CRISPR Therapies for Alzheimer's Disease

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Abstract

Engineered CRISPRs (Clustered Regularly Interspaced Short Palindromic Repeats) have gone from proof-of-concept to an FDA-approved drug in little more than a decade (Fig. 1). This shrunken bench-to-beside timeline was a result of data from clinical trials where single doses lead to effective long-term cures (or predictions for cure) in essentially all treated patients. [1–3] Such remarkable results have been seen for both ex vivo applications in hematologic disorders—where bone marrow–derived cells were gene-edited and reintroduced into the patient [1]—as well as for somatic in vivo gene-editing in systemic protein-folding disorders, where therapeutic components were delivered by intravenous injections [2]. Several other gene-editing clinical trials for chronic bacterial infections, inflammatory diseases, cancers, cardiovascular diseases, HIV/AIDS, diabetes, and autoimmune diseases are ongoing [4], and this new way of treating human disease seems poised to change medical practice. Given this backdrop, it seems imperative to test this new class of medicines in neurodegenerative illnesses such as Alzheimer's disease (AD), where traditional therapeutics have been largely disappointing.

Keywords

Cas9 · CRISPR · Gene-editing · Alzheimer's disease · Amyloid precursor protein · Presenilin · Amyloid beta · CRISPR screen · GWAS

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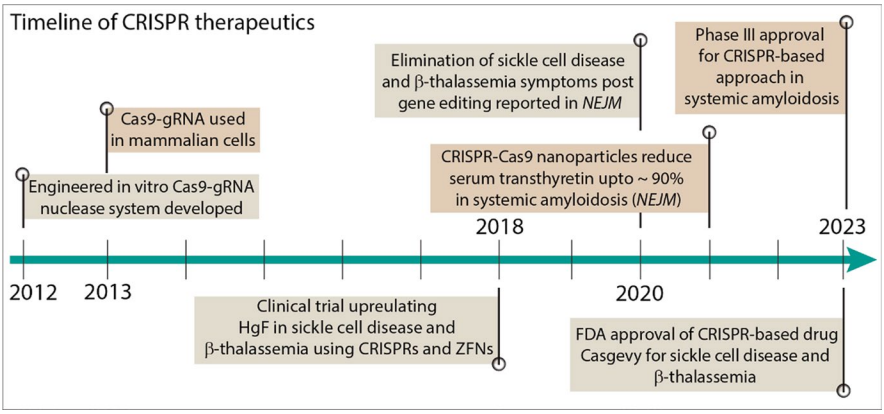

Fig. 1 Timeline of CRISPR-Cas9 development, leading to FDA approval of Casgevy in late 2023

Engineered CRISPRs (Clustered Regularly Interspaced Short Palindromic Repeats) have gone from proof-of-concept to an FDA-approved drug in little more than a decade (Fig. 1). This shrunken bench-to-beside timeline was a result of data from clinical trials where single doses lead to effective long-term cures (or predictions for cure) in essentially all treated patients. [1–3] Such remarkable results have been seen for both ex vivo applications in hematologic disorders—where bone marrow–derived cells were gene-edited and reintroduced into the patient [1]—as well as for somatic in vivo gene-editing in systemic protein-folding disorders, where therapeutic components were delivered by intravenous injections [2]. Several other gene-editing clinical trials for chronic bacterial infections, inflammatory diseases, cancers, cardiovascular diseases, HIV/AIDS, diabetes, and autoimmune diseases are ongoing [4], and this new way of treating human disease seems poised to change medical practice. Given this backdrop, it seems imperative to test this new class of medicines in neurodegenerative illnesses such as Alzheimer's disease (AD), where traditional therapeutics have been largely disappointing.

It is estimated that worldwide, almost 416 million people are affected with some form of AD [5], and this number is rising. Despite intense efforts in developing therapeutics over decades and numerous clinical trials, there are few disease-modifying agents [6]. Recent trials show that monoclonal antibodies to Aβ assemblies can effectively remove Aβ from the brain and slow disease progression, but clinical benefits are modest [7]. Moreover, these therapies come with significant risks of intracerebral hemorrhage (also called "amyloid-related imaging abnormalities" or ARIA), which are seen in up to a third of the treated patients and have led to several deaths [8]. These monoclonal antibodies also need to be repeatedly administered throughout the lifetime of the patient that will likely add to the risk of ARIA, and the need for frequent clinic visits to get these injections may also raise compliance issues. By directly targeting disease etiology and the promise of a "one and done" therapeutic, gene editing–based therapies offer an alternative for the future treatment of AD. This chapter will focus on CRISPR-Cas-based strategies that are being explored for the treatment of AD. We begin by outlining the basic principles

of clinically relevant CRISPR-based technologies, followed by examples of AD-relevant applications. Next, we discuss the unique challenges in developing CRISPR therapies for targeting brain disorders and end with a section highlighting the use of CRISPR technology as a discovery-based tool for AD.

The CRISPR-Cas Therapeutic Toolbox

CRISPR Editing by Exploiting Innate Cellular Mechanisms In nature, the CRISPR-Cas system is a prokaryotic adaptive immunity mechanism that is used to cut and destroy invading nucleic acids [9], and engineered solutions now allow us to implement this system in mammalian cells [10, 11]. The repertoire of available CRISPR tools to edit genes is continually expanding, and only DNA-editing strategies relevant for putative clinical applications are mentioned here. The CRISPR-Cas9 system consists of two basic components—a Cas9 enzyme that generates double-stranded breaks in the DNA and a synthetic guide-RNA (gRNA) that directs the Cas9 to a specific site within the genome (Fig. 2a). The gRNA can theoretically

