Narrative Review Article

The Genetics of Sporadic Alzheimer's Disease and the Potential of CRISPR and Exosome-Based Therapies

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ABSTRACT

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by the accumulation of beta-amyloid plaques and hyperphosphorylated tau tangles. It can be classified as either familial or sporadic, with sporadic AD being the most common. While each type has its own genetic associations, the apolipoprotein E (APOE) gene and its variants are the most strongly linked with sporadic Alzheimer's. Given the genetic component of AD, CRISPR/Cas9 technology has recently emerged as a promising tool for genetic therapy due to its precision and efficiency. However, its clinical translation faces significant challenges, particularly in achieving safe and effective delivery to the brain, which requires penetration of the blood-brain barrier (BBB). Exosomes, small extracellular vesicles capable of naturally crossing the BBB, offer a potential solution for CRISPR/Cas delivery. Advances in engineering have led to the development of "designer exosomes," which can be modified to enhance stability, targeting and cargo capacity. This review covers key genetic risk factors associated with sporadic Alzheimer's and explores how CRISPR/Cas systems, together with exosome-based delivery, have the potential to be applied for therapeutic and diagnostic purposes in AD. By synthesizing recent studies, this review highlights that combination of CRISPR/Cas with engineered exosomes represents a promising strategy for future AD research and therapy.

Keywords: Exosomes; CRISPR/Cas; Cas9; APOE; tau; amyloid; sporadic; Alzheimer's

INTRODUCTION

Alzheimer's disease (AD) is the leading cause of dementia (1) and is widely described as the most common form of the condition (2). It is a progressive neurodegenerative disease characterized by the abnormal accumulation of beta-amyloid (Aβ) peptide plaques and neurofibrillary tangles (NFTs), which are composed of hyperphosphorylated tau protein. These pathological changes contribute directly to neuronal cell death: extracellular Aβ plaques disrupt neuronal signaling from outside of the cell, while intracellular tau tangles interfere with processes inside the cell body (3).

Aβ plaques are made up of fragments of amyloid precursor protein (APP). Under physiological conditions, Aβ peptides are produced in small amounts and naturally cleared from the body. Although the physiological role of these peptides remains unclear, they are not harmful unless they misfold and aggregate into plaques (4). NFTs, by contrast, are composed of the microtubule (MT)-associated protein tau. In healthy neurons, it acts as a support for axonal transport and outgrowth. Normally, tau is phosphorylated at several sites, helping the protein to bind to MTs. However,

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when tau becomes hyperphosphorylated, it detaches from MTs and aggregates into NFTs, which are associated with AD pathology. Although debated by some, evidence suggests that the hyperphosphorylation of tau promotes tau aggregation and neurotoxicity (5). Importantly, abnormal accumulation of Aβ is also thought to, in a currently unclear manner, trigger or accelerate the tau hyperphosphorylation, establishing a toxic feedback loop in which Aβ plaques and NFTs reinforce one another's pathology (Figure 1) (3, 6). This interaction has led to the suggestion that any therapeutic intervention involving only one of these abnormalities may have limited effectiveness (6).

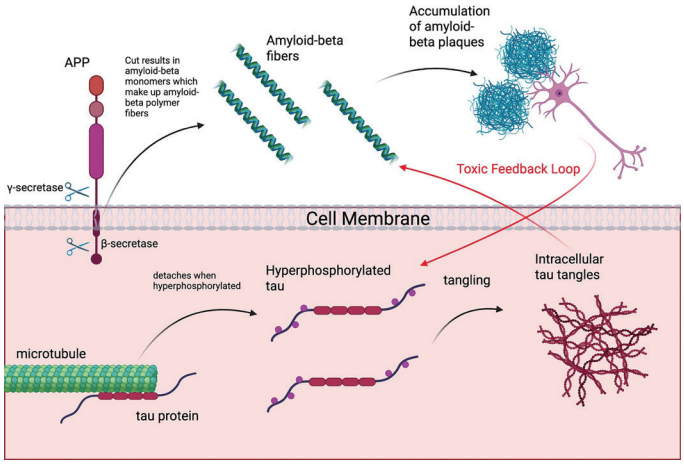

Figure 1. Schematic representation of AD pathology. The proteases γ-secretase and the β-secretase cleave the APP, producing amyloid beta monomers that aggregate into extracellular plaques. In parallel, tau proteins detach from MTs upon hyperphosphorylation and form intracellular NFTs. A proposed toxic feedback loop exists in which tau phosphorylation and Aβ accumulation reinforce one another, exacerbating neuronal dysfunction and degeneration (Created by the author using BioRender).

