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The Potential of Gene Editing for Huntington's Disease

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Abstract

Huntington's disease (HD) is a dominantly inherited neurodegenerative disorder caused by a trinucleotide repeat expansion in the huntingtin gene resulting in long stretches of polyglutamine repeats in the huntingtin protein. The disease involves progressive degeneration of neurons in the striatum and cerebral cortex resulting in loss of control of motor function, psychiatric problems, and cognitive deficits. There are as of yet no treatments that slow disease progression in HD. Recent advances in gene editing using CRISPR-Cas systems and demonstrations of their ability to correct gene mutations in animal models of a range of diseases suggest that gene editing may prove effective in preventing or ameliorating HD. Here we describe potential CRISPR-Cas designs and cellular delivery methods for the correction of mutant genes that cause inherited diseases, and recent preclinical findings demonstrating efficacy of such gene editing approaches in animal models with a focus on HD.

Keywords

base editing; CRISPR; gene therapy; polyglutamine; prime editing; striatal neurons

The genetics and manifestation of HD

Huntington's disease (HD) is caused by trinucleotide (CAG) expansions in the *Huntingtin* (*HTT*) gene resulting in long stretches of the amino acid glutamine in the huntingtin protein. The presence of forty or more CAG repeats leads invariably to HD, while shorter expansions may or may not lead to HD. The symptoms typically manifest between the ages of 30 and 50 but can appear before the age of 20 when there are more than 60 CAG repeats. Mutant huntingtin self-aggregates inside neurons and is associated with mitochondrial dysfunction and impaired autophagy which likely contribute to neuronal death

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[1]. Fast-spiking GABAergic medium spiny neurons with a high energy demand are the first to die in HD and their demise is thought to involve an excitotoxic process [2]. There are no treatments that delay the onset or slow disease progression. Reproductive choices for individuals harboring mutant genes that cause fatal inherited neurological disorders have been described previously [3].

Gene therapy for persons with disease causing mutation, along with the scientific and ethical challenges associated with them, were discussed as early as the 1970s [4]. Since then, several methods for gene therapy have been developed including those that deploy zinc finger proteins (ZFPs), transcription activator-like effector nucleases (TALENs), antisense DNA, or small interfering RNA. The recent development of gene editing methods based on CRISPR (clustered regularly interspaced short palindromic repeats)/Cas9 (CRISPR-associated protein 9) has enabled efficient correction of missense and other gene mutations and has resulted an explosion of translational research studies in animal models of a range of genetic disorders [4]. Here we describe CRISPR-based gene editing technologies, approaches for targeted cellular delivery of the gene editing cargos, and translational studies in experimental models of HD.

CRISPR-base Gene Editing Technologies

CRISPR-Cas9 systems utilize a single guide RNA (sgRNA) that recognizes the target sequence via complementary base pairing (Figure 1A). Cas9 endonuclease uses the sgRNA to form base pairs with DNA target sequences enabling a site-specific double-stranded break in the DNA. The portion of the sgRNA sequence that is complementary to the target sequence is called the ‘spacer’. An additional requirement for Cas9 to function is a specific protospacer adjacent motif (PAM) located directly downstream of the target sequence in the non-target strand. Through RNA-directed Cas9 nucleases this system can modify DNA with greater precision than ZFPs and TALENs. [5]. The double strand break introduced by Cas9 is repaired by either non-homologous end joining (NHEJ) or homology-directed repair (HDR). NHEJ is more efficient than HDR but tends to be error-prone and can produce insertions, deletions, translocations, or other DNA rearrangements [6], making the outcome of the repair process unpredictable. However, progress has been made in predicting the outcome of some types of insertions and/or deletions (InDels) [7]. The end products of the NHEJ repair process often harbor larger DNA alterations that cause disruption of the target gene [8]. In contrast, HDR exhibits high repair precision but low efficiency [7]. Thus, without additional features CRISPR-Cas9 is not suitable for the precise sequence manipulations of genomic DNA required in gene therapy. Recent efforts have therefore focused on developing technologies that enable the precise replacement of individual bases in the DNA without generating double strand breaks and without the need for exogenous repair templates [9, 10].

Base editing

Base editing is a novel technology that can generate gene disruptions or correct DNA mutations. Base editors consist of a Cas nickase (nCas9) fused to a nucleoside deaminase [11,12]. This feature enables construction of fusion proteins that provide CRISPR-Cas

systems with new enzymatic properties, including nucleotide-modifying activities. nCas9 can introduce single strand breaks in a manner that largely prevents the introduction of InDels, while fully retaining the DNA recognition properties conferred by the sgRNA [13].