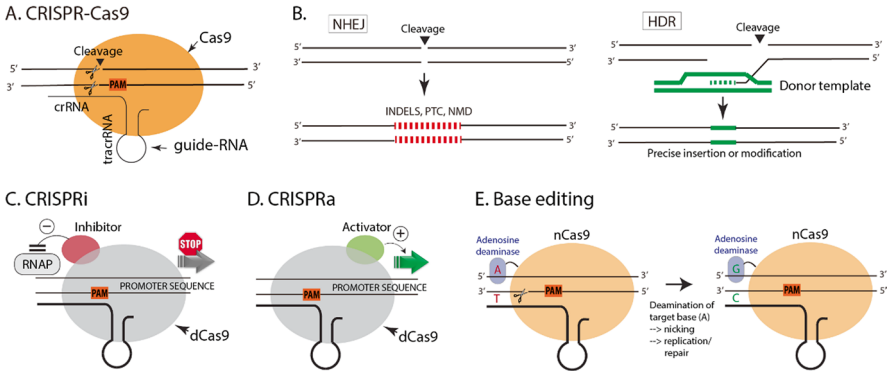

Fig. 2 Schematic showing various forms of CRISPR-Cas9 DNA editing. (**a**) The system consists of a synthetic gRNA that is targeted to a specific site in the genome by recognizing PAM (protospacer adjacent motif) sites—and adjacent ~20 nucleotide sequences; and a Cas9 enzyme that binds to the gRNA and delivers double-stranded cuts to DNA strands (scissors) at specified sites. (**b**) Left: After cleavage, the edited DNA is typically repaired by an inefficient process called non-homologous end joining (NHEJ), which leads to insertions and deletions (INDELs) at the cut site, leading to premature termination codons (PTCs) in the reading frame and subsequent nonsense mediated decay (NMD) of the transcript. Right: An exogenous donor template near the cut site can trigger homology-derived recombination (HDR), which leads to the insertion of the template within the host DNA—a process that occurs most efficiently in dividing cells. (**c**, **d**) In CRISPR-inhibition and CRISPR-activation (CRISPRi/a), a catalytically inactive Cas9—directed by appropriate gRNAs—acts as a shuttle to deliver transcription inhibitors (RNAP) or activators to the promotor region of a gene. (**e**) Base editing is a strategy by which enzymes capable of inducing a single base-pair change—for instance, adenosine deaminase to convert an A to a T—are shuttled to the appropriate location by a nickase Cas9 (nCas9, capable of making only one cut in the DNA). While the enzyme carries out the requisite base-pair change, the non-targeted strand is cut by the nCas9, triggering a repair process that also corrects the mismatched base pair

be directed to any part of the genome, and specificity is dictated by homology of the gRNA to short PAM (protospacer adjacent motif) sequences—that are dispersed throughout the genome—*and* ~ a 20 base-pair sequence adjacent to the targeted PAM sequence. CRISPR editing by non-homologous end-joining (NHEJ, Fig. 2b, left) is the best characterized of all CRISPR applications and is also the conceptual basis for almost all CRISPR trials that have entered the clinic [1, 2, 12]. After Cas-mediated double-stranded breaks in the DNA, both dividing and non-dividing cells undergo error-prone repair mechanisms that trigger insertions and deletions (INDELs) at the cut site that typically lead to premature termination codons (PTCs). These abnormal PTCs usually induce nonsense-mediated decay (NMD) of the transcript and subsequent loss of the protein. Alternatively, in presence of an exogenous donor template near the cut site, a process called homology-derived repair (HDR) can be triggered, which leads to an insertion of the donor template within the host DNA (Fig. 2b, right). Though HDR can potentially allow any change in DNA, the process is inefficient and occurs during mitotic recombination [13], which limits utility in non-dividing cells such as neurons. As such, though HDR is widely used as an experimental tool to study biological effects of mutations/insertions in cells, clinical applications for CNS diseases are limited.

CRISPR-Mediated Modulation of Transcription CRISPR technology can also be used to modulate gene expression by inhibiting or activating endogenous promoters (Fig. 2c, d). In CRISPR inhibition (CRISPRi), a catalytically inactive Cas enzyme (dead-Cas or dCas) is bound to transcriptional inhibitors, and gRNAs are designed to target the dCas-payload to the transcription site [14, 15]. Analogous CRISPR-activation (CRISPRa) is achieved by guiding a transcriptional activator to the promoter region [16–18]. Since genes are driven by their endogenous promoter in CRISPRi/a, this strategy seems intuitively superior to the standard approach of over-expressing therapeutically relevant genes via exogenous promoters, in neurodegenerative diseases where the disease is thought to be a loss of function. Moreover, CRISPRi/a is potentially reversible "scarless" technology, since these manipulations do not lead to DNA-breaks and permanent alterations in the genome.

CRISPR-Mediated Base Editing and Prime Editing Though CRISPR-driven DNA cuts are typically precise, the repair process is not, and the goal of base/prime editing is to introduce more precise changes to the genome. The overall concept is similar to CRISPRi/a approaches, where Cas is used as a shuttle to deliver payloads to a gRNA-targeted site within the genome. However, base/prime editing is designed to alter a single base pair (base editing) or to insert a small stretch of DNA (prime editing) in the targeted region [19, 20]. These techniques use a Cas enzyme that is mutated at one of its two DNA-cleavage sites (called nickase Cas9 or nCas9), which only allows single-stranded cuts in the *non-targeted strand*. In base editing, the nCas9 is fused to deaminase proteins that can convert one base pair to another. For instance, adenine base editors (ABEs) can convert A:T to G:C (Fig. 2e), or cytosine base editors (CBEs) can convert C:G base-pairs to T:A base pairs. After being targeted to the desired DNA strand by a synthetic gRNA, base editors induce the desired nucleotide change in that strand. Subsequently, nCas9 nicks the opposite

non-targeted strand, where endogenous repair mechanisms substitute the existing base pair for a complementary one, completing the repair. Prime editing expands on the concept of base editing by allowing insertion of small stretches of desired DNA sequences at targeted sites [21]. This is achieved by a unique prime-editing gRNA (pegRNA) that has the desired DNA sequence that needs to be inserted at the cut site (in addition to the conventional targeting sequence). Transcription of the desired DNA sequence is triggered by a reverse transcriptase enzyme that is fused to nCas9, ultimately leading to the transfer of encoded genetic information from the pegRNA to the targeted genomic site.

Therapeutic Applications of CRISPR-Cas to Alzheimer's Disease

Mutation-Specific Approaches Familial AD is caused by autosomal-dominant, point mutations in *APP* and presenilin (*PSEN*) genes. Almost all these changes are due to base-pair mutations, and in principle, correction of these mutations would prevent disease in these patients. Though these patients comprise a small fraction of all AD (<1% [22]), the number of affected patients is still significant, and these mutations are almost completely penetrant in large families, with devastating consequences. Since most patients have heterozygous mutations, one option is to inactivate the mutant allele using CRISPR-Cas, while keeping the WT-allele intact. Assuming that the pathology is solely due to a gain of toxic function attributed to the mutant allele, such an approach would be therapeutic. Proof-of-principle of this strategy has been demonstrated for both *APP* and *PSEN1* genes. The *APP* "Swedish" mutation (*APPSwe*)—a double-base change, substituting a pair of nucleotides (GA > TC, see Fig. 3a)—is located at the β-secretase site of APP and leads to an increase in APP β-cleavage and Aβ production [23]. Coincidentally, the mutated nucleotides are close to a suitable *Staphylococcus pyogenes* (SpCas9) PAM site that

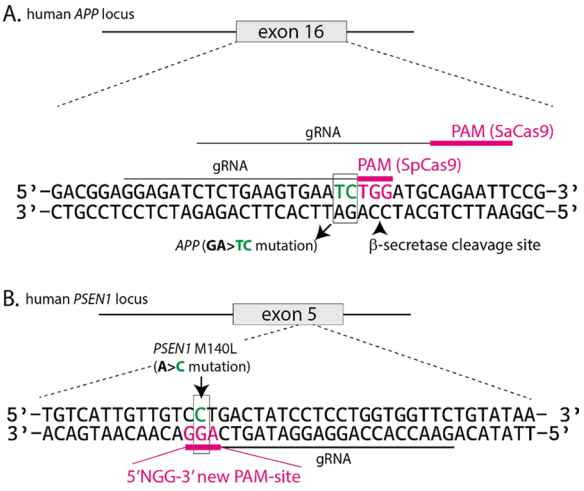

Fig. 3 Strategies for editing mutated *APP* and *PSEN1* alleles using CRISPR-Cas9. Note that the presence of altered nucleotide(s) in the mutated allele generated a unique sequence that could be targeted by a custom-designed gRNA. This allows the CRISPR-Cas9 system to specifically target the mutant allele in *APP* (**a**) or *PSEN1* (**b**), while sparing the WT allele

can be exploited to selectively disrupt the mutant *APPSwe* allele. Such allele-specific editing is possible because even small mismatches between gRNA and the targeted sequences preclude Cas9 editing [24].