AD can be broadly categorized into two distinct types: familial and sporadic. Familial AD (FAD), also sometimes referred to as early-onset AD, is most often caused by mutations in the genes encoding APP, presenilin 1 (PSEN1), and presenilin 2 (PSEN2) (7), and typically follows an autosomal dominant inheritance pattern (8). In contrast, sporadic AD (sAD) has no clear inheritance pattern and usually appears much later in life, typically in individuals over 65 years old. Despite being responsible for about 95% of AD cases, sAD is far less understood than FAD (9). This can likely be attributed to the multifactorial nature of sAD. Although APOE is the best-known genetic risk factor for sAD, more than 60% of sAD cases are not associated with APOE variants, indicating the involvement of additional factors (10). Despite their distinct origins, both types of AD ultimately lead to the same pathological features: Aβ peptide accumulation, tau tangles, and progressive brain atrophy.

Since FAD and sAD arise from different genetic mechanisms, the choice of therapeutic targets is likely to depend on the type of AD. In FAD, mutations in APP, PSEN1 or PSEN2 provide clear genetic targets, whereas in sAD, genes like APOE contribute more indirectly to disease risk. Further, the target gene can also depend on the pathological feature that is being addressed, such as Aβ accumulation or the hyperphosphorylation of tau (11). Consequently, researchers have proposed that methods capable of accurately targeting specific genes may offer a promising direction for future AD therapies. One such method is clustered regularly interspaced short palindromic repeats system, also known as CRISPR, which is used together with CRISPR-associated (Cas) proteins for gene editing (12–14). Originally part of a bacterial immune defense, CRISPR systems recognize and store sequences of foreign DNA to create a targeted response following re-exposure to the same virus (15). Through adaptation of this natural mechanism. CRISPR has been reconstructed to cleave specific DNA sequences. This repurposing has provided a powerful platform for precise, efficient, and relatively affordable gene editing tools compared with earlier technologies (16). Among these systems, CRISPR/Cas9 is one of the most widely used. Cas9 is an endonuclease that cleaves both strands of DNA, guided by a synthetic single guide RNA (sgRNA). Successful cleavage requires the presence of a short protospacer adjacent motif, a sequence of two to five nucleotides, determined by the Cas9's bacterial origin, located at the 3' end of the sgRNA (15). This mechanism has been repurposed for the experimental treatment of disease-related gene mutations, and CRISPR/Cas9 is now recognized as a promising tool for gene therapy in disorders with a genetic component, including AD (16).

In order for CRISPR/Cas9 systems to be effective in AD, however, they must first be successfully delivered into the target organ: the brain. This presents a major challenge, as the brain is protected by the BBB, a semipermeable endothelial membrane that separates neurons from the systemic circulation and maintains the homeostasis of the central nervous system (17,18).

While this barrier provides an essential protective function, it also prevents most therapeutic molecules, including CRISPR/Cas9 components, from entering the brain. To overcome this obstacle, several delivery strategies have been explored.

Among them, exosomes, naturally occurring nanoscopic vesicles, are particularly promising because they can cross the BBB while carrying bioactive molecules and genetic material. Their small size, biocompatibility, and ability to transport genetic materials, make them an appealing delivery vehicle for CRISPR/Cas9 systems (19). Yet, exosomes are not without limitations: they are costly and time-consuming to produce, large-scale manufacturing remains a challenge and their limited size constrains cargo capacity (19). Despite these challenges, many researchers agree that the benefits of exosomes outweigh their shortcomings. Exosomes also offer natural cell-targeting capabilities, a longer half-life than many nanoparticle (NP) alternatives, and reduced risk of immune detection. Moreover, engineering approaches have been developed to expand their cargo capacity. For example, the MAPLEX system uses mMaple3-mediated protein loading, allowing exosomes to fuse with a photocleavable protein and release their cargo in response to blue light (20, 21). Such advances highlight the growing feasibility of using engineered exosomes to deliver therapeutic molecules to the brain. However, it is important to place these developments in the wider context of AD, which remains a multifactorial condition influenced by both genetic and environmental factors (2, 19). The widely-accepted "amyloid-tau" hypothesis may not fully explain its pathology (22), yet the strong genetic associations with these pathways, together with the potential of exosome-mediated CRISPR/Cas9 delivery, justify their continued exploration. This review focuses on the clinical potential of the CRISPR/Cas9 systems and exosomes in AD, with particular attention to how genetic risk factors may guide their application. While acknowledging the role of environmental influences, the discussion is centered on genetic contributors, with special emphasis on APOE, currently the most well-characterized sAD-associated gene (10).

