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A Review of CRISPR Cas9 for Alzheimer's Disease: Treatment Strategies and Could target APOE e4, APP, and PSEN-1 Gene using CRISPR cas9 Prevent the Patient from Alzheimer's Disease?

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Abstract

BACKGROUND: Alzheimer's disease (AD) is a neurodegenerative disorder characterized by the formation of β-amyloid plaques and neurofibrillary tangles (NFTs) from hyperphosphorylated tau. Several studies suggest that targeting the deletion of the APOE e4, PSEN-1, and APP will reduce tau phosphorylation and amyloid-beta (Aβ) protein accumulation, a crucial hypothesis for the causation of AD. APOE e4, PSEN-1, and APP with genome-editing Clustered Regular interspersed Short Palindromic Repeats (CRISPR)-related (CRISPR/Cas9) are thought to have therapeutic promise for AD.

AIM: The purpose of this study was to determine whether targeting APOE e4, PSEN-1, and APP using CRISPR/Cas9 are an effective therapeutic and whether it has a long-term effect on AD.

METHODS: The method used in this study summarized articles by examining the titles and abstracts of specific specified keywords. In this situation, the author picked the title and abstract that matched PubMed, Google Scholar, Science Direct, Cochrane, and the Frontiers in Neuroscience; this was followed by checking to see whether the paper was available in full text. Eventually, the researcher will study the entire article to decide if it is valuable and relevant to the issue.

RESULTS: CRISPR/Cas9 deletion of APOE e4, PSEN-1, and APP in induced pluripotent stem cells and g2576 mice as APP mutant models reduces tau phosphorylation and Aβ protein accumulation from NFTs and prevents cell death, vascular damage, and dementia. Furthermore, CRISPR/Cas9 deletion in APOE e4, PSEN-1, and APP improved neuronal cell resilience to oxidative stress and inflammation.

CONCLUSION: APOE e4, PSEN-1, and APP deletion by genome-editing CRISPR/Cas9 are effective to reduce tau phosphorylation and Aβ protein accumulation from NFTs, cell death, vascular damage, and dementia. However, further research is needed to determine the side effects and safety of its use.

Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder, the most common cause of dementia in the elderly, accounting for 60–70% of all demented cases. According to some, dementia has become one of the most pressing social and economic issues of our day. There are many risk factors for AD, including genetics, cerebrovascular illness, hypertension, type 2 diabetes, obesity, dyslipidemia, stress, smoking, and some preventive factors, such as nutrition, Vitamin D, and estrogen [1], [2], [3], [4], [5].

AD has a worldwide prevalence of 3.9%, with regional prevalences of 1.6% in Africa, 4.0% in China and the Western Pacific regions, 4.6% in Latin America, 5.4% in West Europe, and 6.4% in North America [6]. The prevalence of AD in China between 1990 and 2010 was 1.9%, according to a meta-analysis of 18 research. AD affects more than 25 million people worldwide, with an additional 5 million cases diagnosed each year [7]. Every two decades, the number of people who have dementia is expected to more than double. The prevalence of AD is comparable among areas, despite varying inclusion criteria in meta-analyses and national surveys [8]. Every 5 years after age 65, the incidence of AD almost doubles. Dementia affects roughly one in 10 adults over 65 in industrialized countries, and more than a third of the elderly over 85 show symptoms and indications of dementia [9], [10]. AD and vascular dementia are the two most frequent kinds of dementia, each accounting for 50–70% of all dementia cases and 15–25% of all dementia cases, respectively. Based on data from the WHO, around 1.2 million people in Indonesia were diagnosed with AD in 2016, which is expected to rise to 2 million people by 2030 and 4 million people by 2050 [11].