In recent work, the *APPSwe* allele was selectively deleted (Fig. 3a) in cells, and also in vivo, using mouse models that express this mutation [25, 26], and one of these studies used the compact *Staphylococcus aureus* SaCas9, [27] which can be packaged into a clinically relevant single AAV vector. Both local hippocampal injections of the AAV-CRISPR payload and systemic delivery via intravenous AAV-injections ameliorated neuropathologic, electrophysiologic, and behavioral abnormalities in mouse models carrying the *APPSwe* mutation [26]. The beneficial effects were long-lasting, and no significant off-target or adverse effects were reported. In another proof-of-concept study [28], a mutant *PSEN1* allele was selectively disrupted using a similar allele-specific inactivation strategy (Fig. 3b). There are over 30 pathogenic *APP* mutations and over 300 *PSEN1/2* mutations, and it may be possible to develop niche CRISPR therapies using such allele-excision strategies, though PAM availability and other technical issues related to base-pair matching may be a limiting factor. One potential caveat of this approach is that the pathobiology of the *PSEN* mutations is not well understood [29–31], so it is not clear that eliminating the mutant allele would be the right approach. While most *APP* mutations are located within the β/α/γ-secretase cleavage sites and pathologically alter APP cleavage—advocating a gain of function and justifying allele-deletion—*PSEN* mutations are scattered throughout the gene. While earlier studies suggested a gain-of-function scenario based on evidence that *PSEN* mutations caused a relative increase in the longer, more aggregation-prone Aβ peptides [32], a more nuanced view is emerging (involving both toxic-dysfunction and loss of function), where *PSEN* mutations destabilize the γ-secretase complex, leading to inefficient APP processing and an underproduction of the smaller Aβ peptides, disrupting physiologic ratio of Aβ proteoforms [31]. Notably, disease-causing mutations distributed throughout a gene often lead to a loss of function, as in the case of the *CFTR* gene in cystic fibrosis and the *DMD* gene in muscular dystrophy.

Mutation-Independent Approaches—Targeting the Amyloid Pathway While the mutation-specific methods described above would only be applicable to a small number of AD patients with known mutations in *APP* and *PSEN*, CRISPR technologies can also be used in ways that would be more broadly applicable. In one approach, CRISPR-Cas9 was used to delete segments of the *App* 3'UTR in an *App* knock-in mice ($App^{NL/G/F}$ carrying three familial AD mutations) [33]. Deleting a small 34 base-pair deletion distal to the *App* stop codon decreased *App* transcription, attenuated APP protein, and decreased Aβ deposition in the *App-KI* mouse brains [33]. Though this approach is a promising, the knockdown was achieved by injecting CRISPR-Cas9 directly into mouse zygotes, which limits therapeutic applicability. Safety and efficacy of this approach has also not been evaluated yet. Nevertheless, global attenuation of APP in the brain could be a promising approach, and an antisense oligonucleotide (ASO) based approach is currently in Phase-1 clinical trials [34]. However, the *APP* gene is highly conserved [35], and though

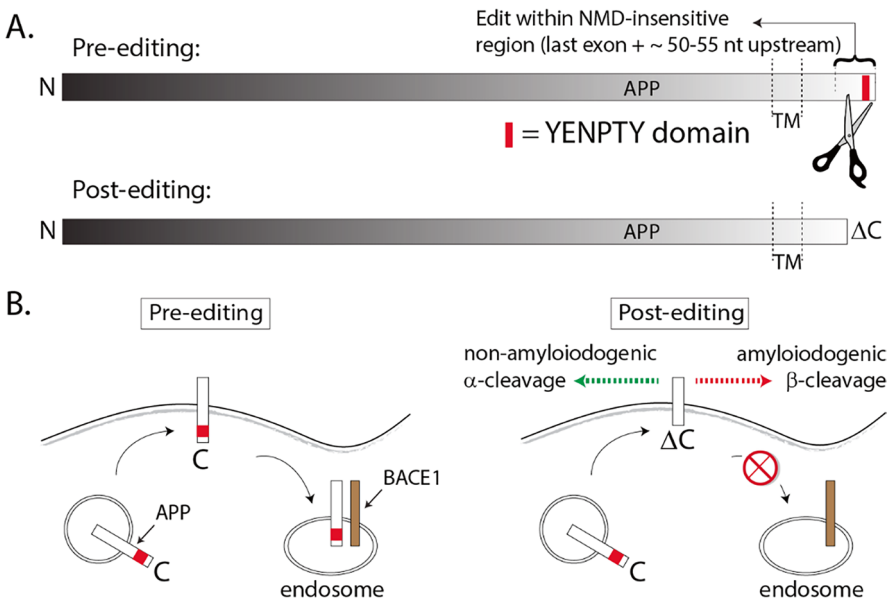

Fig. 4 (**a**) CRISPR-Cas9 (scissors) editing within the NMD-insensitive region of the last *APP* exon leads to effective cutting of the APP C-terminus and eliminates the YENPTY domain. (**b**) Pre-editing: Endocytic YENPTY-domain (red) mediates internalization of APP, which is cleaved by BACE1 in endosomes to trigger the amyloidogenic pathway. Post-editing: APP internalization into BACE1 containing endosomes is inhibited, attenuating amyloidogenic (β-cleavage) pathway. Additionally, APP nonamyloidogenic (α-cleavage) is augmented due surface-retention of APPΔC

there are compensatory *APP* homologues (*APLP*1/2) in mammals, eliminating *App* in mice has deleterious effects, including seizures and learning/memory deficits [36, 37].

In physiologic states, APP is cleaved by an alternate α-secretase pathway that generates neuroprotective and neuroregenerative fragments [38], and removing the entire *APP* gene would eliminate these effects as well. Our group has developed a CRISPR-Cas9 based strategy to shift the balance of APP-cleavage—attenuating toxic APP-β-cleavage products and upregulating protective α-cleavage products [39] (Fig. 4). This is achieved by effective elimination of an endocytic motif (YENPTY) and a few adjacent amino-acids at the extreme C-terminus of APP, a region that is encoded by the last exon (Fig. 4a). Though CRISPR editing typically leads to PTCs that trigger NMD, transcriptional rules dictate that PTCs within the last exon are NMD insensitive (reviewed in [40]), leading to interrupted translation at the PTC and a truncated protein. Removal of the YENPTY-domain attenuates trafficking of APP into endosome containing BACE1 and diminishes APP β-cleavage [41–43], while retention of truncated APP at membranes facilitates α-cleavage—presumably due to the higher activity of α-secretases in membranes [44]. Unlike approaches that eliminate APP, the N-terminus and transmembrane domain is preserved in our strategy, allowing generation of protective APP α-cleavage products.