THE GENETICS OF SAD

APOE in AD

The APOE gene is the most widely studied genetic risk factor for sAD. Among its alleles, APOE4 is associated with substantially increased risk of sAD, whereas APOE2 is generally considered protective, and linked to reduced risk (20). A third variant, APOE3, does not appear to alter risk directly. However, it is the most common APOE allele in the general population and can therefore serve as a reference in studies comparing the effects of APOE2 and APOE4 (20–22). Beyond its role as a 'control' APOE3 has also been explored as a potential therapeutic substitute for APOE4, with the aim of reducing the risk of AD (20, 23).

APOE3 Christchurch Homozygosity Mutation

Certain mutations of APOE3 can also influence disease risk. A notable example is the APOE3 Christchurch (R136S) variant, reported in a 70-year-old Colombian woman who carried the high-risk PSEN1 E280A mutation, typically associated with FAD. Despite this genetic predisposition, she did not develop cognitive impairment until her 70s. Genetic analyses, including whole exome sequencing, whole genome sequencing, and Sanger sequencing, revealed that she was homozygous for the APOE3 Christchurch mutation (20, 24). This finding suggests that the R136S variant could significantly delay the clinical onset of autosomal dominant AD dementia, also known as FAD dementia (24). Subsequent studies have indicated that even a single copy of R136S may delay the age of onset by approximately five years (25). Overall, these findings, based on a limited number of studies, suggest but do not confirm that APOE3 Christchurch may act as a rare protective modifier of AD risk.

APOE4 in AD

The degree of sAD risk associated with APOE4 depends on how many copies of the gene they carry, if any. Individuals carrying two copies of APOE4 have up to fifteen times greater risk of developing sAD, whereas those carrying a single copy, heterozygotes, have up to four times increased risk of sAD (23, 26). APOE4 carriers also tend to experience earlier disease onset (31). Importantly, APOE4 associated risk applies only to late-onset (sAD) and does not cause early-onset (FAD), as even individuals with homozygous APOE4 develop the pathology later in life, from around 55 years onward, which is consistent with the pattern of sAD and later than typical FAD onset (29). Mechanistically, APOE has been linked to impaired lipid transport compared to APOE3 (23, 30). Reduced lipidation capacity compromises neuronal maintenance and is associated with increased Aβ accumulation (30). In APOE4-

targeted replacement (TR) mice (mice with the human APOE4 gene instead of its native mouse APOE4 gene), poorly-lipidated APOE particles were associated with elevated Aβ levels, whereas APOE2 showed the opposite effect (31). APOE is also implicated in tau and amyloid pathology through its effects on neuroinflammation. In the presence of APOE4, microglia release higher levels of pro-inflammatory cytokines, which can activate astrocytes. Activated astrocytes then lose the ability to provide metabolic support to neurons, further exacerbating tau phosphorylation, Aβ aggregation and neurodegeneration (23, 32).

APOE2 in AD

Large-scale imaging studies have strengthened the evidence that APOE2 has a protective effect against Aβ accumulation. One cross-sectional study examined 4,432 cognitively unimpaired adults aged 65-85 who underwent APOE genotyping and amyloid positron emission tomography imaging (33). Within the cohort, carriers of the E2/E4 genotypes showed lower levels of Aβ deposition and a slower age-related increase compared with E3/E4 carriers. These findings indicate that E2 can offset, at least in part, the negative effects of E4 (33).

APOE2 has also been shown to help protect the integrity of the BBB. APOE4 promotes the degradation of the BBB via activation of the cyclophilin A (CypA)-maltrix metalloproteinase-9 (MMP9) pathway (35, 37, 38). In TR-mouse models, APOE2 was associated with intact BBB function, whereas APOE4 and APOE-deficient mice had increased vascular leakage, supporting the idea that APOE2 protects the BBB whereas APOE4 disrupts it (36). Findings from the same study also showed that APOE lacking (APOE-/-) and TR-APOE4 mice had five to six times higher levels of CypA in cerebral microvessels compared to TR-APOE2 mice (36). Elevated CypA levels are associated with greater activation of the CypA-MMP9 pathway, hence explaining the increased BBB degradation in these models.

While gene replacement of APOE4 with APOE2 has become an appealing therapeutic method, it is important to understand that APOE2 is linked with type III hyperlipoproteinemia, due to its decreased affinity for low-density lipoprotein receptors (32). However, hyperlipidemia has only been reported in 5-10% of APOE2 homozygotes. The majority of APOE2 carriers have normal hypolipemic profiles and the mouse models used to study APOE2 (TR-APOE2 mice) have lipid profiles resembling the small minority of APOE2 human carriers who develop hyperlipidemia: making these mouse models unreliable for hyperlipidemia study (37). Additionally, a compiled study of data from the Rotterdam Study and the Dutch Population Study, with randomly selected 35-year-old men, were used to determine the prevalence of APOE2 homozygosity in the Netherlands. The compiled data of 8,888 participants found only 57 which were homozygotic carriers of APOE2 and only 10 of the 57 were type III hyperlipoproteinemia patients (about only 18% of APOE2 homozygotic carriers) (38). Proving, at least within the Netherlands, that type III hyperlipoproteinemia among homozygotic APOE2 carriers may not be a major concern.

