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Alzheimer's disease: A CRISPR/CAS9-mediated therapeutic approach

Mohd Qasid Lari [1, *], Deepak Kumar [1], Yogend Chaurasia [1], Pragati Srivastava [1], Riya Shikha [2, 3], Abhishek Kumar Singh [4] and Amit Kumar [5]

[1] *Sardar Patel College of Pharmacy, Medical College Road, Gorakhpur 273013, (U.P.), India.*
[2] *CSIR-Central Institute of Medicinal and Aromatic Plants, Lucknow 226015. (U.P), India.*
[3] *Integral University, Kursi Road, Lucknow 226015. (U.P), India.*
[4] *Faculty of Pharmaceutical Sciences, Mahayogi Gorakhnath University, Gorakhpur 273007 (U.P.), India*
[5] *Dr. Mahendra Kumar Chhote Lal Bind college of Pharmacy Upardaha handia Prayagraj, (U.P.), India.*

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Abstract

The degenerative nature of Alzheimer's disease (AD) and its severe effects on cognitive function present a major challenge to worldwide healthcare systems. CRISPR/Cas9, one of the most recent developments in gene-editing technology, has created new opportunities to investigate possible AD treatment approaches. The present state of AD research is reviewed in this paper, along with the possibility of using CRISPR/Cas9-mediated methods to target important genetic elements involved in AD pathogenesis. Through targeted gene editing linked to tau protein malfunction, neuroinflammation, and amyloid-beta accumulation, CRISPR/Cas9 presents a viable approach to altering the molecular course of disease. Additionally, using CRISPR/Cas9 in patient-specific induced pluripotent stem cells (iPSCs) may lead to personalized medicine strategies for the treatment of AD. Issues like delivery strategies, off-target impacts, and moral dilemmas are also covered. All things considered, the application of CRISPR/Cas9 technology to AD research is a fresh and potentially revolutionary strategy for creating targeted treatments for this intricate neurodegenerative illness. For the purpose of treating AD, more preclinical and clinical research is necessary to confirm the security and effectiveness of CRISPR/Cas9-based therapies.

With the use of the CRISPR/Cas9 system, precise and effective genome modification is possible, enabling targeted editing of particular genes linked to the pathophysiology of AD. Thanks to this technology, genetic mutations in the presenilin 1 (PSEN1), presenilin 2 (PSEN2), and amyloid precursor protein (APP) genes that are linked to family types of AD can be corrected. It is feasible to restore normal protein function and possibly lessen the pathogenic processes that underlie AD by fixing these mutations.

Keywords: CRISPR/Cas9; Alzheimer's Disease; APP; APOE; PSEN1; PSEN2; Amyloid beta; Tau protein

1. Introduction

For more than a decade, the brain has been solely focused on deleting this residue as a potential therapeutic tool for AD patients. This residue of beta-amyloid plaque elicits the development of various signaling cascades which subsequently affect several intraneuronal and interneuronal dysfunctions that contribute to the formation of neurofibrillary tangles and promote mitochondrial and endoplasmic reticulum stress (1). CRISPR/Cas9, a single guide RNA manipulation technology for the correct and versatile DNA repair tool, has been documented. The double-stranded breakage, particularly non-homologous end joining and homology-directed repair (HDR), can be used for the correct and versatile DNA repair and also as a gene editing tool. Several inhibitory cascades have been designed, implemented, and examined

* Corresponding author: Mohd Qasid Lari

in AD models for knocking out the residue on human neural cells and patients' induced pluripotent cells, either by employing viral vectors or as an untargeted strategy used outside the brain safety of the body (2).

Alzheimer's disease (AD) is one of the most difficult to control neurodegenerative disorders of the aged population and leads to substantial intellectual disability and a decline in various brain functions. It is one of the most substantial chronic disorders leading to dementia, between 64% and 75% of the neurodegenerative disorders (3). Early-onset AD or familial AD is a rare form of AD that begins at the age of 30 to 65 years, accounting for fewer than 5% of all AD cases. The genetic factors responsible for the disease include mutations in genes that are responsible for encoding amyloid precursor protein (APP), presenilin-1 (PSEN1), or presenilin-2 (PSEN2) (4). Polygenic AD or late-onset AD occurs after the age of 65 years in most AD patients. It is influenced by environmental factors and involves the phenotypic heterogeneity in individuals by mutations in over 20 genes, but the major AD risk genes are APP, PSEN1, PSEN2, apolipoprotein E (APOE) ε4 and ε2 alleles. In the early 1990s, the amyloid cascade hypothesis proposed that the intraneuronal acidic cleavages are associated with the formation of beta-amyloid plaque (5).