One potential caveat of CRISPR/ASO based *APP*-targeting approaches (and indeed all Aβ lowering approaches) is that Aβ is normally produced in the brain at very low levels and may have physiologic effects [45]. However, in a clinical context, these methods are expected to diminish—and not eliminate—Aβ in human brains. Hippocampal injections of nanoparticles carrying CRISPR-Cas9 have been used to attenuate BACE1 and diminish AD pathology in an AD mouse model [46]. Though there is broad consensus that BACE1 is not a good therapeutic target [47], and local injections may not be therapeutically relevant for AD that has widespread pathology, delivery of CRISPR components by nanoparticles is an appealing alternative due to lack of immunogenicity and other advantages (discussed later). A nanoparticle-based CRISPRa approach has also been used to over-express *ADAM10*—with the goal of augmenting α-cleavage—but it is unclear if this approach favorably alters the α/β cleavage pattern [48]. Experimental studies have also used germline CRISPR-Cas deletion to attenuate tau in mice [49], but clinical feasibility of this approach has not been explored yet.

Mutation-Independent Approaches—Targeting APOE ε4 The apolipoprotein E (*APOE*) locus is the strongest known genetic risk factor for sporadic, late-onset AD [50, 51]. Three common *APOE* alleles (*APOE ε2, 3, and 4*) are translated to three APOE isoforms (APOE 2, 3, and 4) that differ in only two amino-acid positions (112 and 158), and have differential lipid-binding properties [52, 53]. Carriers of the *APOE ε4* allele have the strongest risk for late-onset AD and make up ~55–70% of all AD cases, though they comprise only ~24% of the healthy population. On the other hand, *APOE ε2* reduces the risk of AD by almost half and is associated with longevity [51]. Thus, conversion of the higher-risk allele (*APOE ε4*) to one of the lower-risk alleles (*APOE ε2/3*)—or reduction of *APOE ε4* altogether—are appealing therapeutic options. While these manipulations may be technically feasible using CRISPR-Cas, there have been few published reports so far, though this is expected to change in the near future. For instance, base editing was used to correct *APOE ε4* to *APOE ε3r*—a rare *APOE* variant—in cells [19]. Though the functional significance of this rare variant is unknown [54], this study highlights the feasibility of such approaches for manipulating APOE4.

Challenges in Developing CNS CRISPR Therapies

Delivery of Gene-Editing Therapeutics in the Human Brain For many neurodegenerative diseases, including AD, it will likely be necessary to deliver gene-editing components diffusely throughout the large human brain, at sufficient concentrations to generate gene editing. Delivery into the cerebrospinal fluid (CSF) space, as well as intravascular delivery, are being explored as options for diffuse brain delivery of gene-editing components. Potential methods for delivering CRISPR-Cas cargoes are shown in Fig. 5. Out of these, recombinant adeno-associated virus (AAV) vectors are currently the most clinically relevant technology to deliver CRISPR-components into the CNS, due to their relatively low immunogenicity and extensive clinical

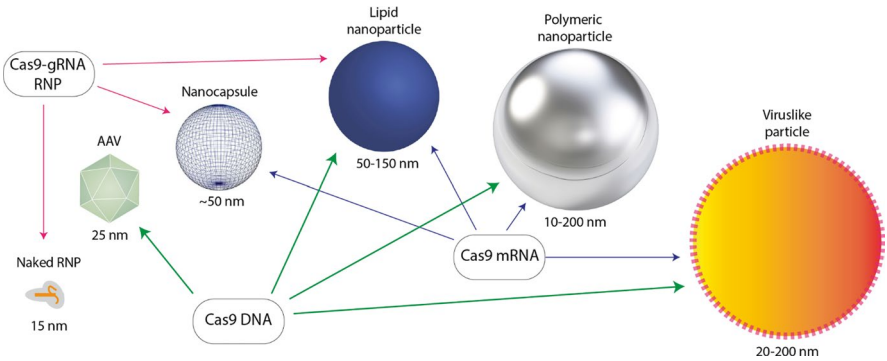

Fig. 5 The CRISPR payload can be packaged as preassembled ribonucleoprotein (RNP), mRNA, or plasmid DNA (e.g., in AAVs), and assembled into a variety of delivery particles, as shown in the schematic. Specific examples relevant to delivery of CRISPR-Cas9 components into the brain are cited in the text

track-record [55, 56]. For instance, ongoing trials are aiming to diffusely deliver transgenes into the human brain using AAVs for various neurodegenerative disorders [57–62], and an AAV-based CNS therapeutic (zogensma or AVXS-101) has been approved by the FDA for spinal muscular atrophy [63]. A clinical trial has also used AAVs and CRISPR-SaCas9 for the inherited eye disease Leber congenital amaurosis type-10 (LCA-10, [12]), and showed clinical efficacy [64] though the trial was halted for commercial reasons [4]. AAVs can also be used to deliver the Cas protein into specific cell types by using cell-defined promoters, which not only improves chances of editing but also mitigates unwanted effects of editing and persistence of Cas protein in untargeted cells. In principle, AAVs can be used to deliver gene-editing components via the CSF space, as well as via intravenous injections—using engineered AAVs—that can cross the blood–brain barrier and diffusely deliver payloads into the brain [65–67]. For instance, newly engineered AAV variants that bind to the human transferrin receptor are expected to cross the human blood-brain barrier and deliver payloads diffusely across the human brain, and further work in his area is eagerly anticipated [68]. However, AAVs also have several disadvantages that are discussed in the next section.

Non-viral modes of delivery using ribonucleoproteins and nanoparticles are promising approaches that allay many of the concerns specific to AAVs, such as limited cargo-capacity, immunogenicity, and cost of manufacturing [69, 70]. The lack of immunogenicity—due to the transient nature of editing and an absence of AAV capsids—is particularly appealing, though developing such delivery agents for the brain is particularly challenging due to size restrictions of the blood–brain barrier (≤60 nm, [71]). Various non-viral preparations have been used to deliver CRISPR payloads to the brain, such as ribonucleoproteins [72], "CRISPR-gold" nanoparticles [73], biodegradable PEGylated nanocapsules [74], and glucose-conjugated silica nanoparticles and AAV9 [75, 76]. Systemic delivery by lipid nanoparticles—widely used to deliver SARS-CoV-2 vaccines [77], and also the basis of delivering gRNA/CRISPR-Cas9 to the liver in a successful clinical trial

[2]—may also be an option, but so far, the particles have not been engineered to cross the human blood–brain barrier. Convection-enhanced delivery into the brain can disperse ribonucleoproteins beyond the site of local injection [70], and this may be a promising approach for delivering non-viral therapies. Though research into non-viral delivery systems is still in the experimental stages, a non-viral delivery system that can safely and efficiently edit neurons throughout the brain with minimal immunogenicity would be a transformative clinical tool.