APOE2 was used as gene replacement for APOE4 in a clinical trial using Aβ pathological 5xFAD mouse models by using an adeno-associated virus-based platform that enabled inducible in vivo replacement of APOE4 with APOE2 (39). As a result of the study, it was discovered that although long-term expression of APOE2 in the mouse models had expected beneficial results on Aβ accumulation, hallmark AD phenotypes, and cognitive decline, short-term APOE2 expression had the exact opposite effect. Although both short-term and long-term APOE4 did prove to be as risk-inducing of AD, as expected (in terms of Aβ accumulation, hallmark AD phenotypes, and cognitive decline), short-term APOE2 increased vascular Aβ accumulation and proved to have no beneficial effects. The study then concluded that these results may show how administration of APOE-based gene therapies at inappropriate times may have negative influences on AD pathology (39).

Other Strongly Associated Genetic Risk Factors for sAD

Although APOE genes are the most commonly AD associated genetic factors, there are other genes that can influence AD pathology. Among them includes bridging integrator 1 (BIN1). BIN1 is a ubiquitously expressed protein which is also considered a major AD risk factor. It is most known for its role in endocytosis: the process by which substances are brought into a cell (40). Some may report that BIN1 is the second most influential risk factor for sAD (second only to APOE) (41). However, researchers looking into BIN1 should note that how BIN1 is involved in AD is relatively unclear (40). A genome-wide association study by proxy (GWAX) analysis was performed in the United Kingdom Biobank

(UKBB) cohort utilizing the family history of AD. This study involved subjects with APOE, BIN1, and TREM2 (another AD risk associated gene). With 55,806 cases and 122,538 controls, grouping subjects using APOE status as a co-variant, BIN1 was the most significantly associated with AD risk among groups (42). The study concluded BIN1 as a prominent gene in AD that can potentially be clinically useful.

Another AD associated gene is the phosphatidylinositol binding clathrin assembly protein (PICALM). PICALM has become associated with AD due to the possibility that it regulates the production, transportation, and clearance of Aβ. It also is associated with the thickness of the entorhinal cortex which is a brain region in the medial temporal lobe near the hippocampus (43). In order to see how PICALM as well as clusterin (CLU), and complement receptor 1 (CR1) (more AD associated genes) are associated with late-onset AD (LOAD) (also known as sAD), a case-control association study was done in replication of studies done by Harold *et al*. (44) and Lambert *et al.* (45). By looking at single nucleotide polymorphisms (SNPs) (a single variation in a DNA nucleotide) of the PICALM gene, the study found that PICALM SNPs had a significant association with reduced risk of LOAD (46). The results of this study reveals another possible protective AD gene other than APOE2 which may also prove clinically useful.

As mentioned in the UKBB GWAX study, the triggering receptor expressed on myeloid cells 2 (TREM2) is another AD associated gene. TREM2 is a single pass transmembrane receptor linked to immune function. Known also to bind to Aβ oligomers, TREM2 R47H variant is associated with a 3 times increased risk of AD or LOAD (47, 48). Other AD risk inducing TREM2 variants include R62H, T66M, H157Y, and D87N. However, some studies suggest that activating TREM2 can actually prove beneficial for Aβ reduction and decreased tau hyperphosphorylation by way of microglial (immune cells in the brain) response (47). Using overexpressed human wild-type TREM2 (TREM2 WT) or TREM2 variant R47H microglia-specific inducible mouse models, is was discovered that TREM2 WT reduces amyloid accumulation and neuritic dystrophy only in the early stages while TREM2 R47H increases amyloid burden during the middle stages of amyloid growth (49). For future research on TREM2 association with AD, these results imply that certain variants of TREM2 are not useful during specific stages of Aβ pathology.

For the genetic therapy of AD, understanding genes that are strongly associated with AD is essential to identify potential gene targets. Gene editing approaches have been explored to modify genes such as APP or APOE4, in order to treat AD and reduce pathological features such as amyloid pathology and neurodegeneration. Among these approaches, the recently developed CRISPR/Cas9 system represents a particularly promising technology that can leverage insights from AD-associated gene identification.