AD is complex, with many contributing causes. Due to the complexity of the human brain and the

appropriate animal models and research tools, and the specific nature of research, the pathogenesis of AD is largely unknown. Many theories about AD have been proposed, including the amyloid-beta (Aβ) hypothesis, Tau, cholinergic neuron injury, oxidative stress, inflammation, and so on. As a result, various attempts have been made based on these theories to determine future therapies for AD [12], [13], [14], [15], [16]. In general, AD is divided into three stages: Preclinical or presymptomatic occurs when people are asymptomatic but have definite laboratory evidence, mild cognitive impairment (MCI) occurs, and MCI occurs when people are asymptomatic but have definite laboratory evidence. Patients in this stage exhibit memory or non-memory domain impairments, such as executive capacity or language function, while dementia is the last stage of AD and occurs when patients have incapacitating memory impairment. Anomia, paraphrasing mistakes, a reduction in spontaneous verbal production, and a proclivity for circumlocution to avoid forgetting words are all examples of language alterations. Wandering in familiar settings and constructional apraxia are symptoms of impaired visuospatial ability [17]. In general, AD can lead to complications include an increased risk of falling, a higher risk of hip fractures from falls, delay some physical changes, losing concentration, pneumonia, malnutrition, and dehydration [18]. Many factors can trigger the occurrence of AD. In terms of genetic factors, it is currently known that studies of twins showed that the risk of AD is 60–80% dependent on heritable factors [4].

Until now, aducanumab is still the main therapy for patients suffering from Alzheimer's. Aducanumab is an amyloid-targeting monoclonal antibody delivered by monthly intravenous infusions. It is titrated to a dose of 10 mg/kg over 6 months. The Expert Panel recommends that the use of aducanumab be restricted to this population in which efficacy and safety have been studied. No contraindications have yet been reported. Meanwhile, the FDA adjusted the indication section from "indicated for the treatment of AD" to "indicated for the treatment of AD should be initiated in patients with MCI or mild dementia stage of the disease, the population in which treatment was initiated in clinical trials" [19], [20].

Treatment trials using stem cells such as embryonic stem cells, mesenchymal stem cells, brain-derived neural stem cells, and induced pluripotent stem cells (iPSCs) are also less effective because only temporary [2].

Strategies for the prevention of AD through non-pharmacological treatments are associated with lifestyle interventions such as exercise, mental challenges, and socialization as well as caloric restriction and a healthy diet [21].

In this case, one solution that can be offered to treat patients suffering from Alzheimer's is gene therapy. Nucleases are the primary too in gene therapy. This modern therapy was done by injecting genetic material into the patient's cells to replace the damaged gene or to insert therapeutic transgenes. The idea in implementing gene therapy is to add normal genes to the genome that is mutated or damaged so that these genes can function properly. This therapy provides the theoretical advantage that a single treatment will achieve lasting clinical benefits. Lately, clinical gene therapy test has proven therapeutic benefits and exceptional security. Furthermore, gene therapy has been widely used to treat cancer, cardiovascular disease, infections, decreased metabolic function, lymphatic diseases, radiation-induced injuries, and post-surgical therapy. Gene therapy can also be a solution to treat Alzheimer's, although the kefficacy and safety of this therapy are still a problem [22], [23].

Until now, there has been no effective treatment for AD. As a result of this literature study, the researchers hoped to get a better understanding of the possibility of gene therapy as a treatment for AD patients with long-term effects.

Methods

In this study, the articles were searched through PubMed, Google Scholar, Science Direct, Cochrane, and the frontiers in Neuroscience. Article searches were performed using keywords AD, Clustered Regular interspersed Short Palindromic Repeats-CRISPR-related (CRISPR-Cas9), genome editing, APOE e4, APP, PSEN-1, aducanumab, Aβ, Tau, cholinergic neuron damage, oxidative stress, inflammation, peripheral nerve injury, ER stress, and mitochondria anomalies. Meanwhile, article screening was done by adjusting the title and abstract to the research topic. When the title and abstract matched, it was then preceded by checking the availability of the article in full text. Eventually, the researcher then read the entire article to determine whether the article is useful and appropriate to the topic.

Results and Discussion

AD

AD is the most common cause of dementia worldwide, with the prevalence continuing to grow in part because of the aging world population. This neurodegenerative disease process is characterized classically by two hallmark pathologies: β-amyloid plaque deposition and neurofibrillary tangles (NFTs) of hyperphosphorylated tau (Figure 1). Clinically, patients initially present with short-term memory loss,