Limitations of AAVs for Brain Delivery of Gene-Editing Therapeutics AAVs have a limited cargo capacity (~4.7 kilobase pairs), which restricts the type of Cas enzymes that can be accommodated into AAV vectors. Though there are several compact Cas proteins that can fit into AAVs along with other required components for gene editing [27, 78]—for example, SaCas9 that was used for the LCA-10 trial [12]—availability of PAM sites at desired targets can be a limiting factor, and the editing efficacies of these compact editors may also be variable. Though AAVs are less immunogenic than most viral vectors, systemically delivered capsids can activate severe host immune responses, particularly at high doses [79]. Delivery into the CSF space can mitigate some of these concerns, as the brain is thought to be an immune-privileged site, but leakage into the periphery is known to occur. AAV-mediated delivery would lead to persistence of the Cas enzyme in the brain, which may be a concern; however, though AAV-Cas9 can trigger systemic immune responses in mice [80], restriction of Cas9 expression in the brain by CSF-mediated delivery may limit potential side effects. Another concern is that AAV genomes can integrate into CRISPR cut sites [81]. Though genomic AAV-integration is very infrequent under normal circumstances, CRISPR editing seems to increase the affinity of AAV genomes to the cut site. Indeed, this phenomenon has been exploited to develop potential therapeutics [82] and also develop assays for determining putative off-target sites [83]. Though the effects of such integration are unknown, they largely occur at specified cut sites and involve small genomic segments, and careful gRNA selection should limit this risk. Finally, cost is likely to be a limiting factor for broadly delivering AAV therapeutics to patients, since they would be custom made for each therapy, and also require a large scale-up of production.

Unwanted Effects of DNA Editing While specificity of the CRISPR-Cas system is dictated by the synthetic gRNA, sequence similarities with other regions of the genome may lead to potential off-target effects such as random mutations, unwanted gene inactivation, deletions, or edits in tumor-suppressor genes that can increase the risk of cancers [82–84]. These concerns can be mitigated by careful gRNA selection, and evaluation of off-target effects by genome-scale assays such as Guide-Seq, Circle-Seq, or ITR-Seq [81, 85, 86]. However, it will probably be impossible to predict all genomic consequence of human brain editing without clinical trials, because of innate genomic diversities between species. Even preclinical testing of gene-editing therapeutics in non-human primates will not reveal the entire repertoire of potential off-target effects in humans due to innate genomic differences in both coding and non-coding regions. Indeed, this new class of genomic therapies has opened a frontier in medicine where the established gameplan of therapeutic

development—in silico/in vitro screens, followed by in vivo studies in mice and non-human primates—is unlikely to answer all the necessary questions before going to human trials.

Discovery-Based Applications of CRISPR-Technology in Alzheimer's Disease

Unbiased CRISPR-based screening tools can also be used to discover new disease mechanisms and identify previously unknown drug targets for AD. In CRISPR screening, large "gRNA libraries" are used to systematically target every gene in the genome—using NHEJ/CRISPR-i/a to inactivate or activate these genes—followed by backend assays to evaluate the functional effects of perturbation and identify genes/pathways involved [87]. Since the vast majority of AD cases are not due to known gene mutations, more mechanistic insight into these cases, and subsequent identification of new therapeutic targets, largely comes from either a deeper understanding of the precise steps that lead to pathology or from studies involving human genetics—such as Genome Wide Association Studies (GWAS)—that can identify potential risk genes in sporadic, late-onset AD populations. GWAS are a particularly rich source of clues into human disease, and insights from such studies have transformed the course of AD research in recent years, for instance, by highlighting the importance of microglia in the progression of AD. However, though the list of genes identified by GWAS continues to grow, our understanding of the putative roles played by these genes lags far behind. CRISPR screens can be valuable for identifying genetic modifiers of known AD-relevant pathologies—that can reveal new drug targets—and also for deciphering putative functions of GWAS hits.

Unbiased CRISPR screens have been used to identify previously unknown genetic modifiers of AD-relevant cellular phenotypes. Following the lead of cell-based RNAi screens that identified PAFAH1B2 and Rab11 as modifiers of Aβ generation [88, 89], CRISPR screens for Aβ production in mouse neuronal cell lines have also found a role for calcium and integrin-binding protein 1 (CIB1) in upregulating Aβ levels by interacting with the γ-secretase complex [90]. Several CRISPR screens have also identified cellular modifiers for tau aggregation. For example, CRISPR screen in cell lines has identified genes involved in tau aggregation and phosphorylation [91, 92]. Tau aggregates also spread between cells, triggering accumulation and aggregation of native tau in host cells, and this "prion-like" propagation is thought to play a role in disease [93]. In one study, CRISPRi and FRET biosensors were used to screen for genes involved in tau endocytosis and propagation [94]. CRISPR-knockdown of several components in the endosome-sorting ESCRT (EndoSomal Complexes Required for Transport) enhanced tau propagation, and the authors propose a model where deficits in the ESCRT machinery (which are linked in human tauopathies) damage endolysosomal membranes and facilitate the leakage of tau into the cytosol, where it is more prone to aggregate [94]. Another study combined CRISPRi-based screening with cellular models where tau propagation was triggered by both free tau aggregates and tau carried within exosome-like

vesicles; identifying novel genes that regulated tau seeding [95]. Unbiased CRISPR screening also holds great promise for deciphering putative phenotypic consequences of the many AD risk loci that have been identified by human GWAS studies (reviewed in [87]). In principle, hits from GWAS can be mimicked in functionally relevant cellular assays, followed by CRISPR screens and a systems-genetics approach to identify putative genes and pathways that are affected by the GWAS-hits—though there are several challenges to this approach [87, 94]. Nevertheless, recent studies using massively parallel reporter assays followed by CRISPRi screens are starting to provide insight into the functional significance of some of the GWAS variants [96], and this is likely to be an active area of future research.