Two different nucleoside deaminases are used for base editing (Figure 1B). Adenine base editing (ABE) uses adenosine deaminase fused to nCas9 and cytosine base editing (CBE) uses cytidine deaminase fused to nCas9 [10,12]. In ABE, the adenosine deaminase catalyzes the oxidative deamination of deoxyadenosine to deoxyinosine. Because deoxyinosine mimics deoxyguanosine, this conversion causes A-to-G transitions. CBEs cause C-to-T transitions by deaminating deoxycytidine to deoxyuridine. ABEs and CBEs enable all four base transitions (A to G, T to C, C to T, and G to A). These base editors do not require provision of a template or additional protein factors for nucleotide modification to occur [9,14]. However, because uracil and hypoxanthine are both alien bases in DNA they are recognized and removed by the base excision repair pathway. This makes the correction of the mutation introduced by base editing dependent on improper repair or lack of repair prior to DNA replication and poses a significant limitation on the efficiency of the editing. Additional strategies for improving the efficiency of deaminase-based base editing are therefore being developed [15].

During complex formation between the base editing fusion protein and sgRNA with the target DNA sequence an R-loop is formed by annealing of the sgRNA with the complementary DNA strand. The displaced single-stranded DNA provides the substrate for the deaminase. The sequence stretch of the displaced strand that is accessible to the deaminase domain of the fusion protein is referred to as the ‘base editing window’. Since base editing depends on direct interaction between the catalytic domain of the deaminase and the substrate nucleotide, Cas protein variants differing in their PAM sequence requirements provide different editing windows. The base editing window can also be modified by the orientation of the domains within the base editor fusion protein. The linkers that connect the different domains of base editing fusion proteins can also influence the location and width of the editing window as well as editing activity. However, ABE and CBE may induce RNA editing in an sgRNA-independent manner because of their affinity to RNA which in the case of ABEs is the natural substrate of the deaminase.

The editing activities of all currently available base editors is not strictly limited to the intended target site, and off-target DNA editing can occur in either an sgRNA-dependent or sgRNA-independent manner. Guide RNA-dependent off-target editing can be largely avoided by using high fidelity Cas variants [16–18], careful sgRNA design, use of truncated sgRNAs [19, 20], and/or delivery of the base editor as a preassembled ribonucleoprotein particle [21, 22]. Guide RNA-independent off-target mutations can lead to deamination of accessible nucleotides preferentially in coding regions. This phenomenon is predominantly observed in CBEs and less so in ABEs [22, 23]. Base editing can also cause unwanted bystander mutations within the editing window. Bystander mutations occur when a C or A nucleotide other than the targeted one is deaminated, or multiple Cs or As within the editing window undergo editing [24].

A major potential application of base editing is the reversion of gain of function disease-causing point mutations [25]. However, a major limitation of base editing is the ability to generate precise base-edits beyond the four transition mutations. Recently, prime editing has been developed to overcome this shortcoming [26].

Prime editing

Prime editing can mediate targeted insertions, deletions, and all possible base-to-base conversions without double strand breaks or donor DNA templates (Figure 2).An engineered prime editing guide RNA (pegRNA) that specifies the target site and contains the desired edit(s) engages the prime editor protein consisting of a nCas9 fused to a reverse transcriptase (RT). The nCas9 is guided to the DNA target site by the pegRNA. After nicking by nCas9, the RT domain uses the pegRNA as a template for reverse transcription of the desired edit directly polymerizing DNA onto the nicked target DNA strand. The edited DNA strand replaces the original DNA strand creating a heteroduplex containing one edited strand and one unedited strand. The prime editor then guides the heteroduplex to copy the edit onto the unedited strand.

The pegRNA is a guide RNA with a 3′ end that contains two additional functional domains: a primer-binding sequence (PBS) complementary to the 3′ end of the nicked DNA strand, and an upstream RNA sequence that serves as a template for reverse transcription (RT) (Figure 2). Thus, peg RNA not only guides the prime editor to its target site but also facilitates the annealing of the primer-binding sequence with the nicked DNA strand. The 3′ end of the DNA is extended by reverse transcription of the RNA template containing the sequence to be introduced by prime editing. cDNA synthesis leads to the formation of a branched intermediate at the nicked site consisting of two DNA flaps, the extended and edited DNA (3′ flap), and the original DNA strand (5′ flap). Hybridization of the two flaps to the uncut DNA strand occurs in an equilibrium that is then resolved by the DNA repair machinery. Removal of the unedited 5′ flap results in formation of a DNA heteroduplex consisting of one edited and one unedited strand. The cellular DNA mismatch repair system subsequently replaces the original sequence with the edited sequence.

Prime editing is less constrained by PAM sequence and more versatile and precise than base editing, and is more efficient than HDR [26]. Prime editing can incorporate point mutations into relatively large sequence stretches and can enable multiple mutations up to 30 nucleotides away from the cut site in the DNA strand. Prime editors can target about 90% of the pathogenic gene variants that are currently listed in the ClinVar database[i] [27–29]. The pegRNA is believed to be the main component limiting prime editing efficiency [26] but other factors such as the orientation of the RT relative to the Cas9 nickase and the lengths of both the primer-binding sequence and RT sequences of the pegRNA may also affect editing efficiency [18,26]. Additionally, the cellular DNA mismatch repair system lowers the efficiency of prime editing, but this can be alleviated by co-expression of an engineered mismatch repair-inhibiting protein [30].