Currently, there is no known cure for AD. There is already a wide range of symptomatic treatments on the market to treat those symptoms of AD or to slow disease progress (6). These medicines are capable of reducing the effectiveness of or delaying symptoms to some extent in some patients, although they are regarded as effective therapeutic approaches to improve cognitive functions in managing AD patients (7). AD condition is a heterogeneous neurodegenerative disease, for which no particular target has been found. Appropriate therapy procedure to decompose Aβ or Tau aggregates in AD would necessitate a precise genetic intervention (8). The scarcity of these interventions suggests the need to change the current treatment methodology.

Alzheimer's disease (AD) is the most common and progressive neurodegenerative disorder among a wide neurodegenerative ailment (NDD) spectrum. First characterized by the German physician Alois Alzheimer in 1906, this condition has gained increasing interest from both the public and the scientific community over the past twenty years due to its root of toxicity and its being an important cause of dementia (9). Competitive inhibition of β-secretase (BACE1) or γ-secretase to prevent Aβ aggregation and tau phosphorylation inhibition or tau kinase inactivation can serve as a major therapeutic strategy to manage AD (10). Early identification of pathology that supports the course of disease advancement would require an efficient genetic diagnostic approach. Variants of familial AD genes (APP, PSEN1, and PSEN2) in the early-onset AD were identified, associating Aβ processing to familial AD mutations. On the other hand, the gene variant that conveys the most common risk factor with late onset AD is APOE. The built APOE gene showed two heritable alleles (ε2, ε3, and ε4) that encode the APOE protein. APOE-ε4 is well-known as the most important AD genetic risk factor (11).

The breakthrough of CRISPR/Cas9 technology three to four years ago brought a revolutionary change in the scientific world, enthusing, reanalysing, assessing, redefining, and reimagining numerous biological aspects of the organism (12). Alzheimer’s disease (AD) is one of the most prevalent forms of age-related dementia and the root cause of most human memory loss. Several lines of evidence suggest that specific alleles in the gene for β-amyloid precursor protein (APP) are related to its onset or progression of AD. The currently approved therapeutic options are symptomatic and have proven to be of modest clinical benefit (13). The impact of the first CRISPR-derived therapeutic, which treats a rare form of blindness, is still the focus of global attention. AD is expected to be the focus of future CRISPR/Cas9-based gene therapy trials (2). Therefore, this review discusses CRISPR/Cas9-generated gene regulation and gene editing in the context of idiopathic AD, with the aim of providing an accessible starting place for biologists who want to switch to reverse engineering and gene editing of cells derived from AD patients. Information is also provided in a format that allows for easy accessibility of novices and advanced practitioners alike with the central dogma of molecular biology.

2. Molecular basis of Alzheimer’s Disease

Amyloid beta (Aβ) and Tau proteins are connected to the onset and progression of Alzheimer's, and both of their metabolic pathways are related to genetic deregulation (14). The basis of the current review focuses on the significance and possibilities of the recombinant CRISPR/Cas9 system in the processing of amyloid beta plaques and Tau protein. Although other genes are engaged in the metabolic processing of amyloid beta and Tau proteins, we primarily focused on genes for CRISPR/Cas9 therapeutic approaches that have been implicated in Alzheimer's disease progression.

2.1. Amyloid beta and Tau protein

AD is a common type of dementia that impacts millions of people's health worldwide. Even after more than a century of research, many mysteries regarding its pathophysiology remain unknown (15). Diminished cognitive functions, such as memory, recognition, judgement, and problem solving, are one of the standard clinical characteristics of AD (16, 17).

Research on the brain of AD patients has shown neuropathological alterations that are indicative of the disease, including the build-up of extracellular Aβ plaques and intracellular neurofibrillary tangles (NFTs) made of hyperphosphorylated Tau (18-20). More than 92% of documented cases of early-onset AD show that the disease is predominantly hereditary and manifests symptoms in people between the ages of 30 and 65. In contrast, late-onset AD manifests symptoms beyond the age of 65. >5.8 million people in the United States (USA) alone have been diagnosed with AD, according to a 2019 report, with 45% of cases falling into the 75–84 age range (21). By 2050, there will likely be about 14 million impacted patients in the United States due to the steadily rising number of cases (22). The majority of the time, factors other than genetic predisposition are thought to be responsible for the disease. The brain's Aβ production and aggregation are explained by the amyloid hypothesis. This theory proposes that the coordinated actions of α-, β-, and γ-secretases cause amyloid beta precursor protein (APP) to be proteolyzed. AD results from the aggregation of Aβ monomers into oligomers, which leads to the formation and deposition of Aβ plaques. This process is caused by an increase in β-secretase 1 (BACE1) activity. Moreover, once APP is cleaved by BACE1, a C99 fragment is formed, which is then further cleaved by γ-secretase to yield the Aβ monomers Aβ40 and Aβ42. Remarkably, APP can also be cleaved by α-secretase at different locations, which reduces the production of Aβ monomers (23).