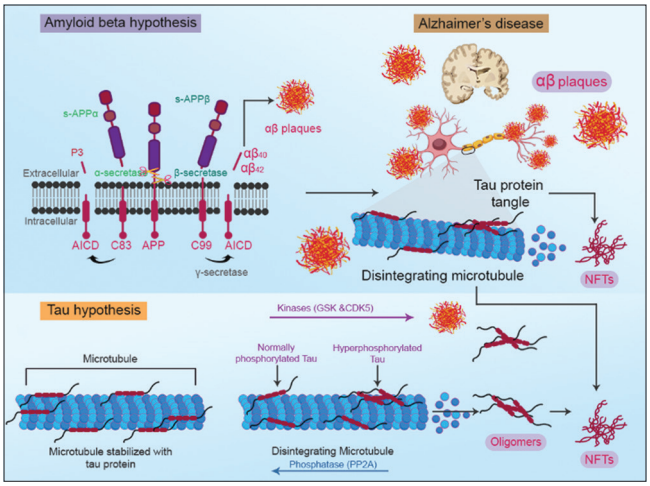
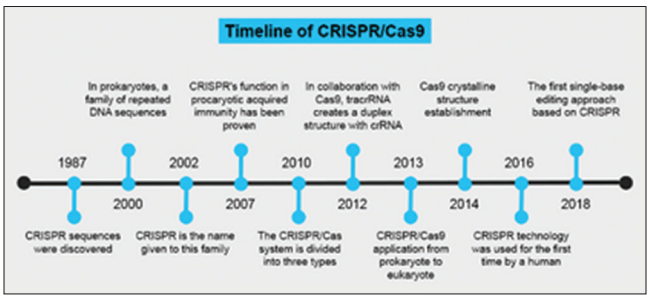


Figure 1: Alzheimer's disease schematic theory The amyloid-beta and tau theories have been offered to explain the most general features of Alzheimer's disease. GSK: Glycogen synthase kinase, CDK5: Cyclin-dependent kinase 5, PP2A: Protein phosphatase 2A



AQ11 *Figure 2: The CRISPR/Cas9 chronology is shown below. CRISPR/Cas9 stands for clustered regularly interspaced short palindromic repeats/CRISPR-associated proteins nine systems. crRNA stands for CRISPR-derived RNA*

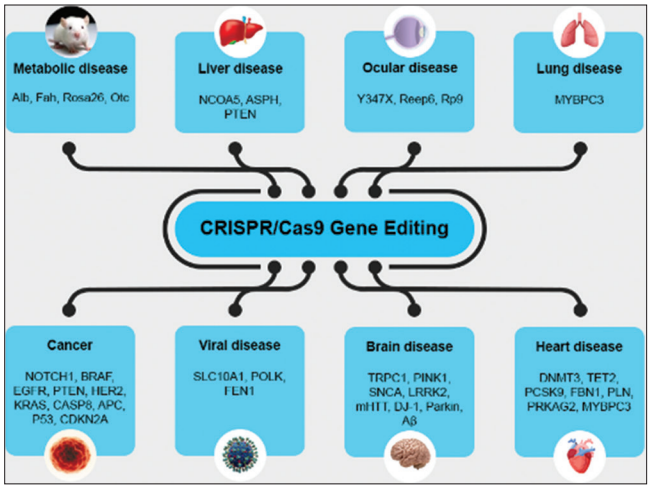


Figure 3: The CRISPR/Cas9 technique's numerous applications in human diseases. We have highlighted various disease-associated genes and proteins in this schematic as possible targets for this gene-editing strategy. MYBPC3: Myosin-binding protein C3, BRAF: B-Raf proto-oncogene, serine/threonine kinase, PTEN: Phosphatase and tensin homolog, EGFR: Epidermal growth factor receptor, HER2: Human epidermal growth factor receptor 2, CASP8: Caspase-8, CDKN2A: Cyclin-dependent kinase inhibitor 2A, SLC10A1: Solute carrier family 10-member 1, TRPC1: Canonical transient receptor potential, PINK1: PTEN-induced putative kinase 1, LRRK2: Leucine-rich repeat kinase 2, SNCA: A-synuclein, mHTT: Mutant huntingtin protein, DJ-1, PARK7; DNMT3A: DNA methyltransferase 3a, FBN1: Fibrillin-1, PCSK9: Proprotein convertase subtilisin/Kexin type 9, PLN: Phospholamban, PRKAG2: Kinase AMP-activated non-catalytic subunit-2, ASPH: Aspartate beta-hydroxylase, KRAS: Kirsten rat sarcoma viral oncogene homolog, APC: Adenomatous polyposis coli, p53: Tumor suppressor gene, FEN1: Flap endonuclease 1, TET2: Epigenetic modifier enzyme, NCOA5: Nuclear receptor coactivator 5, Y347X: Nonsense point mutation, Reep6: Receptor expression-enhancing protein 6, Rp9: Pre-mRNA splicing factor, Alb: Albumin, Fah: Fumarylacetoacetate hydrolase, Otc: Ornithine transcarbamylase, POLK: DNA polymerase kappa