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References

1. Frangoul H, et al. CRISPR-Cas9 gene editing for sickle cell disease and beta-thalassemia. N Engl J Med. 2021;384:252–60. https://doi.org/10.1056/NEJMoa2031054.
2. Gillmore JD, et al. CRISPR-Cas9 in vivo gene editing for transthyretin amyloidosis. N Engl J Med. 2021;385:493–502. https://doi.org/10.1056/NEJMoa2107454.
3. Wong C. UK first to approve CRISPR treatment for diseases: what you need to know. Nature. 2023;623:676–7. https://doi.org/10.1038/d41586-023-03590-6.
4. Henderson, H. CRISPR Clinical Trials: A 2024 Update. Innovative Genomics Institute News Published Mar 13, 2024. https://innovativegenomics.org/news/crispr-clinical-trials-2024/. Accessed 22 Apr 2024
5. Gustavsson A, et al. Global estimates on the number of persons across the Alzheimer's disease continuum. Alzheimers Dement. 2023;19:658–70. https://doi.org/10.1002/alz.12694.
6. Cummings J, et al. Alzheimer's disease drug development pipeline: 2023. Alzheimers Dement (N Y). 2023;9:e12385. https://doi.org/10.1002/trc2.12385.
7. van Dyck CH, et al. Lecanemab in early Alzheimer's disease. N Engl J Med. 2023;388:9–21. https://doi.org/10.1056/NEJMoa2212948.
8. Honig LS, et al. ARIA in patients treated with lecanemab (BAN2401) in a phase 2 study in early Alzheimer's disease. Alzheimers Dement (N Y). 2023;9:e12377. https://doi.org/10.1002/trc2.12377.
9. Barrangou R, et al. CRISPR provides acquired resistance against viruses in prokaryotes. Science. 2007;315:1709–12. https://doi.org/10.1126/science.1138140.
10. Jinek M, et al. A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. Science. 2012;337:816–21. https://doi.org/10.1126/science.1225829.
11. Pickar-Oliver A, Gersbach CA. The next generation of CRISPR-Cas technologies and applications. Nat Rev Mol Cell Biol. 2019;20:490–507. https://doi.org/10.1038/s41580-019-0131-5.
12. Maeder ML, et al. Development of a gene-editing approach to restore vision loss in Leber congenital amaurosis type 10. Nat Med. 2019;25:229–33. https://doi.org/10.1038/s41591-018-0327-9.
13. Gutschner T, Haemmerle M, Genovese G, Draetta GF, Chin L. Post-translational regulation of Cas9 during G1 enhances homology-directed repair. Cell Rep. 2016;14:1555–66. https://doi.org/10.1016/j.celrep.2016.01.019.
14. Qi LS, et al. Repurposing CRISPR as an RNA-guided platform for sequence-specific control of gene expression. Cell. 2013;152:1173–83. https://doi.org/10.1016/j.cell.2013.02.022.

15. Gilbert LA, et al. CRISPR-mediated modular RNA-guided regulation of transcription in eukaryotes. Cell. 2013;154:442–51. https://doi.org/10.1016/j.cell.2013.06.044.
16. Chavez A, et al. Highly efficient Cas9-mediated transcriptional programming. Nat Methods. 2015;12:326–8. https://doi.org/10.1038/nmeth.3312.
17. Maeder ML, et al. CRISPR RNA-guided activation of endogenous human genes. Nat Methods. 2013;10:977–9. https://doi.org/10.1038/nmeth.2598.
18. Konermann S, et al. Genome-scale transcriptional activation by an engineered CRISPR-Cas9 complex. Nature. 2015;517:583–8. https://doi.org/10.1038/nature14136.
19. Komor AC, Kim YB, Packer MS, Zuris JA, Liu DR. Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage. Nature. 2016;533:420–4. https://doi.org/10.1038/nature17946.
20. Gaudelli NM, et al. Programmable base editing of A*T to G*C in genomic DNA without DNA cleavage. Nature. 2017;551:464–71. https://doi.org/10.1038/nature24644.
21. Anzalone AV, et al. Search-and-replace genome editing without double-strand breaks or donor DNA. Nature. 2019;576:149–57. https://doi.org/10.1038/s41586-019-1711-4.
22. Holstege H, et al. Exome sequencing identifies rare damaging variants in ATP8B4 and ABCA1 as risk factors for Alzheimer's disease. Nat Genet. 2022;54:1786–94. https://doi.org/10.1038/s41588-022-01208-7.
23. Mullan M, et al. A pathogenic mutation for probable Alzheimer's disease in the APP gene at the N-terminus of beta-amyloid. Nat Genet. 1992;1:345–7. https://doi.org/10.1038/ng0892-345.
24. Gao X, et al. Treatment of autosomal dominant hearing loss by in vivo delivery of genome editing agents. Nature. 2018;553:217–21. https://doi.org/10.1038/nature25164.
25. Gyorgy B, et al. CRISPR/Cas9 mediated disruption of the Swedish APP allele as a therapeutic approach for early-onset Alzheimer's disease. Mol Ther Nucleic Acids. 2018;11:429–40. https://doi.org/10.1016/j.omtn.2018.03.007.
26. Duan Y, et al. Brain-wide Cas9-mediated cleavage of a gene causing familial Alzheimer's disease alleviates amyloid-related pathologies in mice. Nat Biomed Eng. 2022;6:168–80. https://doi.org/10.1038/s41551-021-00759-0.
27. Ran FA, et al. In vivo genome editing using Staphylococcus aureus Cas9. Nature. 2015;520:186–91. https://doi.org/10.1038/nature14299.
28. Konstantinidis E, et al. CRISPR-Cas9 treatment partially restores amyloid-beta 42/40 in human fibroblasts with the Alzheimer's disease PSEN 1 M146L mutation. Mol Ther Nucleic Acids. 2022;28:450–61. https://doi.org/10.1016/j.omtn.2022.03.022.
29. Shen J, Kelleher RJ 3rd. The presenilin hypothesis of Alzheimer's disease: evidence for a loss-of-function pathogenic mechanism. Proc Natl Acad Sci USA. 2007;104:403–9. https://doi.org/10.1073/pnas.0608332104.
30. Devkota S, et al. Familial Alzheimer mutations stabilize synaptotoxic gamma-secretase-substrate complexes. Cell Rep. 2024;43:113761. https://doi.org/10.1016/j.celrep.2024.113761.
31. De Strooper B, Karran E. New precision medicine avenues to the prevention of Alzheimer's disease from insights into the structure and function of gamma-secretases. EMBO J. 2024;43:887–903. https://doi.org/10.1038/s44318-024-00057-w.
32. Sun L, Zhou R, Yang G, Shi Y. Analysis of 138 pathogenic mutations in presenilin-1 on the in vitro production of Abeta42 and Abeta40 peptides by gamma-secretase. Proc Natl Acad Sci USA. 2017;114:E476–85. https://doi.org/10.1073/pnas.1618657114.
33. Nagata K, et al. Generation of App knock-in mice reveals deletion mutations protective against Alzheimer's disease-like pathology. Nat Commun. 2018;9:1800. https://doi.org/10.1038/s41467-018-04238-0.
34. A Study to Evaluate the Safety and Tolerability of ALN-APP in Patients with EOAD. ClinicalTrials.gov identifier: NCT05231785. Updated Feb 13, 2024. https://classic.clinicaltrials.gov/ct2/show/results/NCT05231785. Accessed 22 Apr 2024
35. Muller UC, Zheng H. Physiological functions of APP family proteins. Cold Spring Harb Perspect Med. 2012;2:a006288. https://doi.org/10.1101/cshperspect.a006288.