Aβ formation is explained by the amyloid hypothesis, and NFT formation in AD brains is explained by the widely accepted Tau hypothesis. A well-known microtubule related protein called tau is essential for the development and maintenance of the microtubule cytoskeleton (24). According to reports, 3R and 4R are the two most prevalent Tau isoforms in adult human axons out of the six. Tau is the target of several kinases and phosphates. If found in the axons and bodies of neuronal cells, Tau isoforms 3R and 4R can accumulate in the AD brain in a hyperphosphorylated form, resulting in the formation of NFT in neuronal tissues and Tau pathology. According to current research, tau oligomers may act as molecular initiators in AD or as microstructures that mediate neuropathology (25). Additionally, a potential biochemical connection between Aβ deposition and NFT production has been documented. The latter has been shown to impair mitochondrial transport along microtubules, neuronal survival, neuroplasticity, and modified microtubule assembly. Thus, Tau neurotoxicity may be the source of an event that occurs downstream of Aβ polymerization and is accountable for the neurotoxicity resulting from Tau (26).

2.2. Genetic Factors in Alzheimer's Disease

The primary genetic link to Alzheimer's is an increase in the variety of allele genes that produce γ-amyloid proteins or reduce the clearance of γ-amyloid proteins. In this regard, γ-amyloid proteins disrupt synaptic functions and communication between nerve cells. The genetic factors for this disease are organized in different groups in terms of protein function (27). Several researchers believe that the generation protein activity is affecting γ-secretase, such as the less beneficial proteins that are developed. In this regard, it is realized that the same sites of amyloid mutated proteins are responsible for intellectual retardation compared to Alzheimer's disease (28, 29). It is shown that γ-secretase is functional in developing this disease when PSEN2 mutated proteins are compared to the PSEN1 mutated protein (30). In reality, the two working areas are affected by this disease. Interestingly, 31 Alzheimer's disease mutations result in disrupted proteolytic processes of PS1 (31). The two mutations show symmetry in the generation of amyloidβ production, which means Aβ42/Aβ40. The neuroanatomically diverse plaque organization develops different presenilin numerical changes. The Aβ42 plaque grows significantly through genetic and biochemical factors, which appear in the infected brain (32).

Surprisingly, γ-amyloid and tau proteins are both genetic as well as non-genetic factors present in Alzheimer's. After a lot of research, it is found that non-genetic factors are involved in the deposition of both γ-amyloid and tau proteins, while genetic factors are responsible for infections, types, and timing of deposition of these proteins (33) So, it is very hard to say whether γ-amyloid or tau pathology is involved in Alzheimer's. About 1% of Alzheimer's cases occur due to genetics; they suffer from an inherited form of Alzheimer's. It occurs because parents are carrying the significant genes APP, PSEN1, and PSEN2 (34).