CRISPR/CAS9 SYSTEM USES FOR SAD

CRISPR/Cas9 technology is a versatile gene editing tool that enables precise modification of specific DNA sequences. Since its development, it has been applied widely in biomedical research to investigate mechanisms of disease and explore potential therapeutic options. Given that AD involves well-characterized genetic components, CRISPR/Cas9 has attracted growing interest as a possible tool for developing gene-based interventions. To date, no clinical trials using CRISPR for AD have been conducted, current findings are limited to pre-clinical or proof of concept studies in cellular and animal models. This is not unexpected, as the technology is still relatively new and presents unique challenges for delivery to the brain. Nonetheless, researchers are actively investigating how CRISPR could be used to knockout (KO) or replace AD associated genes, with promising results from preclinical studies.

At the Alzheimer's Association International Conference (AAIC) in 2023, several groups presented early applications of CRISPR technology in AD research (50). For example, Brent Aulston, PhD., and colleagues in the Subhojit Roy lab at University of California San Diego, described a strategy targeting APP to reduce Aβ plaque formation in mouse models (50). In another study, presented at the same meeting, Boris Kantor, PhD., and Ornit Chiba-Falek, PhD., reported a CRISPR-based approach that selectively reduced APOE4 expression levels while preserving levels of other APOE variants (50). These findings provide proof-of-concept evidence that CRISPR could be applied in the context of AD therapy. Beyond these examples, additional studies have demonstrated the utility of the CRISPR/Cas9 system in generating disease models of AD, identifying biomarkers and testing potential therapeutic strategies.

CRISPR/Cas9 Systems for AD Modeling

CRISPR/Cas9 technology, often used in combination with human-induced pluripotent stem cells, provides a powerful platform for generating AD models. These models are in turn valuable for testing genetic-targeting approaches and studying the molecular mechanisms of AD. In particular, CRISPR/Cas9 can be used to introduce AD-associated mutations into cell lines allowing a more in-depth study of the molecular mechanisms of AD, within relatively short experimental timeframes (51, 52). In a recent study, a TREM2 H157Y knock-in (KI) mouse model was developed via CRISPR/Cas9 technology in order to test TREM2 H157Y risk association with AD (53). In 2024, Zhong *et al*. did a review on mouse models for AD. In that review, Zhong *et al*. mentions the utilization of CRISPR/Cas systems to create a new generation of mouse models using KI or KO technology (54). As many current studies on AD involve KI or KO mouse models, CRISPR/Cas9 systems provide immense support in their creation. As CRISPR/Cas9 technology continues to develop, CRISPR-generated AD models will likely continue to become better. While these approaches provide valuable modeling systems, therapeutic application of CRISPR in humans faces distinct barriers including off-target risk, editing efficiency, and optimal delivery timing.

CRISPR For Identifying Neuronal Aging

Although less significant than its applications in AD modeling and therapy, CRISPR technology can also be used to identify biomarkers relevant to AD, including neuronal aging. In 2024, Saurat *et al.* developed a CRISPR screen to identify regulators of neuronal age. Their work showed that blocking nddylation (a process that marks proteins for degradation) increased cellular hallmarks of aging and promoted tau aggregation and phosphorylation in neurons carrying the APP Sweden mutation. Using a human stem cell model, the study demonstrated that cellular aging can reveal LOAD phenotypes, such as tau pathology and neurodegeneration (55). These findings suggest that CRISPR-based screening can expand AD research into new pathways.

CRISPR/Cas9 For sAD Therapy

Among Cas proteins, Cas9 currently shows the greatest clinical potential for AD. CRISPR/Cas9 has enabled the generation of improved cellular and molecular models, functional KOs, and precise genome insertions (51). Previous studies have used CRISPR/Cas9 to target genes such as APP, BACE1 (sequential in APP modification or processing (51), and APOE4 in mouse models (56). Although genetic therapy for sAD remains debated, both FAD and sAD involve alteration in Aβ metabolism, making genetic correction of Aβ production a compelling therapeutic approach. Thus, while APP and BACE1 mutations are more strongly associated with FAD, they remain worthwhile CRISPR/Cas9 targets in sAD because of their central role in Aβ processing (56).

Another therapeutic strategy under investigation is the conversion of APOE4 to either APOE3 or APOE2. Given the established protective effects of these alleles (20, 23, 31, 36, 39), several groups have explored CRISPR/Cas9-mediated APOE gene replacement. For example, Rottner *et al*. investigated prime editing as a means of generating optimized APOE4 induced pluripotent stem cells models (57). Their approach used a Cas9- nickase fused to a reverse transcriptase along with a prime editing guide RNA (pegRNA).