36. Steubler V, et al. Loss of all three APP family members during development impairs synaptic function and plasticity, disrupts learning, and causes an autism-like phenotype. EMBO J. 2021;40:e107471. https://doi.org/10.15252/embj.2020107471.
37. Lee SH, et al. APP family regulates neuronal excitability and synaptic plasticity but not neuronal survival. Neuron. 2020;108:676–690.e8. https://doi.org/10.1016/j.neuron.2020.08.011.
38. Muller UC, Deller T, Korte M. Not just amyloid: physiological functions of the amyloid precursor protein family. Nat Rev Neurosci. 2017;18:281–98. https://doi.org/10.1038/nrn.2017.29.
39. Sun J, et al. CRISPR/Cas9 editing of APP C-terminus attenuates beta-cleavage and promotes alpha-cleavage. Nat Commun. 2019;10:53. https://doi.org/10.1038/s41467-018-07971-8.
40. Kurosaki T, Maquat LE. Nonsense-mediated mRNA decay in humans at a glance. J Cell Sci. 2016;129:461–7. https://doi.org/10.1242/jcs.181008.
41. Das U, et al. Visualizing APP and BACE-1 approximation in neurons yields insight into the amyloidogenic pathway. Nat Neurosci. 2016;19:55–64. https://doi.org/10.1038/nn.4188.
42. Perez RG, et al. Mutagenesis identifies new signals for beta-amyloid precursor protein endocytosis, turnover, and the generation of secreted fragments, including Abeta42. J Biol Chem. 1999;274:18851–6.
43. Citron M, Teplow DB, Selkoe DJ. Generation of amyloid beta protein from its precursor is sequence specific. Neuron. 1995;14:661–70. https://doi.org/10.1016/0896-6273(95)90323-2[pii].
44. Rajendran L, Annaert W. Membrane trafficking pathways in Alzheimer's disease. Traffic. 2012;13:759–70. https://doi.org/10.1111/j.1600-0854.2012.01332.x.
45. Abramov E, et al. Amyloid-beta as a positive endogenous regulator of release probability at hippocampal synapses. Nat Neurosci. 2009;12:1567–76. https://doi.org/10.1038/nn.2433.
46. Park H, et al. In vivo neuronal gene editing via CRISPR-Cas9 amphiphilic nanocomplexes alleviates deficits in mouse models of Alzheimer's disease. Nat Neurosci. 2019;22:524–8. https://doi.org/10.1038/s41593-019-0352-0.
47. Zhang Y, Chen H, Li R, Sterling K, Song W. Amyloid beta-based therapy for Alzheimer's disease: challenges, successes and future. Signal Transduct Target Ther. 2023;8:248. https://doi.org/10.1038/s41392-023-01484-7.
48. Park H, Hwang Y, Kim J. Transcriptional activation with Cas9 activator nanocomplexes rescues Alzheimer's disease pathology. Biomaterials. 2021;279:121229. https://doi.org/10.1016/j.biomaterials.2021.121229.
49. Tan DCS, et al. Generation of a new Tau knockout (tauDeltaex1) line using CRISPR/Cas9 genome editing in mice. J Alzheimers Dis. 2018;62:571–8. https://doi.org/10.3233/JAD-171058.
50. Corder EH, et al. Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. Science. 1993;261:921–3. https://doi.org/10.1126/science.8346443.
51. Corder EH, et al. Protective effect of apolipoprotein E type 2 allele for late onset Alzheimer disease. Nat Genet. 1994;7:180–4. https://doi.org/10.1038/ng0694-180.
52. Raulin AC, et al. ApoE in Alzheimer's disease: pathophysiology and therapeutic strategies. Mol Neurodegener. 2022;17:72. https://doi.org/10.1186/s13024-022-00574-4.
53. Blumenfeld J, Yip O, Kim MJ, Huang Y. Cell type-specific roles of APOE4 in Alzheimer disease. Nat Rev Neurosci. 2024;25:91–110. https://doi.org/10.1038/s41583-023-00776-9.
54. Seripa D, et al. The missing ApoE allele. Ann Hum Genet. 2007;71:496–500. https://doi.org/10.1111/j.1469-1809.2006.00344.x.
55. Ginn SL, Amaya AK, Alexander IE, Edelstein M, Abedi MR. Gene therapy clinical trials worldwide to 2017: an update. J Gene Med. 2018;20:e3015. https://doi.org/10.1002/jgm.3015.
56. Sun J, Roy S. Gene-based therapies for neurodegenerative diseases. Nat Neurosci. 2021;24:297–311. https://doi.org/10.1038/s41593-020-00778-1.
57. Hinderer C, et al. Adeno-associated virus serotype 1-based gene therapy for FTD caused by GRN mutations. Ann Clin Transl Neurol. 2020;7:1843–53. https://doi.org/10.1002/acn3.51165.