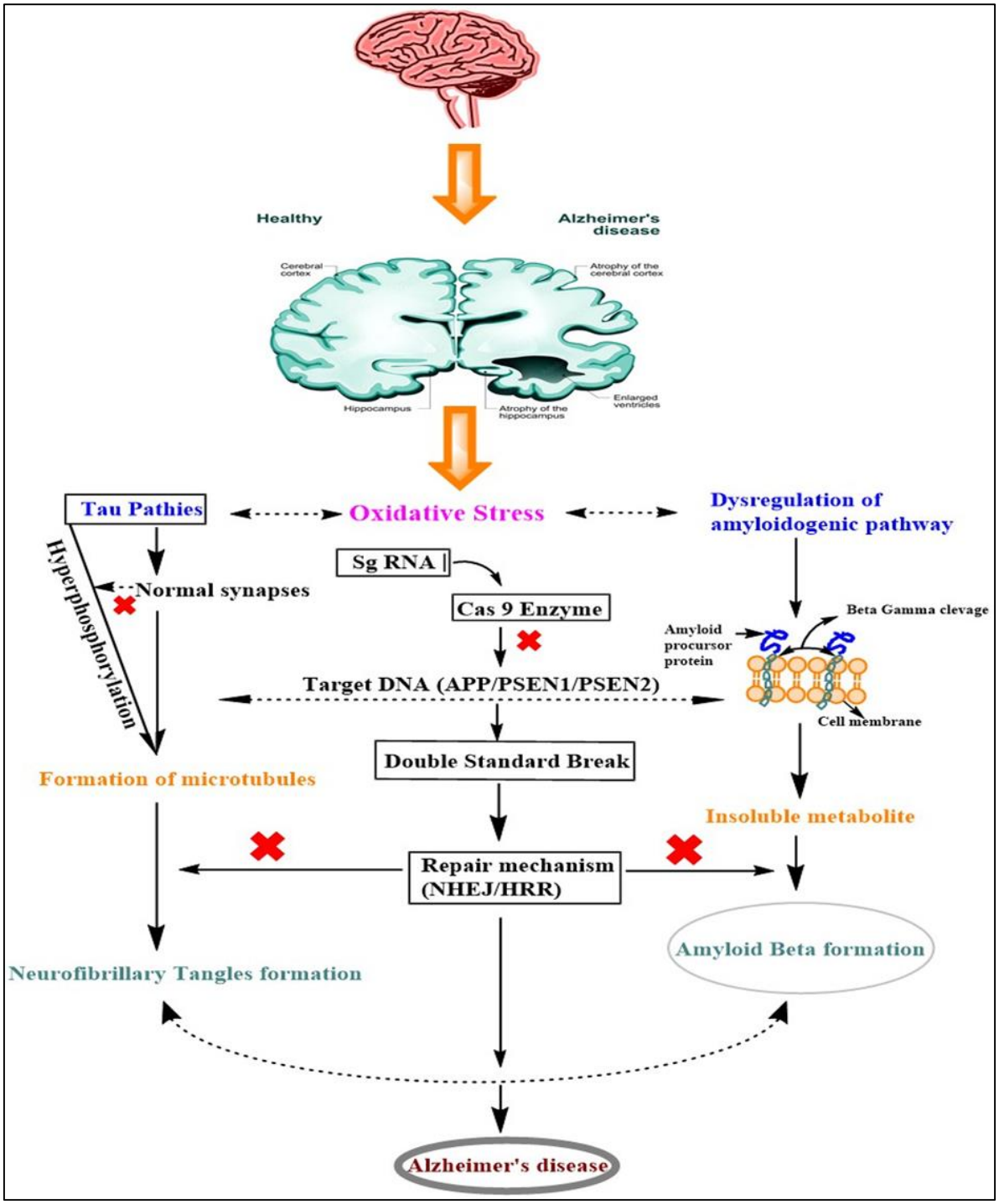

Figure 1 CRISPR/Cas mechanism and its impact on Alzheimer's Disease pathophysiology

3. CRISPR/Cas9: An overview

Although the accumulation of amyloid plaques is one of the chief causes of Alzheimer's disease, multiple challenges have puzzled the clinical trials of recent therapies for controlling amyloid β toxicity, and the number of promising results abound.

The single-guide RNA (sgRNA) and the Cas9 enzyme are both primary parts of the CRISPR/Cas9 system. While the Cas9 protein is an endonuclease that functions as blades to break DNA double strands, the sgRNA recognises the targeted DNA pattern; hence, during the design process, many criteria must be taken into account to enhance specificity. CRISPR/Cas systems come in two varieties: Class 1 (types I, III, and IV) and Class 2 (types II, V, and VI). Class 2 systems

employ a single Cas protein, making them simpler and better for genome editing than Class 1 systems, which require many Cas proteins cooperating. The type II CRISPR/Cas9 system is the most researched and utilised of Class 2 (35).

When the Cas9 protein identifies the desired genomic sequence, it breaks the strands twice. After then, non-homologous end joining (NHEJ) or homology directed repair (HDR) might be started with the objective to fix this break. Insertions and deletions (InDel) caused by the NHEJ pathway cause premature stop codons, DNA frameshifts, and ultimately, gene inactivation. Conversely, the HDR pathway facilitates the substitution of an accurate sequence for the defective or altered one. With the aid of a donor DNA template, the proper DNA sequence is inserted into the intended location to start HDR (36). While HDR is limited to the S or G phases of the cell cycle, NHEJ can occur in any phase. Even though the NHEJ process is more effective, the HDR pathway is often the more dependable DNA repair mechanism.

The CRISPR/Cas9 system can be applied in three different methods to edit a target gene: plasmid-borne CRISPR/Cas9 system, purified Cas9/sgRNA complexes, or a combination of Cas9 mRNA and sgRNA. Every tactic has benefits and drawbacks of its own (37).

4. Management of alzheimer's disease applying CRISPR/CAS9 technique

Genome editing therapy for AD can target mutations in various genes like APP, PSEN1, and PSEN2, as well as disrupt the synthesis of Aβ.