Although the APOE4-to-APOE3 conversion encountered technical challenges, such as incomplete stem loop formation, which limited editing efficiency, the authors proposed that further refinement of pegRNA design, combined with sgRNA application, could overcome these barriers (57). While the study was aimed at creating improved APOE4 models, it also provides crucial insight into how CRISPR/Cas9 might be harnessed for allele conversion.

CRISPR/Cas systems hold great potential for AD. However, for CRISPR components to carry out their function, they first need to reach their target tissue. A major challenge lies in developing efficient and reliable delivery methods. In the context of AD, where the primary target is the brain, exosomes have emerged as a highly promising option.

EXOSOMAL DELIVERY FOR CRISPR/CAS9 SYSTEMS IN ALZHEIMER'S THERAPY

Exosomes As Natural Carriers

Although substantial progress has been made in CRISPR/Cas9 research, no clinical studies have yet applied this technology to AD. One of the key barriers to translation is the challenge of delivering CRISPR/Cas9 systems efficiently and safely into the brain. Exosomes, small extracellular vesicles (EVs), have emerged as a promising solution. As naturally occurring, biocompatible, and easy to handle carriers of nucleic acids, exosomes are being investigated as

potential vectors capable of partial or selective BBB passage under specific physiological or engineered conditions. However, their delivery efficiency remains heterogeneous and depends on factors such as targeting strategy, cargo size, and yield (58) Another major limitation is clearance by phagocytes in peripheral tissues and by microglia within the CNS, which can engulf exosomes, and thereby reduce their circulation time and delivery efficiency (Figure 2) (59).

Although their small size (30-150nm) makes membrane diffusion easy, this also limits how much nucleic cargo unmodified exosomes can carry (Figure 2). Other challenges include the unpredictability of exosomal release from stem cells, the presence of biomolecules with unknown effects, the varying exosome yields based on demographics, and unreliable characterization techniques that often produce inconsistent results (60). To address these challenges, researchers have implemented strict quality control protocols to ensure homogeneity, isolating exosomes from other bodily fluids, optimizing isolation protocols for each varying exosome type, and ensuring proper storage (60). Overall, exosomes remain challenging to work with. But, they remain a prominent option as a potential nucleic delivery vehicle due to their amenability to future modifications.

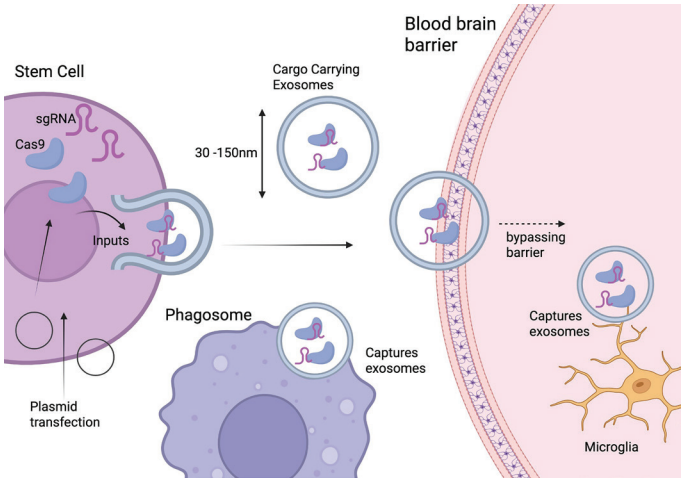

Figure 2. Exosome-mediated delivery of CRISPR/Cas components across the BBB. Cas proteins and sgRNAs are expressed via plasmid transfection in donor stem cells and subsequently packaged into exosomes. These cargo carrying exosomes are then carried past the blood brain barrier into the brain for target editing (Created by the author using BioRender).

Another challenge surrounding exosome use is that they are rapidly cleared from circulation by the immune system reducing the likelihood of reaching the target tissue (59). To address this, researchers have investigated exosomes derived from mesenchymal stem cells (MSCs), which display protective surface signals that extend their circulatory half-life to several hours (59-62). In recent years, advances in bioengineering have produced modified exosomes with improved properties, such as increased cargo capacity, an important improvement given the small size of natural exosomes, and enhanced targeting abilities (60, 61). Numerous techniques have been developed to facilitate the loading of cargo into exosomes. Electroporation, for example, allows larger cargo to be incorporated (57, 59). Ongoing research and improvement of exosomal delivery techniques point towards a promising future for CRISPR/Cas9 delivery, offering potential for safer and more precise therapeutic interventions. As exosomes have many challenges, the need for further modification is critical.

