletal muscles[56]. Studies have demonstrated that base and prime editing can effectively modify exon skipping, splice sites, or reframe the open reading frames in the dystrophin gene, restoring dystrophin expression and alleviating symptoms in preclinical models[56-57].

3.2 Liver diseases

Correcting mutations through gene editing techniques holds great promise for effectively treating liver diseases. In recent years, development of precise gene editing tools demonstrates significant potential in addressing liver diseases.

Hereditary tyrosinemia type 1 (HT1) is a fatal genetic disease caused by a deficiency in fumarylacetoacetase (FAH), which leads to the accumulation of toxic metabolic intermediates and

severe liver damage or liver failure. CBE has been utilized to disrupt the hydroxyphenylpyruvate dioxygenase (Hpd) gene by inducing a premature termination codon in Fah knockout mice. In utero, Hpd base editing improves liver function and rescues the lethal phenotype of Fah knockout mice, implying a potential new therapeutic approach for selected congenital genetic disorders[58]. In another mouse model harboring a homozygous mutation that causes enon skipping of *Fah* gene, tail vein injection of plasmid DNA encoding ABE partially corrects the mutant site, restores splicing and Fah expression, and rescues liver disease phenotype[59].

Phenylketonuria (PKU) is a widely studied autosomal recessive metabolic liver disease resulting from mutations in the phenylalanine hydroxylase (*PAH*) gene, the deficiency of which leads to decreased metabolism of phenylalanine (L-Phe) and systemic hyperphenylalaninemia[60]. AAV-mediated delivery of intein-split CBE systems through intravenous injection achieves correction and restoration of PAH enzyme activity, and blood L-Phe levels as well as the reversion of the light fur phenotype in Pahenu2 mice harbor a *Pah* p. F263S mutation[61]. The humanized mice with *Pah* p. R408W mutation exhibit a phenotype resembling that of PKU patients. LNP-mediated delivery of a selected ABE mRNA/guide RNA combination into mice is sufficient to edit *Pah* p. R408W in the liver, normalizing blood L-Phe levels within 48 h[62]. Prime editing has also been verified to be effective in treating PKU. By developing a size-reduced PE lacking the RNaseH domain for adeno-associated virus-mediated delivery into the liver, It is shown that average correction efficiencies of 11.1% in neonate Pahenu2 mice leads to therapeutic reduction of blood L-Phe[63].

BEs are also applied to correct other types of liver diseases. Mutation of telomerase reverse transcriptase (TERT) is associated with hepatocellular carcinoma (HCC). Injection of AAV expressing sgRNA-guided CjABE can effectively inhibit the growth of liver tumors harboring TERT promoter-124 C→T mutation and prolong the survival time of mice, suggesting the potential of base editing-mediated correction of TERT promoter mutations in treating HCC[64]. Chronic hepatitis B virus (HBV) infection remains a global health problem that lacks effective treatments. CBEs have been used to abrogate hepatitis B virus (HBV) replication and antigen production in cell culture and in preclinical mouse models, indicating the effective abrogation of HBV replication and silencing of viral protein expression[65].

3.3 Hereditary hearing loss

Hereditary hearing loss affects 1−3 per 1 000 newborns worldwide, severely impairing children's communication and cognitive development. Gene editing has shown promise in correcting genetic mutations responsible for hearing loss. In 2020, a base editing strategy was developed to treat Baringo mice, which carry a recessive, loss-of-function point mutation p. Y182C in transmembrane channel-like gene family 1 (Tmc1) that causes deafness. Delivery of CBE by dual AAVs results in up to 51% correction of the mutation and restoration of sensory conduction function and morphology in some inner hair cells in mice[66]. Mutations in the *PCDH15* gene can cause usher syndrome type 1F (USH1F), characterized by congenital lack of hearing and balance, as well as progressive loss of vision. Delivery of dual AAVs carrying a split-intein ABE into a humanized mouse model for USH1F (*Pcdh15* p. R245X) is capable of reversing the R245X mutation to restore the *Pcdh15* sequence and hearing function, demonstrating the ability of an ABE to treat USH1F[67].

Table 1 Applications of precise gene editing in disease treatment

Mutation	Disease	Base editor	Delivery carrier	Injection method	Reference
Rbm20 p.R636Q	DCM	ABEmax	AAV	Intraperitoneal injection	［50］
Myh6 p.R404Q	HCM	ABEmax-NG	AAV	Vitelline vein injection	［51］
Myh6 p.R403Q	HCM	ABE8e-NG	AAV	Thoracic cavity injection	［52］
Myh6 p.R403Q	HCM	ABEmax-VRQR	AAV	Thoracic cavity injection	［53］
Scn5a p.T1307M	LQT3	ABEmax	AAV	Intraperitoneal injection	［54］
Camk2d p.M281V	IR	ABE8e-SpRY	AAV	Intracardiac injection	［55］
Dmd^{E4*}	DMD	eTAM	AAV	Intraperitoneal injection	［56］