4.1. CRISPR/Cas9 Targets APP Gene Mutations

The APP gene mutation results in enhanced β-secretase cleavage of the amyloid-β (Aβ) precursor protein, which in turn causes most significantly inherited AD. The APP mutation KM670/671NL, which is native to Sweden (with APPsw for both the mutation and the mutant allele), causes β-secretase to cleave more enzymatically, which raises the levels of Aβ protein. Researcher found that employing CRISPR/Cas9 to delete out APP alleles results in a decrease in Aβ protein expression in a first proof of concept research. Thus, for AD patients with APP mutations, the CRISPR/Cas9 technology may therefore offer gene therapy options (38). Furthermore, a different study discovered potential protective deletion mutations in the mouse APP gene's 3′-UTR. When approximately 700 bp of the 891 bp APP 3′-UTR in the mouse model zygotes were removed using CRISPR/Cas9 technology, they discovered a significant decrease in Aβ buildup. It's important to note that an Icelandic population that did not exhibit symptoms of AD at an elderly age can be attributed to the A673T mutation. This mutation might cause a 40% reduction in β-secretase cleavage (39, 40). The insertion of this mutation in patients' neurons may therefore be a viable and efficient way to slow down or perhaps prevent the advancement of AD, according to a different scientist's theory (41). In order to achieve this, scientists inserted a novel mutation into HEK293T and SH-SY5Y cells (which had the APP gene with deaminated cytosine1 and cytosine2 locations) by changing the alanine codon to a threonine. Due to the effective incorporation of the A673T mutation together with a new mutation (E674K) in 53% of HEK293T cells, the level of accumulation of Aβ peptide has further decreased (41). Similarly, scientists modified the amyloid pathway in return by specifically editing endogenous APP at its extreme C-terminus in cell and animal models through the use of a CRISPR/Cas9-based approach. Thus, attenuating APP-β-cleavage and increasing neuroprotective APP-α-cleavage have lowered the generation of Aβ (42).

4.2. CRISPR/Cas9 aims for crucial Aβ protein enzymes

BACE1 and γ-secretase sequentially modify APP to create the Aβ protein. Thus, one possible therapeutic approach for the treatment of AD is to target BACE1. According to a recent study, using Cas9 nanocomplexes—which are made by combining the amphiphilic R7L10 peptide with Cas9-sgRNA—was successful in reducing the levels of BACE1 in two mouse models of AD (43). A significant intramembrane protein complex called γ-secretase protease, which is controlled by γ-secretase activating protein (GSAP), is another potential target of gene therapy in AD. There is a report that suggests a large decline in Aβ levels occurs when GSAP expression is reduced (44, 45). According to the claims, a researcher also used CRISPR-Cas9 technology to wipe out GSAP in HEK293 cells that express APP steadily (HEK-APP), which resulted in a notable decrease in γ-secretase efficiency and Aβ production (46).

It was found that γ-secretase is regulated by the expression of the GSAP, and PSEN1 and PSEN2 are the essential components of the γ secretase complex. As a result, PSEN1 mutations would result in AD and be connected to the majority of familial AD cases (47, 48). The majority of these mutations affect amyloid metabolism, which can lead to decreased synthesis of Aβ40 and/or an increase in the Aβ42/40 ratio and Aβ42 concentration (49). Furthermore, it has been established that PSEN1 gene mutations are associated with most cases of early-onset familial AD (50).

4.3. CRISPR/Cas9 is applied to APOE genotype editing

The most potent genetic risk factor for AD is the APOE4 isoform (51). As far as our knowledge, the primary source of APOE expression in the central nervous system is astrocytes. However, the presence of APOE expression in neurons will indicate the incidence of various conditions, such as neurodegeneration, age-related cognitive decline, and neurological damage. According to a study on the target of therapy for APOE4, E3 neurons in iPSCs taken from two AD patients with the E4 allele corrected to the E3/E3 genotype using the CRISPR/Cas9 technique showed reduced tau phosphorylation and were less vulnerable to ionomycin-induced neurotoxicity (52). Additionally, the function of APOE4 was determined utilising CRISPR/Cas9 and hiPSC technology; the results demonstrated that APOE4 affected the Aβ metabolism in a way that was distinct to each cell type. More encouraging findings revealed that attenuating several AD-related diseases can be achieved through isogenic conversion of APOE4 to APOE3. These results also demonstrated the potential of APOE4 as an AD therapeutic target (53).