While prime editing is currently the most versatile method for DNA editing several challenges remain. The efficiency of prime editing still varies greatly depending on the

type of edits to be made, the surrounding sequence context, and the target cell type. The large size of the prime editor complex also poses a challenge for packaging and cell delivery.

Delivery and Cell Targeting

Gene editing cargos can be delivered as DNA, RNA, or ribonucleoproteins (RNP). Delivering DNA encoding Cas9 (or related endonucleases) and a sgRNA results in stable expression of these gene editing cargos which can be advantageous for some applications but also increases the probability of off-target effects. Delivering mRNAs encoding Cas9 proteins in combination with sgRNA results in transient expression of the gene editing machinery. Because RNA is rapidly degraded by RNAses, methods of chemical modification of the RNA to increase stability have been developed [31]. For RNP, recombinant Cas protein is combined with sgRNA.

There are several features of a vehicle for gene editing cargos that are required for effective delivery to the desired cells [32, 33]. First, the vehicle must contain the cargoes and protect them from degradation prior to their delivery to the target cells. This is typically accomplished by enclosing the cargos in a lipid or protein shell. Second, the delivery vehicle must bind to the surface of the target cells. Typically this is mediated by an antibody or glycoprotein on the surface of the delivery vehicle that recognizes a molecule on the surface of the target cell. Third, the delivery vehicle must enter the cell, for example by receptor-mediated endocytosis. Fourth, the gene editing cargos must be released into the cytosol and trafficked to the cell nucleus; to accomplish this, vehicles typically include features that exploit the acidic pH of endosomes resulting in release of cargos into the cytosol.

The technologies used for delivery of the gene editing cargos can be categorized as physical, chemical, or biological [32,33]. Physical methods include microinjection, electroporation, and ultrasonication. Physical methods are useful for delivery of cargos into cultured cells but are generally impractical for in vivo applications. The most commonly used chemical delivery methods are incorporation of the gene editing machineries into nanoparticles comprised of lipids or gold. Lipid nanoparticles (LNP) consist of a combination of cationic lipids, polyethylene glycol, ‘helper’ phospholipid, and cholesterol (Figure 3a). This combination enhances cell entry and intracellular release of the gene editing cargos. LNP are useful for delivery of gene editing cargos to cultured cells and have also been used to correct genetic defects in mouse models of neurological disorders [34]. For example, in a mouse model of hereditary deafness caused by a missense mutation in the *Tmc1* gene, injection into the cochlea of LNP with Cas9-sgRNA complexes targeting the mutant allele reduced hearing loss and prevented degeneration of auditory hair cells [35]. LNP delivery has several advantages over viral delivery of gene editing cargos. LNPs have a much larger cargo capacity, result in transient expression of the gene editing cargos which reduces the probability of off-target editing.

CRISPR-gold nanoparticles consist of 15 nanometer diameter gold particles conjugated to oligonucleotides with Cas9 RNP incorporated into the oligonucleotides. The nanoparticles are coated with polyaspartic acid which is degraded upon entry of the nanoparticles into

endosomes thereby releasing the CRISPR cargos to the cell cytoplasm. Gold nanoparticle delivery of CRISPR-Cas9 RNP cargos targeting the metabotropic glutamate receptor 5 gene ameliorated behavioral abnormalities in a mouse model of fragile X syndrome [36].

Viral vector-based delivery of gene editing tools has been widely used in translational neuroscience research. More recently, this approach has been incorporated into various clinical trials as well. It is important to note that viral delivery methods carry several limitations that require careful consideration in any potential clinical application, including: (i) limited cargo capacity; (ii) potential viral-mediated toxicity; (iii) immune responses, which may limit the number of viral delivery administrations and/or the unity of virus-like particles; (iv) decreased efficiency and/or off-target effects due to in-cell generation of active Cas9 enzyme from nucleic acids; (v) side effects of long term expression of endonucleases following viral delivery; (vi) risk of oncogenesis, particularly for lentiviruses, due to integration. We refer the readers to prior articles for more detailed discussion of the advantages and limitations of potential clinical applications of viral vectors [37]. Adeno-associated viruses (AAVs) have been widely employed as vectors for the delivery of genes and small interfering RNAs to neurons [38]. AAVs are small (~4.8 kilobase) linear single-stranded DNA viruses that are replication-defective. Although AAV remains episomal in infected target cells it is stabilized by circularization and concatemer formation which enables long-term transgene expression in non-mitotic cells such as neurons. Importantly, in contrast to other viruses, AAV elicits limited immune responses compared to other viral vectors[39].