Loading Strategies

Researchers have found several efficient methods of loading genetic cargo into exosomes. One such method is electroporation: a commonly used technique for loading RNA cargo into exosomes. By applying an electric field, temporary pores are formed in the exosome membrane, allowing the cargo to enter. Once the membrane is restored, the RNA is encapsulated, and the exosome can subsequently deliver this genetic material to target cells (58, 63, 64). In the context of CRISPR/Cas9 delivery, mRNA encoding Cas proteins and gRNAs can be introduced into exosomes via electroporation, enabling the system to be directed to specific genomic locus (58). Importantly, RNA cargo delivered via exosomes has demonstrated improved stability in vivo, prolonged circulation time in the blood, and enhanced cell-targeting efficiency. Further, among the studies involved to prove that RNA delivered by exosomes improves overall efficiency, one study was done using BACE1 siRNA as a therapeutic exosomal cargo for Alzheimer's therapy (64).

Another potential loading strategy is preloading. Preloading aims to increase the payload of exosomes. Unlike post-loading strategies (which include passive and active packaging), preloading relies on co-incubation with donor or parent cells that are modified and loaded with exogenous cargo, thereby producing exosomes containing the desired material in situ (64, 65). This approach allows for continuous production

of cargo-loaded exosomes without compromising exosomal membrane integrity (65).

Although preloading is not feasible for all types of cargo (64), it represents an appealing method for generating "designer exosomes" that could potentially prove useful in AD therapy.

Further, sonication is another loading strategy which is sometimes preferred over electroporation due to its high loading efficiency. Sonication uses ultrasound to temporarily deform exosome membranes, enabling cargo to enter (65, 66). Recovery of the exosome membrane can be accomplished via incubation right after sonication (66). As an active packaging method, sonification has been associated with exosomes that display improved stability and anti-inflammatory effects (66, 67).

Important to note, however separate from loading strategy but relevant to it, is the cargo type loaded into the exosomes. Cargo type can influence exosome modification. For example, Hade *et al.* reported that in their system, loading capacity increased with longer incubation times at room temperature when exosomes were combined with their developed simple peptide-equipped technology: YARA-miR-21-5p (a cell penetrating peptide with a mammalian mRNA (66, 68)). This effect was not observed in other studies using different exosome variants and cargo, suggesting that loading efficiency may depend on the interaction between cargo type and exosome origin.

Engineering "Designer Exosomes" for Stability And Targeting

The modifications of exosomal loading strategies can fall into a category labeled as "designer exosomes." The term "designer exosomes" has an ambiguous origin, but was used in a 2025 paper by Dr. Han-Mo Yang to describe exosomes modified to achieve a higher payload with reduced immunogenicity (61). In this paper, however, the term will be defined more broadly as exosomes that have been engineered to possess more advantageous properties. Another modification strategy for designer exosomes involves reducing phagocytosis-mediated clearance, which is considered the primary cause of the short circulation time of exosomes (**Figure 2**). Phagocytosis is the process by which phagocytes engulf bacteria or other particles, and macrophage-mediated phagocytosis plays the most significant role in limiting exosome half-life in vivo. To address this, researchers have proposed a "camouflaged cloak" strategy, in which exosomes are modified with antiphagocytic molecules to evade clearance (59). In a study looking into exosomes for targeted cancer therapy, artificial chimeric exosomes were generated by incorporating the transmembrane protein CD47, a candidate antiphagocytic molecule, into their phospholipid bilayer using red blood cell membranes. In animal models, these modified exosomes demonstrated reduced interception by the mononuclear phagocyte system (69). These findings suggest that the "camouflaged cloak" strategy could be integral to the development of "designer exosomes" enabling longer circulation times and, in turn, improving the potential utility for genetic therapies in AD.

Designer exosomes can also benefit from CRISPR/Cas9 assisting technology. CRISPR/Cas9 systems have their own struggles. Although exosomes provide a gateway into the brain (by passing the BBB), it cannot provide an efficiently guided pathway with tissue specificity. As such, CRISPR/Cas9 systems, with or without exosomes, are in need of support or modification to yield that greater efficiency and specificity. Further, because the brain is a particularly challenging organ for CRISPR/Cas9 delivery, improvements in targeting methods could substantially enhance therapeutic outcomes. Selective Organ Targeting (SORT), a strategy introduced by Cheng *et al*., involves engineering lipid NPs (LNPs) with an additional SORT molecule to direct editing activity to extrahepatic tissues (70). In this study, researchers used the LNPs to deliver Cas9 and RNA complexes into organs other than the liver such as the spleen and lungs in their mouse models. Although this technique has not yet been applied to the brain, the underlying concept suggests potential relevance for AD therapy. If exosomes were used in place of LNPs, the SORT principle could, in theory, improve the efficiency of CRISPR delivery to neural tissue. In LNPs, the SORT molecule is a lipid tailored to the target tissue when administered intravenously (70). For exosomes, an analogous strategy might involve modifying surface proteins, which naturally carry information about the exosome's tissue of origin (71). By engineering these proteins to mimic the targeting function of SORT lipids, it may be possible to create an exosome version of Cheng *et al.* 's lipid SORT molecules. Designer exosomes can then benefit from CRISPR/Cas9 intended SORT technology to overcome specificity challenges: improving overall target specificity.