Table 1 Overview of CRISPR/Cas9 Applications in Neurodegenerative Diseases: Targets, Experimental Models, and Key Finding

Disease	Target	Experimental model	Key findings	References
Alzheimer's disease	APP*swe*	Transgenic APP*swe* mice	Decrease Aβ	(54)
	Glia maturation factor	BV2 microglial cell line	Inhibition of pp38 MAPK	(55)
	Beta-secretase 1 (*Bace1*)	Alzheimer's disease mouse model	Reduction in $A\beta_{42}$ plaque accumulation	(56)
	γ-secretase activating protein (GSAP)	HEK-APP cell lines, Human neuroblastoma, SH5YSY cell lines	Reduction in Aβ secretion	(57)
	APP	*In vitro* model involving the AlphaLISA assay	Mutations near the Aβ42 cleavage site reduce Aβ40 production, Mutations beyond the Aβ42 cleavage site increase the Aβ42/Aβ40 ratio by raising Aβ42 levels and lowering Aβ40 levels	(58)
	APPSwe, PSEN1M146V	Human induced pluripotent stem cells	Enhances the accuracy of homology-directed repair (HDR) for introducing specific mutations using CRISPR/Cas9, generates isogenic human induced pluripotent stem cell (IPS) lines with heterozygous and homozygous early onset Alzheimer's disease mutations in APP and PSEN1, allowing the derivation of cortical neurons to study disease-associated phenotypes.	(59)
	PSEN2N141I	Human basal forebrain cholinergic neurons	Insulin may act as a mediator of resilience by counteracting specific metabolic and molecular features of Alzheimer's disease, chronic insulin treatment reduces the Aβ42/40 ratio	(60)
	Human CD33 (hCD33), murine CD33(mCD33)	RAW264.7 cells, BV-2 cultured microglia, hCD33 transgenic mice	Phagocytosis regulatory role of hCD33 and mCD33 in management of AD, its impact on microglial activity and Aβ clearance.	(61)

	APP	*App* knock-in mice model	3′-UTR disruption results in reduced Aβ pathology through transcriptional and translational regulation of APP expression, Reduced Aβ pathology on 34-bp deletion near the APP gene's stop codon	(52)
	APOE E4	Induced pluripotent stem cell (iPSC) model	Neuronal expression of E4 alone can alter AD-related cellular pathways	(62)
	Cysteinyl leukotrienes receptor 1 (CysLT1R)	APP/PS1-CysLT1R knockout mouse model	Decreased amyloid processing, reduced neuroinflammation, and suppression of the kynurenine pathway. Genetic or pharmacological depletion of CysLT1R could be a potential therapeutic strategy for ADS	(63)
Parkinson's disease	Soluble RAGE	Rotenone-induced PD mouse model	Identification of novel neuroinflammatory pathways, including PKCδ signalling, and protective pathways like Prokineticin-2 signaling, Reduced neuronal death in Corpus Striatum and Substantia Nigra, Inhibition of AGE-RAGE binding, a theraputic approach against PD	(64)
	Leucine-rich repeat kinase 2 (LRRK2)	Marmoset stem cells	CRISPR/Cas9-induced LRRK2 G2019S mutation in marmoset stem cells confirmed the common marmoset as a valid model for Parkinson's disease, mutation increases LRRK2 kinase activity	(65)
Huntington's Disease	Mutant Huntingtin gene (mHTT)	YAC128 mouse model	CRISPR/Cas9 targeting distrupt the translation of mHTT, reduces the production of mutant huntingtin protein	(66)
	mHTT gene	HD140Q-knockin mice	CRISPR/Cas9 mediated gene editing inhibits expression of mutant protein in HD	(67)
	mHTT gene	Personalized allele-selective CRISPR/Cas9 model	Prevents the production of mutant HTT mRNA and protein, demonstrating precise allele-specific inactivation	(68)
	mHTT gene	Patient-derived cells with expanded CAG repeats in HTT	Preventing transcription of mutant HTT mRNA without affecting normal HTT, Selective genomic deletion	(69)
Amyotrophic lateral sclerosis	SOD1-G93A and SOD1-A4V	Human induced pluripotent stem cells	Cell-autonomous proteinopathy, axonopathy,synaptic pathology, and aberrant neurotransmission in human motor neurons caused by SOD1 mutations, Decreased spiking activity and network bursting, but increased burst duration in mutant motor neurons	(70)

5. CRISPR/Cas9 delivery in Alzheimer's disease

The CRISPR/Cas9 technique holds potential for the creation of innovative therapeutic strategies for the management of AD. To put this genome editing technology to use in practical applications, it still needs to be delivered in a way that is

safe, effective, and efficient. In general, there are two ways to deploy the CRISPR/Cas9 system: viral and non-viral. The CRISPR approach being used and whether in vitro or in vivo administration is planned determine which delivery vehicle is best. For instance, the Cas9 protein is positively charged, yet the Cas9/sgRNA complex and oligonucleotides are negatively charged (71).