subsequently followed by executive dysfunction, confusion, agitation, and behavioral disturbances. Three causative genes have been associated with autosomal dominant familial AD (*APP, PSEN1*, and *PSEN2*) and one genetic risk factor (*APOE*ε4 allele) [24], [25].

The accretion of tau proteins correlates very closely with cognitive decline and brain atrophy, including hippocampal atrophy. In the neuropathology of AD, there is a loss of neurons and atrophy in the temporofrontal cortex, which causes inflammation and deposit the amyloid plaques and an abnormal cluster of protein fragments and tangled bundles of fibers due to this, there is an increase in the presence of monocytes and macrophages in the cerebral cortex and it also activates the microglial cells in the parenchyma. A rare mutation paralyzes the regular functioning of microglial surface receptors, contributing to AD intensification [26], [27].

Alzheimer's is divided into three stages. Stage 1 in Alzheimer's is known as MCI which may represent a transitional stage from normal aging to early dementia. MCI is diagnosed when the patient has an objective memory impairment but no significant change in daily functioning. Then, at Stage 2, the patient's disease has now progressed to moderate stage AD. In Stage 3 (severe AD), although it is difficult to predict how long an individual patient will continue to live with severe AD, usually, the prognosis is between months and 2–3 years. This stage can be particularly difficult for caregivers, as the patient may not show signs of recognition and cannot verbally express his or her needs, and thus is completely dependent [28].

The hypothesis about AD

There are various ideas on what causes AD, including the Aβ hypothesis, Tau, cholinergic neuron damage, oxidative stress, and inflammation. These numerous hypotheses will aid in the discovery of medicines for the treatment of AD. The key pathogenic hallmark of AD is extracellular Aβ peptide deposition accompanied by a plaque, intraneuronal NFTs, and large-scale neuronal cell death, according to one hypothesis that causes AD. As a consequence, peptide Aβ has long been thought to be a potential target for AD, which has dominated research into novel therapies over the past two decades [12]. The most direct anti-Aβ therapeutic method is to inhibit Aβ production by targeting - and -secretase [12], [13]. In reality, the original amyloid cascade theory proposed

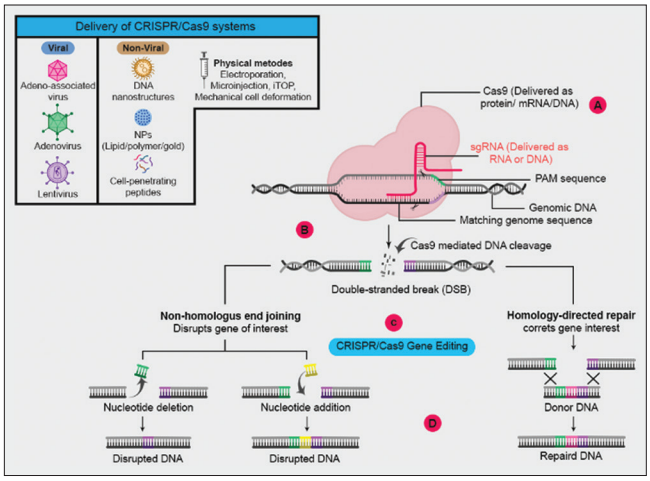


Figure 4: A diagram depicting the stages involved with the CRISPR/Cas9 method. (a) A specially designed sgRNA (guide RNA) binds to Cas9 (CRISPR-associated endonuclease), a DNAase capable of inducing a double-strand break, forming the Cas9-sgRNA complex. (b) The Cas9-sgRNA complex associates with the target genomic DNA. Cas9 searches for and recognizes appropriate sequences in target DNA using sgRNA. PAMs (protospacer adjacent motifs) sequences, which are typically 2–6 base pairs long and located 3–4 nucleotides downstream from the cut site, serve as a tag. (c) DNA cleavage mediated by Cas9 results in the formation of a double-strand break (DSB). (d) The formation of a DSB triggers the DNA repair mechanism, which attempts to close the gap either through non-homologous end joining (NHEJ) or homology-directed repair (HDR)