58. Rosenberg JB, et al. AAVrh.10-mediated APOE2 central nervous system gene therapy for APOE4-associated Alzheimer's disease. Hum Gene Ther Clin Dev. 2018;29:24–47. https://doi.org/10.1089/humc.2017.231.
59. A Study of PBFT02 in Patients With Frontotemporal Dementia and Progranulin Mutations (FTD-GRN) (upliFT-D). ClinicalTrials.gov identifier: NCT04747431. Updated April 18, 2024. https://clinicaltrials.gov/study/NCT04747431. Accessed 22 Apr 2024
60. Phase 1/2 Clinical Trial of LY3884963 in Patients With Frontotemporal Dementia With Progranulin Mutations (FTD-GRN) (PROCLAIM). ClinicalTrials.gov identifier: NCT04408625. Updated Jan 17, 2024. https://clinicaltrials.gov/study/NCT04408625. Accessed 22 Apr 2024
61. Gene Therapy for APOE4 Homozygote of Alzheimer's Disease. ClinicalTrials.gov identifier: NCT03634007. Updated Nov 15, 2023. https://classic.clinicaltrials.gov/ct2/show/NCT03634007. Accessed 22 Apr 2024
62. Phase 1/2a Clinical Trial of PR001 (LY3884961) in Patients With Parkinson's Disease With at Least One GBA1 Mutation (PROPEL). ClinicalTrials.gov identifier: NCT04127578. Updated Mar 13, 2024. Accessed 22 Apr 2024.
63. Mendell JR, et al. Single-dose gene-replacement therapy for spinal muscular atrophy. N Engl J Med. 2017;377:1713–22. https://doi.org/10.1056/NEJMoa1706198.
64. Pierce EA, et al. Gene editing for CEP290-associated retinal degeneration. N Engl J Med. 2024;390:1972–84. https://doi.org/10.1056/NEJMoa2309915
65. Goertsen D, et al. AAV capsid variants with brain-wide transgene expression and decreased liver targeting after intravenous delivery in mouse and marmoset. Nat Neurosci. 2022;25:106–15. https://doi.org/10.1038/s41593-021-00969-4.
66. Chuapoco MR, et al. Adeno-associated viral vectors for functional intravenous gene transfer throughout the non-human primate brain. Nat Nanotechnol. 2023;18:1241–51. https://doi.org/10.1038/s41565-023-01419-x.
67. Doudna JA. The promise and challenge of therapeutic genome editing. Nature. 2020;578:229–36. https://doi.org/10.1038/s41586-020-1978-5.
68. Huang Q, et al. An AAV capsid reprogrammed to bind human transferrin receptor mediates brain-wide gene delivery. Science. 2024;384:1220–7. https://doi.org/10.1126/science.adm8386
69. Betzer O, et al. The effect of nanoparticle size on the ability to cross the blood-brain barrier: an in vivo study. Nanomedicine (Lond). 2017;12:1533–46. https://doi.org/10.2217/nnm-2017-0022.
70. Staahl BT, et al. Efficient genome editing in the mouse brain by local delivery of engineered Cas9 ribonucleoprotein complexes. Nat Biotechnol. 2017;35:431–4. https://doi.org/10.1038/nbt.3806.
71. Lee B, et al. Nanoparticle delivery of CRISPR into the brain rescues a mouse model of fragile X syndrome from exaggerated repetitive behaviours. Nat Biomed Eng. 2018;2:497–507. https://doi.org/10.1038/s41551-018-0252-8.
72. Metzger JM, et al. Efficient in vivo neuronal genome editing in the mouse brain using nanocapsules containing CRISPR-Cas9 ribonucleoproteins. Biomaterials. 2023;293:121959. https://doi.org/10.1016/j.biomaterials.2022.121959.
73. Wang Y, et al. Overcoming the blood-brain barrier for gene therapy via systemic administration of GSH-responsive silica Nanocapsules. Adv Mater. 2023;35:e2208018. https://doi.org/10.1002/adma.202208018.
74. Yan S, et al. Cas9-mediated replacement of expanded CAG repeats in a pig model of Huntington's disease. Nat Biomed Eng. 2023;7:629–46. https://doi.org/10.1038/s41551-023-01007-3.
75. Hou X, Zaks T, Langer R, Dong Y. Lipid nanoparticles for mRNA delivery. Nat Rev Mater. 2021;6:1078–94. https://doi.org/10.1038/s41578-021-00358-0.
76. Hou Z, et al. Efficient genome engineering in human pluripotent stem cells using Cas9 from Neisseria meningitidis. Proc Natl Acad Sci USA. 2013;110:15644–9. https://doi.org/10.1073/pnas.1313587110.
77. Wilson JM, Flotte TR. Moving forward after two deaths in a gene therapy trial of Myotubular myopathy. Hum Gene Ther. 2020;31:695–6. https://doi.org/10.1089/hum.2020.182.

78. Chew WL, et al. A multifunctional AAV-CRISPR-Cas9 and its host response. Nat Methods. 2016;13:868–74. https://doi.org/10.1038/nmeth.3993.
79. Hanlon KS, et al. High levels of AAV vector integration into CRISPR-induced DNA breaks. Nat Commun. 2019;10:4439. https://doi.org/10.1038/s41467-019-12449-2.
80. Wolter JM, et al. Cas9 gene therapy for Angelman syndrome traps Ube3a-ATS long non-coding RNA. Nature. 2020;587:281–4. https://doi.org/10.1038/s41586-020-2835-2.
81. Breton C, Clark PM, Wang L, Greig JA, Wilson JM. ITR-Seq, a next-generation sequencing assay, identifies genome-wide DNA editing sites in vivo following adeno-associated viral vector-mediated genome editing. BMC Genomics. 2020;21:239. https://doi.org/10.1186/s12864-020-6655-4.
82. Yeh CD, Richardson CD, Corn JE. Advances in genome editing through control of DNA repair pathways. Nat Cell Biol. 2019;21:1468–78. https://doi.org/10.1038/s41556-019-0425-z.
83. Park SH, et al. Comprehensive analysis and accurate quantification of unintended large gene modifications induced by CRISPR-Cas9 gene editing. Sci Adv. 2022;8:eabo7676. https://doi.org/10.1126/sciadv.abo7676.
84. Papathanasiou S, et al. Whole chromosome loss and genomic instability in mouse embryos after CRISPR-Cas9 genome editing. Nat Commun. 2021;12:5855. https://doi.org/10.1038/s41467-021-26097-y.
85. Tsai SQ, et al. GUIDE-seq enables genome-wide profiling of off-target cleavage by CRISPR-Cas nucleases. Nat Biotechnol. 2015;33:187–97. https://doi.org/10.1038/nbt.3117.
86. Tsai SQ, et al. CIRCLE-seq: a highly sensitive in vitro screen for genome-wide CRISPR-Cas9 nuclease off-targets. Nat Methods. 2017;14:607–14. https://doi.org/10.1038/nmeth.4278.
87. Kampmann M. CRISPR-based functional genomics for neurological disease. Nat Rev Neurol. 2020;16:465–80. https://doi.org/10.1038/s41582-020-0373-z.
88. Page RM, et al. Loss of PAFAH1B2 reduces amyloid-beta generation by promoting the degradation of amyloid precursor protein C-terminal fragments. J Neurosci. 2012;32:18204–14. https://doi.org/10.1523/JNEUROSCI.2681-12.2012.
89. Udayar V, et al. A paired RNAi and RabGAP overexpression screen identifies Rab11 as a regulator of beta-amyloid production. Cell Rep. 2013;5:1536–51. https://doi.org/10.1016/j.celrep.2013.12.005.
90. Chiu YW, et al. Identification of calcium and integrin-binding protein 1 as a novel regulator of production of amyloid beta peptide using CRISPR/Cas9-based screening system. FASEB J. 2020;34:7661–74. https://doi.org/10.1096/fj.201902966RR.
91. Sanchez CG, et al. Genome-wide CRISPR screen identifies protein pathways modulating tau protein levels in neurons. Commun Biol. 2021;4:736. https://doi.org/10.1038/s42003-021-02272-1.
92. Duan L, et al. Arrayed CRISPR reveals genetic regulators of tau aggregation, autophagy and mitochondria in Alzheimer's disease model. Sci Rep. 2021;11:2879. https://doi.org/10.1038/s41598-021-82658-7.
93. Dujardin S, Hyman BT. Tau prion-like propagation: state of the art and current challenges. Adv Exp Med Biol. 2019;1184:305–25. https://doi.org/10.1007/978-981-32-9358-8_23.
94. Chen JJ, et al. Compromised function of the ESCRT pathway promotes endolysosomal escape of tau seeds and propagation of tau aggregation. J Biol Chem. 2019;294:18952–66. https://doi.org/10.1074/jbc.RA119.009432.
95. Polanco JC, et al. CRISPRi screening reveals regulators of tau pathology shared between exosomal and vesicle-free tau. Life Sci Alliance. 2023;6:e202201689. https://doi.org/10.26508/lsa.202201689.
96. Cooper YA, et al. Functional regulatory variants implicate distinct transcriptional networks in dementia. Science. 2022;377:eabi8654. https://doi.org/10.1126/science.abi8654.