Table 2 Types of method use in genomic transmission of CRISPR

Method type	Description	Reference
Viral Vectors	High specificity and effectiveness, adeno-associated virus (AAV) is widely employed for brain in vivo administration.	(72)
	Lentivirus: Adaptable for long-term expression in both dividing and non-dividing cells, capable of integrating into the host genome.	(73)
Non-Viral Methods	Lipid Nanoparticles: Vesicles made of lipids that have the ability to enclose CRISPR elements, providing a less dangerous option to viral vectors.	(74)
	By electroporation, CRISPR components can enter cells by temporarily piercing cell membranes with electrical pulses.	(75)
Physical Methods	Microinjection: A highly accurate yet labor- and technically-intensive method of directly injecting CRISPR components into cells or embryos.	(76)
	Rapidly injecting a sizable volume of solution into the bloodstream is known as hydrodynamic injection, and it is mostly utilized in animal models.	(77)
Chemical Methods	Polymer-based Delivery: To improve cellular absorption and stability, polymers are combined with CRISPR components to produce complexes.	(71)
Biological Methods	Short peptides known as "cell-penetrating peptides" help move CRISPR components across cell membranes.	(78)
Plasmid-based Delivery	Usually employed for in vitro research, plasmids are circular DNA molecules that can be inserted into cells to express CRISPR components.	(79)

5.1. Viral Delivery Method

Because of their effectiveness and long-term stability, viral vectors are a traditional method that has been used to deliver CRISPR/Cas9 in vitro and in vivo in the past. Adeno-associated virus (AAV) and lentivirus are the two most commonly used viruses. Viral vectors are by far the most effective means of delivering plasmid-based CRISPR/Cas9. They may, however, cause unintentional mutations with detrimental side effects. Moreover, they may trigger deadly immunological reactions.

Because AAVs do not generally integrate into the human genome and have an elevated rate of infection and minimal immunogenicity (80), they are the most often utilised viral vector (81). It can enter cells and cause little to no immunological response. The single-stranded DNA that makes up the AAV genome has more than 200 variations. According to one investigation, two distinct AAV vectors were used to package gRNA specific to APPsw and Cas9 that targets the APP mutation KM670/671NL, which causes AD (82). The viruses were examined both in vivo, using intrahippocampal injections in Tg2576 mice, and in vitro, using primary nerve cells from embryos of Tg2576 mice. With this therapy, the human-derived fibroblasts produced 60% less Aβ (38). Lentivaun virus integrates into the human genome more efficiently and is more probable to elicit immunological responses than AAV. It is also more challenging to purify in huge quantities (81). The co-injection of two viruses may be required since AAV has a smaller packaging capacity of just 4.7 kb. This delays the procedure because both viruses may not infect the same cell at the same time. Longer DNA inserts (8–10 kb) can be added to lentiviruses, albeit their efficacy in propagating throughout the brain is reduced (83).

5.2. Plasmid based delivery method

One appealing method of introducing the CRISPR/Cas9 machinery into cells is the delivery of DNA encoding the Cas9 protein. This approach has several advantages, including the following: first, it is relatively easy to synthesise the gene; second, it does not require integration of the synthesised gene into the host genome after being transferred into the host cell through a plasmid; third, it is possible to constantly express the gene; and, fourth, organ-specific delivery of the

CRISPR/Cas9 system is crucial for further utilisation. An additional benefit of plasmid-based delivery is the possibility of incorporating tissue- or cell-specific targeting into the plasmid itself (84).

Even though CRISPR/Cas9 has been shown to be useful for in vivo gene editing, getting high molecular weight DNA into cells is a major implementation challenge. In order to transport Cas9 into primary bone marrow-derived macrophages, Scientists currently developed a PLGA nanoparticle fluorescently labelled with the dye 6, 13-bis (triisopropylsilylethynyl) pentacene (TIPS pentacene). Remarkably, after 24 hours, the Cas9 protein was first discovered to be expressed, and TIPS fluorescence was seen in the majority of cells (85). Furthermore, an additional study developed a method of delivering Cas9-sgPlk-1 plasmids (CPs) via electrostatic interactions, succeeding lipid-encapsulated and laser-controlled AuNPs/CP, ACP, which are condensed on TAT peptide-modified Au nanoparticles. This is a really effective way to administer CRISPR/Cas9 (86). These findings suggest that the plasmid coating technique using nanoparticles is probably going to work well for CRISPR/Cas9 in vivo therapeutic uses. Additionally, a multifaceted nucleus-targeting "core-shell" artificial virus (RRPHC) has been created by researchers to deliver the CRISPR/Cas9 plasmid. More targeted gene disruption can be induced by the method than by conventional transfection reagents (like Lipofectamine 3000). Even more intriguingly, the synthetic virus can efficiently target ovarian cancer through endocytosis mediated by dual receptors. As a result, this will offer the perfect concept for effective CRISPR/Cas9 delivery and localization (87).