In applications where a single engineered AAV is insufficient to deliver all of the desired cargos, two AAV constructs have been employed (Figure 3b). Alternatively, AAV systems with smaller Cas9 orthologues and minimal promoters can be used. For example, Cas9 dual AAV gene editing approach incorporating a protein auto-processing domain (intein) was used to introduce a nonsense coding sequence into transgenic mice expressing a mutant SOD1 that causes familial ALS [40]. Expression of CRISPR components using such AAVs has also proven effective in generating transgenic mouse models of several different neurological disorders and for the rapid generation of brain cell type-specific gene knockout mice [41–44].

Lentivirus readily infects neurons, provides sustained expression of cargos, and has a carrying capacity (8.5 kilobases) that is greater than AAV (4.7 kilobases). For example, inactivation of the synaptotagmin gene with a lentivirus CRISPR interference method resulted in a decrease in excitation of glutamatergic neurons in the hippocampus [45]. In another study, lentivirus was used for CRISPR activation and interference systems engineered with intron-containing Cre recombinase to drive gRNAs targeting several genes including *Fos*, *GRM2*, and *Gadd45b* in neurons [46]. However, among the major drawbacks of lentiviruses is that they can induce unwanted immune responses.

Another vehicle for delivery of gene editing cargos that is being developed is virus-like particles (VLP) [32]. VLP are assemblies of viral proteins in which desired gene editing cargos are packaged (Figure 3c). VLP have several advantages including: transient expression of gene editing cargos which reduces the chance of off-target editing. VLP have

been used extensively for the preparation of vaccines including SARS-CoV2 vaccines [47] but neuroscientists have yet to exploit their advantages for gene editing.

The most common routes of delivery of gene editing cargos into the central nervous system in animal studies are stereotaxic injection into desired locations, intrathecal injection or intraventricular injection into the cerebrospinal fluid. In addition, AAV variants that efficiently infect axons and are retrogradely transported to neuronal cell bodies have been developed and shown to express cargos in projection neurons in the mouse brain [48]. Intravenous delivery suffers from several problems including dilution of the CRISPR cargo vehicles, limited access to the central nervous system, and increased chance of adverse effects on peripheral tissues.

Preclinical studies of gene therapy for HD

Gene editing using CRISPR-Cas9 techniques have been used to correct gene mutations in cell culture and animal models of several neurological disorders (Table 1). One common approach is to first establish the efficacy of a CRISPR-Cas9 construct designed to target a gene mutation in cultured neurons derived from pluripotent stem cells (iPSC) generated from fibroblasts taken from a human carrier of that mutation. Once the efficacy and specificity of a gene editing system is established in cultured cells, the system can be tested in transgenic mouse models.

The therapeutic potential of CRISPR-Cas9 based gene therapy for HD has been tested in several preclinical models. In HD140Q knock-in mice expressing human *HTT* exon-1 with 140 CAG repeats, a nonallele-specific targeting strategy repressed mutant human *HTT* expression, and was associated with reduced mutant huntingtin protein aggregates and improved motor function [49]. In a study from our group using the same Cas9 system, altered arteriolar cerebral blood volumes were restored and striatal atrophy and the progression of motor phenotype were slowed by CRISPR-Cas9-mediated mutant *HTT* silencing in striatal neurons of zQ175 knock-in HD mice [50].

Because the difference between normal and mutant *HTT* alleles is the size of CAG expansions, classical CRISPR-Cas9 systems that target CAG will not achieve allele-specific silencing or excision. Discrimination between the two CAG tracts therefore requires specificity for repeat expansions greater than ~35 CAGs, corresponding to about 105 base pairs, which is not achievable using current methods. In addition, other genes containing repetitive CAG tracts would likely be affected by targeting the CAG expansions.

One study deployed CRISPR-Cas9 designed to selectively target human mutant *HTT* in a transgenic mouse model expressing exon-1 of the human HD gene, the R6/2 model. This study demonstrated reduction of mutant huntingtin protein and improved lifespan and motor symptoms in mice receiving the CRISPR-Cas9 therapy [51]. But this selectivity was possible only because the sgRNA selectively targeted a non-CAG sequence specific for the human *HTT* allele thereby leaving the endogenous mouse *Htt* allele unaffected.

Wild type huntingtin is critical for normal brain development and adult neuroplasticity. Because people with HD have one normal and one mutant allele, it is critical to develop

gene editing systems that target only the mutant allele. Several such approaches have been evaluated in cultured cells derived from HD patients. For example, a paired nCas9 strategy selectively excised expanded CAG repeats in exon 1 of the *HTT* gene in three different HD patient fibroblast lines, inactivating only mutant *HTT* [52]. A strategy using CRISPR-Cas9 and piggyBac transposon was also successfully used in HD human iPSCs, correcting the mutant *HTT* allele and amending HD mediated phenotypic abnormalities [53]. So far, to our knowledge, these allele-selective approaches have only been applied to cultured cells.