AQ12 **Table 1: An overview of clinical trials on AD therapies that use the CRISPR/Cas9 method**

Target genes	Clinical outcomes	References
Amyloid precursor protein (APP)	Reduction APP and Aβ	[57]
3'-UTR APP	Reduction APP and Aβ	[58]
Beta-secretase 1 (BACE1)	Reduction Bace1, Aβ, memory impairment Considerable downregulation of Ab42 plaque aggregation in mice	[59]
γ-Secretase activating protein (GSAP)	Reduction GSAP, γ-Secretase activity, and Aβ	[60]
APOE	Increased turning APOE4 to APOE3 reduction hyperphosphorylation tau and protein deposition of amyloid	[61]
CD33	hCD33mC+hCD33MA - Increased b1–42 phagocytosis in microglia	[62]
Glia maturation factor (GMF)	Reduction GMF and p38 MAPK	[63]
CysLT1R	CysLT1R -/- Increased hippocampal synaptic plasticity Reduction amyloidogenesis and neuroinflammation in the hippocampus	[64]
PSEN2	Reduction Ab42/40 ratio	[65]
MAPT	Production of new tau knockout strain (tauDe×1) in mice	[66]
PSEN1M1	Disease models generated by CRISPR	[67]
APPS	Disease models generated by CRISPR	[67]
APP	Attenuation of b-cleavage and Ab production	[68]
PSEN2	Normalization of enhanced levels of Ab42/40 through CRISPR/Cas to correct the mutation in PSEN2N141I	[69]
PSEN1	Establishment of homozygous and heterozygous mutations	[70]
APOE	Arg158 converted to Cys158 in 58–75%	[71]
APP	A model to investigate outcomes of APP mutations in cleavage of c-secretase and notch signaling	[72]

that "Aβ" is the causal agent in AD pathology, and that "Aβ" causes NFTs, cell death, vascular damage, and dementia [14], [16].

Contrary to the Tau idea, Tau is a scaffolding protein found in axons and associated with microtubules. Tau aggregation causes neurodegeneration in abnormal situations by impairing the axons of neurons. Following the failure of multiple Aβ targeting therapies for AD, greater attention is being paid to the therapeutic potential of tau targeting, especially since biomarker studies reveal that tau pathology is more directly associated with the course of the disease. Phosphorylation, arginine monomethylation, lysine acetylation, lysine monomethylation, lysine dimethylation, lysine ubiquitylation, and serine are just some of the alterations that tau goes through [29]. The absence of strong and reliable biomarkers for diagnosis and response tracking, as well as the constriction of the blood–brain barrier, makes tau-targeting therapy hard in general [30]. Even though the idea of inflammation induced by the hallmarks of AD includes reactive gliosis and neuroinflammation, emerging genomic and transcriptome investigations have reinforced the notion that microglia-related pathways are crucial to AD risk and pathogenesis [31], [32], [33]. Microglia are emerging as key participants in AD, according to mounting data. Microglia, TREM2, and the complement system are responsible for synaptic pruning at an early stage [34], [35]. Recent improvements in our knowledge of the mechanisms driving microglia malfunction in pruning, regulating plasticity, and neurogenesis are paving the way for novel therapeutic and diagnostic options for AD [36]. Targeting these abnormal microglial processes and restoring homeostasis might lead to new therapeutic paradigms for AD. Given the complexity and diversity of roles of microglia in health and illness, novel biomarkers representing the activity of distinct microglia are urgently needed [37], [38].