5.3. Strategies for CRISPR/Cas9 using proteins

Ribonucleoprotein (RNP), a potent technique for genome editing in CRISPR/Cas9 modifying genes technology, is made up of Cas9 protein and sgRNA. It has several benefits, like being quick and safe, having less off-targeting, and having a greater modifying efficiency. Furthermore, a variety of model organisms and cell types, including stem cells (88), immune cells (89), primary cells (90), etc., can be used with the Cas9 RNP system.

Presently, there are numerous methods available for the administration of the Cas9 RNP system, including synthetic transporters (lipid nanoparticles) and physical means (such as microinjection, electroporation, biolistic, and microfluidic techniques). However, microinjection comes with a long list of prerequisites, a laborious procedure, and a steep price tag. Therefore, more improvements to the approach are required. More encouragingly, a subsequent study by the same team demonstrated an easy and affordable technique based on electroporation to deliver the Cas9 RNP system (91). CRISPR RNP Electroporation of Zygotes (CRISPR-EZ) is an effective way to boost the survival of the embryo as compared with microinjection (91). Regarding the delivery of Cas9 RNP using lipid nanoparticles, a lab has published a study on the use of LNPs in conjunction with microfluidic techniques to deliver both Cas9 and Cpf1 RNPs through CRISPR/RNP. When applied to HBV-infected human liver cells, the optimised formula delivery method effectively suppresses both covalently closed circular DNA (cccDNA) and HBV DNA (92). These increasingly compelling results have had a major impact on the growth of the medicinal and clinical uses of CRISPR/Cas9 delivery system.

6. Ethical issues with Crispr technology

The discipline of molecular biology is rapidly changing as a result of the CRISPR/Cas9 genome-editing technique, which enables researchers to make desired changes to a variety of animal genomes. Due to its straightforward design and simplicity of use, it was quickly welcome after its release in 2012. It is being researched for a variety of purposes, such as human germline modifications to address genetic issues and agricultural and pharmaceutical therapies (93).

6.1. Human Germline Editing

Because of the possibility of heritable modifications in subsequent generations, the capacity to edit the human germline using CRISPR/Cas9 poses ethical concerns. When germline cells, like embryos or sperm and egg cells, are edited, questions arise regarding safety, unforeseen repercussions, and the possibility of producing "designer babies" with desired characteristics. In particular, when it comes to human germline editing, the international scientific community has been involved in discussions and initiatives to control and harmonize the use of genome editing technology (94, 95).

6.2. Informed Consent and Autonomy

When thinking about the application of CRISPR technology in human patients, the ethical precept of informed permission is essential. Ensuring that individuals possess the necessary knowledge and liberty to make well-informed decisions regarding their participation in genome editing interventions is imperative, given the risks, benefits, and potential implications of such interventions. The ethical application of CRISPR technology must take into account a number of factors, including preserving individual rights and welfare, supplying accurate information, and guaranteeing transparency (94, 96).

6.3. Environmental and Ecological Impact

The release of genetically modified organisms without adequate containment mechanisms or the loss of control over their spread could have unintended consequences for ecosystems and biodiversity. Such are the ethical concerns raised by the use of CRISPR technology in non-human organisms (97).

6.4. Scientific Responsibility and Oversight

Because of the CRISPR technology's quick development, there are now concerns regarding the scientific community's duty to control its application. Preventing misuse, unethical behaviours, and the spread of false information requires adequate surveillance, adherence to ethical standards, and responsible research conduct (98).

7. Conclusion

In the field of neurodegenerative illnesses, investigating CRISPR/Cas9-mediated therapeutic methods for AD is a potential area of research. The ability of gene-editing tools to specifically target important genetic variables linked to the pathophysiology of AD presents new opportunities for the development of accurate and individualised treatments for this intricate illness. CRISPR/Cas9 has the ability to modify certain genes linked to tau protein malfunction, neuroinflammation, and amyloid-beta accumulation. This means that it can act at the molecular level and change the course of disease.

Furthermore, the combination of patient-specific induced pluripotent stem cells (iPSCs) with CRISPR/Cas9 technology opens doors for personalized medicine methods in the treatment of AD, enabling customised therapies based on unique genetic profiles. Innovation in AD therapies is still being driven by continuous research and breakthroughs in CRISPR/Cas9 technology, despite obstacles including off-target effects, delivery strategies, and ethical considerations that must be addressed.

Subsequent preclinical and clinical investigations are crucial to confirm the safety, effectiveness, and enduring consequences of CRISPR/Cas9-driven therapies within the framework of AD. Regulatory agencies, physicians, and researchers working together will be essential to bringing these promising medicinal techniques from the lab into the real world. In the end, the possibility that CRISPR/Cas9-mediated therapeutics will change how AD is treated emphasises the significance of ongoing research and funding for cutting-edge approaches to treat neurodegenerative illnesses.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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