One promising method for selectively targeting mutant *HTT* is to take advantage of allele-specific single nucleotide polymorphisms (SNP) which allow for allele discrimination based on the creation or destruction of a PAM sequence by a particular SNP, leaving a PAM solely on the mutant *HTT* allele at a given location [54]. The CRISPR-Cas9 system has been used to achieve silencing of the mutant *HTT* gene through heterozygous PAM sites in patient-derived fibroblast cells significantly reducing expression of the mutant huntingtin protein [55]. In this approach, two SNPs and two cut sites were required to excise large portions of the gene that included the promoter and exon 1. A similar CRISPR-Cas9 system requiring a single SNP and a shared target site in an intronic region selectively targeted the disease allele and reduced mutant *HTT* expression in neural cells of the BACHD mouse model [56]. These approaches require at least two target sites in the genome, which may increase the risk of off-target side effects.

But not all people with a mutant *HTT* gene contain SNPs. Therefore, haplotypes should be examined to identify gRNAs which lead to allele-specific silencing of the mutant *HTT* gene. A potential challenge is that screening patients for SNPs and determining on which allele the SNP is present requires specific sequencing techniques [57]. Additionally, the mutation that causes HD involves expanded CAG repeats that contain a larger number of nucleotides than the gRNA construct for Cas9 can recognize. In those instances, indirect methods have to be used to specifically identify a target sequence outside of the mutation. The success of these approaches relies on SNPs within both the promoter region and just beyond exon 1, which significantly limits the targetable SNPs because there are 67 exons in the *HTT* gene. A more robust approach would allow for the use of SNPs outside of the promoter and first intron.

Prime editing may overcome the difficulties in targeting mutant *HTT* because prime editing has the potential to insert or delete short sequences to disrupt the promoter and alter splicing sites which could lead to a selective reduction in expression of the mutant HTT gene. Indeed, prime editing technology was applied to target progerin, a dominant negative mutant protein that accumulates in Hutchinson-Gilford Progeria, a rare form of accelerated aging. Encouragingly, in a mouse progeria model, there was a correction of the disease-causing mutation and median lifespan was extended [58].

Another CRISPR-based approach for HD is to target the mutant huntingtin mRNA rather than the mutant *HTT* gene. The efficacy of this approach preclinically was recently reported in a study that employed a CRISPR-Cas13d system designed with a gRNA-Cas13d that selectively targets and cleaves CAG-expanded huntingtin mRNA (CRISPR-Cas13d-HTT^{EX}) [59]. When packaged in a constitutive lentiviral vector and delivered into cultured HD patient fibroblasts and neurons derived from HD iPSC, CRISPR-Cas13d-HTT^{EX} eliminated

the accumulation of mutant huntingtin mRNA and protein. AAV-mediated delivery of CRISPR-Cas13d-HTT^{EX} into the striatum of mutant *HTT* transgenic mice reduced the accumulation of mutant huntingtin aggregates, reduced neuronal degeneration, and improved motor function. These phenotypic improvements lasted for at least eight months without noticeable adverse effects and with minimal off-target transcriptomic effects.

Concluding Remarks

Base and prime editing are currently the most promising approaches for correcting mutations that cause disease. Methods for delivery of gene editing cargos to desired cellular targets in vivo have been developed, and a rapidly growing number of preclinical studies are testing the efficacy of these gene editing approaches for correction of gene mutations in animal models of a wide range of inherited diseases including neurological disorders.

In the instances of gain of function missense mutations, correcting a single-base on one allele is sufficient. However, because HD results from CAG expansions in the *HTT* gene, the CAG repeat region cannot be directly targeted without also altering the wildtype *HTT* allele. To overcome this problem HD requires a different gene editing approach (see Outstanding Questions). One promising approach to selectively disrupt the mutant *HTT* gene is to target a SNP that is specific for the mutant allele. While the results of recent preclinical tests of such gene editing systems for HD are promising, clinical trials in HD patients will require considerable further refinement of the methods in animal models.

Recent clinical trials have deployed CRISPR-Cas9 systems for delivery of gene therapy cargos in cells from a few patients with sickle cell anemia, beta thalassemia, lymphocyte cancers, inherited childhood blindness, and transthyretin amyloidosis [60–63]. These trials have provided evidence that gene editing is feasible and can be efficacious in cells in human patients. However, so far clinical trials have only targeted cells located in readily accessible compartments (blood and eye) and it will likely prove more difficult to deliver gene editing cargos into the brain cells affected in HD.

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Resources

i. https://www.ncbi.nlm.nih.gov/clinvar/

Outstanding questions

How can gene-editing technologies be further refined in ways that eliminate or greatly reduce off-target side effects?

At what time in relation to the onset of HD symptoms should gene-editing therapy be administered?

Can cell delivery platforms be developed that selectively target the striatal and cortical neurons that are most severely affected in HD?