Cholinergic theory and oxidative stress have a part in AD suspicion. Acetylcholine (ACh) is a neurotransmitter that is produced by cholinergic neurons and is involved in a variety of physiological activities including attention, learning, memory, stress response, wakefulness and sleep, and sensory information [39], [40], [41], [42]. Damage to cholinergic neurons was thought to be a crucial pathogenic alteration linked to cognitive impairment in AD. As a result, the cholinergic theory was initially investigated in the treatment of AD using cholinesterase inhibitors. Tacrine, a cholinesterase inhibitor, was the first anti-AD medicine to enter clinical trials [43], [44], but it was pulled from the market in 2012 due to significant adverse effects. Although blocking cholinesterase is a symptomatic relief medication with minimal advantages, it is now the most widely accessible treatment, providing a ray of hope to desperate AD patients. There has been some research on other neurotransmitter dysfunctions, such as dopamine and 5-hydroxytryptamine, but not nearly as many as ACh in AD. The pathogenesis of AD is thought to be influenced by oxidative stress. The brain, in particular, consumes more oxygen than other organs and conducts mitochondrial respiration, increasing the risk of reactive oxygen species (ROS) exposure. AD is strongly linked to cellular oxidative stress, which includes increased protein oxidation, protein nitration,

AQ12 **Table 2: Comparison CRISPR versus amyloid beta-directed monoclonal antibody as a preventing therapy in Alzheimer disease**

Indicator	CRISPR (Clustered Regular interspersed Short Palindromic Repeats)	Amyloid-beta-directed monoclonal antibody	References
Definition			
	Technology offers great potential in genome editing and gene therapy for treating cancer, infections, and genetic abnormalities	Immunotherapy includes both active vaccinations that encourage the immune system to manufacture its antibodies and passive immunization with external antibodies	[106], [107]
Type			
	Cas8a2, Cas8b, Cas8c, Cas5, Cas10d, Cse1, Cse2, Cys1, Csy2, Csy3, Cas6f, Csy1/Csy2 fusion, PBPRB1993, PBPRB1992, Csn2, Cas9, Csm2m Cs×10, Cmr5, Cas10/Cs×11, Csf4, RHA1_ro10070	Bapineuzumab, solanezumab, gantenerumab, crenezumab, ponezumab, aducanumab	[107], [108]
Function			
	Endogenous gene expression regulation, epigenome editing, live-cell chromosomal locations labeling, single-stranded RNA editing, and high-throughput gene screening	Enter the brain, bind parenchymal Aβ, and reduce soluble and insoluble Aβ in a dose-dependent manner	[109], [110]
Types of molecules involved			
	Genes – DNA or mRNA	T cells, modified T cells, or monoclonal antibodies	[107], [108]
Effectiveness			
	Enables the efficient modification of endogenous genes in various species and cell types	Given the clinical therapeutic effects of anti-Aβ immunotherapies for AD, aducanumab, and solanezumab improve cognitive function, while aducanumab and bapineuzumab may increase the risks of amyloid-related imaging abnormalities (ARIA)	[111], [112]
Side effect			
	Mutations and production of undesired proteins	Headache, urinary system, upper respiratory tract infection, and autoimmune disease	[107], [108], [111]

glycoxidation, and lipid peroxidation, as well as Aβ buildup, since Aβ may cause oxidative stress [45], [46]. In principle, therapy with antioxidant chemicals would protect patients against oxidative stress and Aβ damage. However, since oxidative stress is just one of the symptoms of AD, antioxidant treatment has been tested for its efficacy in slowing the disease's development, and it is now recommended as part of combination therapy [47].

Genes associated with AD

Genetic factors are one of the causes of Alzheimer's. The first gene associated with early-onset AD (EOAD) was the *APP* gene. At present, three causative genes have been associated with autosomal dominant familial AD (APP, PSEN1, and PSEN2) and one genetic risk factor (APOEε4 allele). Investigators also reported 45 different AD mutations in *PS1* but only two in *PS2*. Meanwhile, instead of acting deterministically like the three early-onset genes, *APOE-4* seems to act as a risk factor for the disease, especially in *APOE-4/4* homozygotes (i.e., individuals with two copies of this allele) [25], [48].

One of the reasons for AD is genetic factors [49], [50]. EOAD and late-onset AD (LOAD) are two types of AD (LOAD). A family history of autosomal dominant, familial, or sporadic AD may also be present [51]. It has been identified by genome-wide association studies that dozens of genes influence AD. There were 43 gene loci associated with AD including APP, PSEN1, PSEN2, ABCA7, SORL1, BIN1, CASS4, CD33, CD2AP, CELF1, CLU, CR1, DSG2, EPHA1, FERMT2, HLA-DRB5-HLA-DRB1, INPP5D, MEF2C, MS4A6A/MS4A4E, NME8, PICALM, PTK2B, SLC24A4, ZCWPW1, TREM2, APOE, ADAM10, APH1B, ADAMTS4, CLNK, KAT8, ALPK2, AC074212.3, HESX1, CNTNAP2, ADAM10, IQCK, WWOX, ACE, HESX1, CNTNAP2, ADAMTS1, and NIN1 [52], [53], [54].