Continued to Table 1

Mutation	Disease	Base editor	Delivery carrier	Injection method	Reference
Dmd $^{\Delta E51}$	DMD	ABEmax-NG	AAV	Intramuscle injection	［57］
Fah ^{5981SB}	HTI	ABE6.3	LNP	Tail vein injection	［59］
Pah p.F263S	PKU	nSaKKH-BE3	AAV	Tail vein injection	［61］
Pah p.R408W	PKU	ABE8.8-SpRY	LNP	Retro-orbital injection	［62］
Pah ^{enu2}	PKU	PE2-P.1153	ADV	Tail vein/temporal vein injection	［63］
Tmc1 p.Y182C	HL	AID-BE3.9max	AAV	Inner-ear injection	［66］
Pcdh15 p.R245X	USHIF	ABE8e/ABEmax	AAV	Round window membrane injection	［67］

Rbm20, RNA binding motif protein 20; *Myh6,* myosin heavy chain 6; *Scn5a*, sodium channel protein type 5 subunit alpha; *Dmd*, dystrophin; DMD, Duchenne muscular dystrophy; *Fah*, fumarylacetoacetate hydrolase; *Pah*, phenylalanine hydroxylase; ADV, adenovirus; *Tmc1*, transmembrane channel-like 1; *Pcdh15*, protocadherin-15; DCM, dilated cardiomyopathy; HCM, hypertrophic cardiomyopathy; LQT3, long QT syndrome type 3; IR, ischemia reperfusion; HTI, hereditary tyrosinemia type I; PKU, phenylketonuria; HL, hearing loss; USHIF, Usher syndrome type 1F; AAV, adeno-associated virus; LNP, lipid nanoparticle.

4 Conclusion

This review summarizes the evolution of precise gene editing technologies and their applications in disease research and therapy. Compared to traditional gene editing methods, novel CRISPR-based precise gene editing technologies do not necessitate the introduction of DSBs or donor templates at the target site, thus achieving more accurate and safer editing, reducing adverse effects, and significantly impacting life sciences and medicine. However, despite the great potential of these technologies in therapeutic applications, several challenges must be addressed for their successful clinical implementation.

One of the most pressing concerns is the safety of these technologies, particularly the risk of off-target effects, which could lead to unintended genetic modifications with potentially harmful consequences, such as chromosomal alterations or the disruption of essential genes[68]. Prolonged *in vivo* expression of gene editing tools further increases the likelihood of genomic damage, metabolic dysfunction, and the risk of tumorigenesis[69]. Additionally, the efficiency of current gene editing tools, such as PEs and CASTs, still requires significant improvement to meet therapeutic standards. Another critical area that needs advancement is the delivery systems for gene editors. Despite progress in viral and non-viral delivery methods, achieving safe, efficient, and tissue-specific delivery remains a significant hurdle, particularly for organs and tissues that are difficult to target.

Meanwhile, continuous efforts have been made to develop new and more precise gene editing tools. Click Editor (CE) couples DNA polymerases with Cas nickases, allowing for programmable and precise genomic engineering using simple DNA templates to enable substitutions, insertions, and deletions in the genome[70]. Another promising tool, en-R2Tg, based on the R2 retrotransposon, can efficiently integrate large gene fragments (over 1.5 kb in length) into various mammalian cells. This breakthrough lays a foundation for advancing the research and application of next-generation innovative gene therapies[71].

In summary, although there is still a long way to go in overcoming the existing challenges, the unique advantages of precise gene editing offer promising prospects for transforming various aspects of human life.

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精准基因编辑技术的发展及治疗应用*

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摘要　基因编辑技术是生命科学领域最具变革的突破性技术之一，它的出现使基因组的遗传操作变得极为便捷。传统的CRISPR/Cas基因编辑通过引发DNA双链断裂（DSB）发挥作用，特别适合基因失活的研究，但在基因的精准修饰方面略有不足。新的基因编辑技术可以不依赖DSB和同源重组修复（HDR），达到了前所未有的精准度，目前已经开发了包括碱基编辑、引导编辑和CRISPR相关转座酶（CAST）在内的多种编辑系统，实现了从单个核苷酸到DNA大片段范围内的精准编辑。近年来，这些精确基因编辑工具的优化大大提高了编辑的效率、特异性和多功能性，如能够实现颠换的碱基编辑器、更高效和精准的增强型引导编辑系统，以及用于大段DNA精确插入的CAST系统，极大扩展了这些工具的应用范围。同时，体内递送系统的持续发展也极大推动了精准基因编辑工具的治疗应用，有效的递送系统对于基因治疗的成功至关重要，近年来在病毒和非病毒载体方面的进展提高了体内基因编辑的有效性和安全性。精确基因编辑技术在治疗遗传性疾病方面潜力巨大，本综述讨论了精准基因编辑工具和递送系统的最新进展，及其在心肌病、肝病和听力疾病等遗传性疾病中的临床前治疗应用，也讨论了技术在安全性和特异性的优化、递送系统的改进等方面存在的挑战，凸显了其从根本上治愈疾病的巨大潜力。

关键词　精准基因编辑，CRISPR/Cas系统，碱基编辑，引导编辑，基因治疗

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