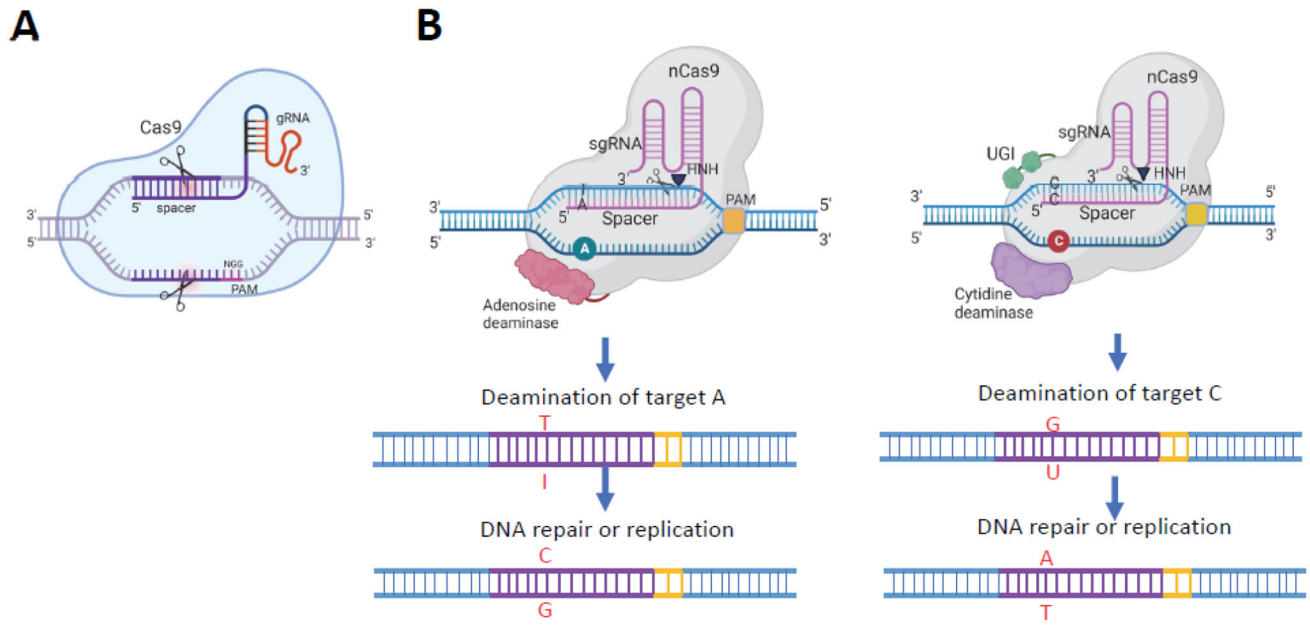

Figure 1.

Illustration of CRISPR-Cas9 and base editing. **A**. CRISPR-Cas9-mediated gene editing. **B**. Adenine base editing (left) and cytosine base editing (right) are illustrated. Adenine base editors consist of Cas9 nickase (nCas9) and an adenosine deaminase that mediates deamination of adenosine (A) to inosine (I). Inosine is recognized as guanosine (G) upon DNA replication, thus resulting in fixation of an A-to-G mutation. Cytosine base editors consist of a nCas9, a cytidine deaminase, and a uracil DNA glycosylase inhibitor (UGI). The UGI protects the uracils arising from cytidine deamination by preventing their excision by uracil DNA glycosylase. The nick in the unedited strand is recognized by the cellular mismatch repair pathway that then can use the edited strand as a repair template to form a U:A base pair, which can become fixed as a T:A base pair by DNA repair and/or DNA replication. Figure created using BioRender.com