Photo Influenced Exosomes

An interesting new addition to the category of "designer exosomes" are photoinducible, photo-

cleavable, or photo-influenced exosomes. These approaches use light to modify exosomes, either within animal models or in experimental systems (72–74). One example is MAPLEX, a photocleavable protein platform to load protein cargo into exosomes and then expel them. In a recent study, MAPLEXs loaded with a BACE1 targeting sgRNA coupled with the catalytic domain of DNA methyltransferase 3A achieved significant reduction in BACE1 expression, cognitive impairment, and Aβ pathology (72). In another study, researchers developed photoactivatable synthetic exosomes capable of encapsulating RNA and enabling paracrine (hormone influence within a small vicinity) signaling between artificial and living cells. This work provided insight into how light-sensitive exosomes could be used for RNA communication (74). Similarly, photobiomodulation (PBM), a form of light therapy that applies visible or near-infrared light for cellular activation, was shown to influence microglia behavior. Specifically, PBM promoted a shift from the pro-inflammatory M1 phenotype to the anti-inflammatory M2 phenotype, leading to the secretion of microRNA-containing exosomes (73). Unlike other approaches, this study modified exosome release indirectly by influencing the stem cells that produced them rather than the exosome themselves. Overall, these studies show the ways in which light can be harnessed to engineer exosomes.

Utility of Exosome Origin in Alzheimer's Therapy

The cellular origin of exosomes plays an important role in determining their function, and leveraging exosomes derived from different sources can broaden their clinical application. MSC derived exosomes are particularly attractive because they are secreted in abundance, easily obtained from many tissue types and have demonstrated therapeutic effects across a wide range of diseases, including neurological ones (75). MSC-derived exosomes have been extensively researched for AD therapy and have shown promise in reducing AD-related neuropathology, improving cognitive performance, and slowing down AD pathogenesis. These MSC-exosome treatment studies don't involve loaded exosomes or specific cargo but they do reveal the benefits of MSC-exosomes that can be used along with delivered cargo for AD (76). In a study looking to explore the safety and efficiency of allogenic human adipose MSC-exosomes (ahaMSCs-Exo) in patients with mild to moderate AD, subjects were assigned to three dosage groups with one group receiving ahaMSCs-Exo twice weekly for 12 weeks (77). After four more follow-ups beyond week 12, the treatment was found to be safe and associated with improved cognitive function, as assessed by AD Assessment Scale-Cognitive (ADAS-cog) and Montreal Cognitive Assessment scores.

Although no effects were observed on tau or Aβ accumulation (77), these findings suggest that MSCs-Exos hold potential as therapeutic carriers for AD.

Another potentially promising exosome type for AD therapy is derived from microglial cells. Although microglia are often associated with AD pathology, microglia-derived exosomes may have beneficial roles, particularly by mediating communication between glial cells and neurons (78). Studies in rat models have shown that these exosomes can secrete microRNAs in response to disease conditions (79). Collectively, research indicates that microglia-derived exosomes may help reduce Aβ pathology and other biomarkers of AD by mitigating neuroinflammation and facilitating neuronal dendritic spine plasticity (73, 78, 79).

Looking forward, tailoring exosome sources may become essential for optimizing therapeutic outcomes in AD. Understanding the distinct properties of MSC- versus microglia-derived exosomes represents an important step toward defining how cellular origin influences their role in AD therapy.

CONCLUSION

AD remains a complex neurological disease with limited treatment options. This review has discussed how CRISPR/Cas technologies may provide new opportunities for precise genetic interventions and how exosomes, particularly "designer exosomes," offer a promising means of overcoming current delivery barriers. In the future, exosomes are likely to become versatile genetic carriers, with different variants tailored to distinct therapeutic functions. When combined with CRISPR/Cas technology, they may enable more accurate gene editing, improved biomarker detection and earlier diagnosis. Although significant challenges remain, including safety, efficiency and large-scale production, ongoing advances in both CRISPR/Cas technology and exosome modification suggest that these obstacles can be overcome. By integrating insights into the genetics of AD with innovations in gene editing and delivery CRISPR/Cas-based exosome therapy holds considerable promise for transforming the therapeutic management of AD.

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CONFLICT OF INTERESTS

The author declares that there are no conflicts of interest regarding the publication of this article.

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