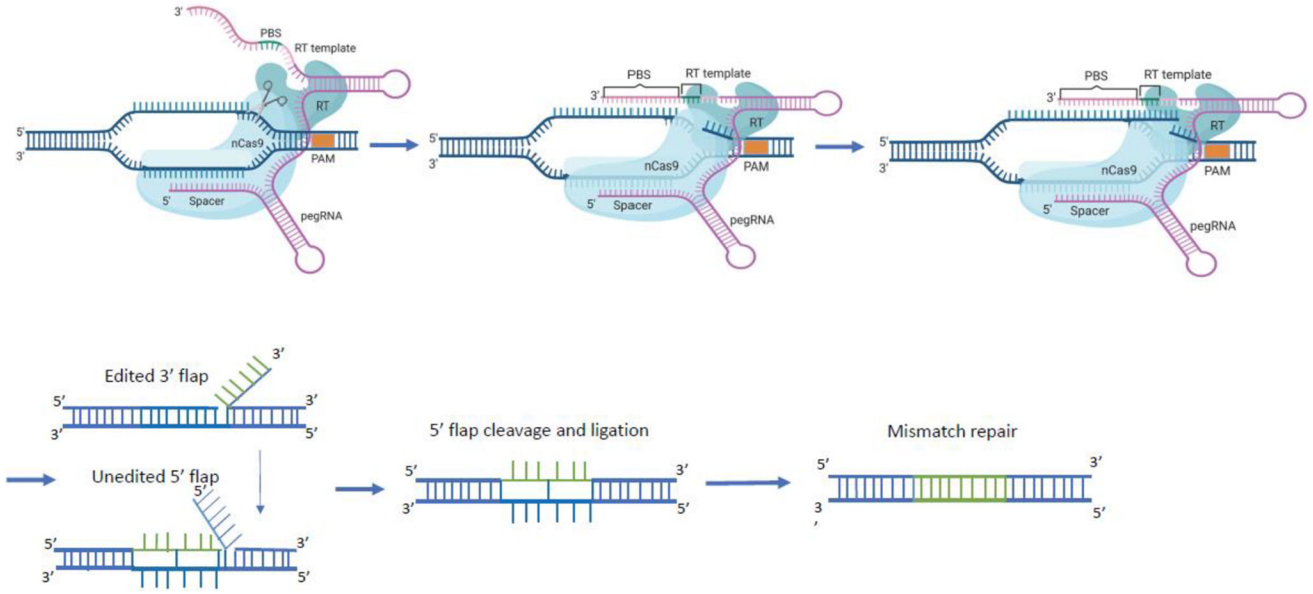

Figure 2.

Prime editing components are comprised of a fusion protein of nCas9 (neon blue) with a reverse transcriptase (RT; turquoise) domain and an engineered prime editing single guide RNA (pegRNA; purple). The pegRNA harbors a primer binding site (PBS) and a reverse transcription template (RT) as 3′ extension of the sgRNA scaffold. The pegRNA guides the nCas9 domain to the target site to cut the protospacer adjacent motif (PAM)-containing strand and directs the synthesis of an edited DNA strand starting from the 3′ end of the nicked strand and using the RT sequence (green) as template. The 3′ newly synthesized edited DNA strand can displace the 5′ unmodified DNA strand, resulting in a hybridization of the uncut strand with two DNA flaps. The 3’ flap contains the newly synthesized (edited) sequence and the 5’ flap contains the dispensable, unedited DNA sequence. The 5’ flap is then cleaved by structure-specific endonucleases or 5’ exonucleases. The edited 3’ flap proceeds to ligation and forms a heteroduplex DNA composed of one edited strand and one unedited strand. The reannealed double stranded DNA contains nucleotide mismatches at the location where editing took place. The mismatch repair mechanism is initiated and the information in the edited strand is copied into the complementary strand. Figure created using BioRender.com

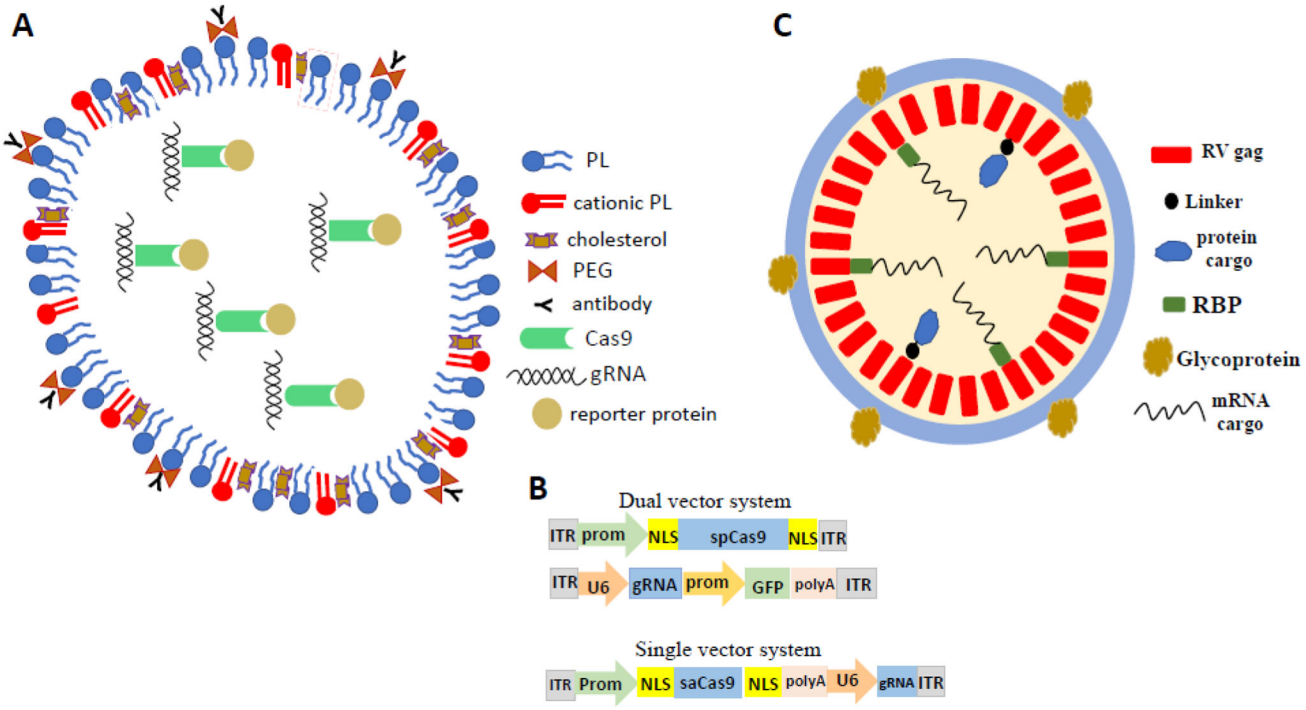

Figure 3.

Lipid nanoparticles (LNP), adeno-associated virus (AAV), and virus-like particles (VLP) as systems for delivery of CRISPR-Cas9 gene editing cargos. **A**. Molecular composition of a LNP for delivery of CRISPR-Cas9 gene editing cargos (see [34]). gRNA, guide RNA; PEG, polyethyleneglycol; PL, phospholipid. **B**. Examples of AAV constructs for gene editing. GFP, green fluorescent protein; ITR, inverted terminal repeat; NLS, nuclear localization sequence; prom, promoter; saCas9, Cas9 from staphylococcus aureus; spCas9, Cas9 from streptococcus pyrogenes; U6, an RNA polymerase II promoter. **C**. An example of a VLP containing CRISPR-Cas9 cargos. The viral gag protein contains the protease required for cleaving polyproteins into subunits and also provides structural stability to VLP. RBP, RNA-binding protein; RV, retroviral.

Table 1.

Preclinical gene-editing studies in iPSC and animal models of neurological disorders.

Disease	Method	Model	Major findings	Reference
ALS	CRISPR/Cas9	SOD1 G93A mice	Reduction of mutant SOD1 protein in the lumbar and thoracic spinal cord improves motor function and reduces muscle atrophy	[64]
ALS	CRISPR/Cas9	SOD1 G93A mice	A nonsense-coding insertion into a mutant SOD1 gene prolongs survival and markedly slows disease progression	[40]
SCA3	CRISPR/Cas9	Human iPSC	iPSC derived from an SCA3 patient retain pluripotency and neural differentiation following deletion of the expanded polyQ-encoding region of ataxin 3	[65]
Fragile-X syndrome	CRISPR/Cas9	Human iPSC	After removal of the CGG repeats, the upstream CpG island of the FMR1 promoter shows extensive demethylation, an open chromatin state, and transcription initiation	[66]
ALS	CRISPR/Cas9	Human iPSC	Excision of the large C9orf72 repeat expansion mutation rescues RNA foci formation and promoter hypermethylation without altering C9orf72 transcript and protein expression	[67]
HD	CRISPR/Cas9	Human iPSC	Excision of *mHtt* on the disease chromosome prevents the generation of mutant huntingtin mRNA and protein	[55]
HD	CRISPR/Cas9	HD140Q-KI mice	Permanent suppression of mutant *Htt* expression in the striatum depletes huntingtin aggregates and attenuates early neuropathology	[49]
HD	ZFN	R6/2 mice	One-time striatal AAV-ZFP infusion can correct histopathological, electrophysiological and biomarker deficits that are characteristic of human HD pathology	[68]
AD	CRISPR/Cas9	C57Bl/6J mice	Genome editing of intron −1/exon 1 of Mapt in C57Bl/6J mice generates a strain that shows a significantly reduced susceptibility to excitotoxic seizures, with normal learning and memory	[69]
PD	CRISPR/Cas9	Synuclein A53T rats	Gene deletion of A53T-SNCA significantly ameliorates α-synuclein pathology, dopaminergic neurodegeneration, and parkinsonian motor symptoms	[70]
HD	CRISPR/Cas9	Human iPSC	CAG repeat deletion ameliorates mitochondrial phenotypes in motor neurons	[71]
ALS	CRISPR/Cas9	Human iPSC	Normal cellular phenotypes are restored following focused deletion of the G4C2 hexanucleotide repeat expansion in C9orf72	[72]
ALS	CRISPR/Cas9	G93ASOD1 mice	The deletion of mutant SOD1 prolongs survival	[73]
ALS/FTD	CRISPR/Cas9	Human iPSC	The excision of the repeat expansion in CRISPR/Cas9 MNs reverses several physiological defects in Ca^{2+} homeostasis	[74]
ALS	CRISPR/Cas9	SOD1 G93A mice	hSOD1-G93A transgene editing in SOD1-linked ALS mice prevents the development of ALS-like disease	[75]
AD	CRISPR/Cas9	5XFAD mice	The deposition of amyloid-beta, as well as microgliosis, neurite dystrophy and cognitive performance impairments are ameliorated using brain-wide selective disruption of a mutant APP allele	[76]
Fragile-X Syndrome	CRISPR/Cas9	FMR1KO mice	Reduction of local mGluR5 levels in the striatum can rescue mice from the exaggerated repetitive behaviours	[77]
HD	CRISPR/Cas9	Huntingtinz Q175 mice	Non-allele-specific HTT silencing in striatal neurons restores altered arteriolar cerebral blood volumes in premanifest mice, delays onset of striatal atrophy, and slows the progression of motor phenotype and brain pathology	[50]