The APOE 4 allele remains the strongest genetic risk factor for sporadic AD, and it is a major risk factor for AD in all ethnic groups analyzed, as well as in men and women of all ages between 40 and 90 years. However, there are currently no APOE-targeted treatments available. There are presently no APOE-targeted medicines available [8], [55], [56].

African-American and Hispanic APOE 4 carriers are at a lesser risk than white APOE 4 carriers, but Asian (i.e., Japanese) APOE 4 carriers had the greatest ORs. If a person with a PSEN1 or APP mutation lives a typical lifespan, they will get AD. Not everyone with a PSEN2 mutation, on the other hand, will acquire AD [51].

AD: Roles for mitochondrial anomalies express the APP gene

Abnormalities in the electron transport chain inside the mitochondria are substantial contributors to free radical generation. Many studies have found a low degree of oxidative phosphorylation in AD, which manifests as both energy deficiencies and potentially hazardous free radical generation. It is important to note that the electron transport chain that results in the formation of ATP by reducing oxygen to water is a complex enzymatic system composed of five distinct phases: Complex 1 (NADH dehydrogenase), complex 2 (succinate dehydrogenase), complex 3 (ubiquinol-cytochrome-c reductase), complex 4 (cytochrome-c oxidase), and complex 5 (ATP synthase) [73].

What role do mitochondrial abnormalities have in the development of AD? In principle, they can cause two negative events: The formation of damaging free radicals and a decrease in energy supplies. In pyruvate dehydrogenase (lipoamide), oxoglutarate dehydrogenase (lipoamide), and oxidative phosphorylation enzymes, the slowdown of energy metabolism in AD has been widely described. In cultured AD fibroblasts, oxoglutarate dehydrogenase (lipoamide) activity was reduced to 50% of normal. *In vivo* observations in AD patients were also made, with positron emission tomography employed to

evaluate glucose metabolism in patients. At rest, there was a decrease in regional cerebral metabolic glucose concentration. This was observed across the neocortex and was discovered to be related to the degree of dementia. Despite this lower rest metabolism, which is frequently accompanied by left-right asymmetry, some cortical areas of AD patients' brains can still be active during specific cognitive activities. Rapoport *et al*. interpreted these data to imply the presence of a downregulation mechanism in AD that is reversible in the early stages. This notion is supported by research into gene expression. There is a decrease in mRNA in the mitochondrial genes of the cytochrome-c oxidase complex, as well as in the mRNA of the cytochrome-c oxidase complex 4 component expressed by a nuclear gene. Rapoport *et al*. maintained that cytochrome-c oxidase subunit gene expression may be downregulated in AD due to the reduced neuronal energy need induced by synaptic loss. Reduced brain glucose consumption, as evaluated by positron emission tomography in the early stages of AD, would represent this downregulation. If this was the case, free radicals would not be the source of the process, but rather a result of it [73].

AD: Roles for ER stress express the PSEN-1 gene

According to prior research, PSEN1 mutation alters the UPR response, increasing the sensitivity to ER stress [74]. Additional studies have discovered that exposing cells to Aβ activate caspase-12 in mice (analogous to caspase-4 in humans), a proapoptotic caspase whose activation leads to the activation of other caspases and, ultimately, neuronal cell death [75], [76]. Furthermore, this conclusion was validated by additional investigations. Caspase-12 mutant animals exposed to Aβ protein did not develop ER stress and were resistant to cell death in one of these investigations. It has been demonstrated that A-induced ER stress and cytotoxicity are caused by mitochondrial malfunction and ROS. The suppression of cytochrome oxidase-mediated mitochondrial damage reduces cellular damage caused by A-induced ER stress [77]. Barbero-Camps *et al*. discovered that an increase in mitochondrial cholesterol transport accelerates the course of AD in APP/PS1 mice. In addition to Ca2+ imbalance, data support a link between ER stress and APP mutation [77], [78]. Synthesis and accumulation outside the cell are connected to a mutation in familial AD (FAD)-linked APP. The E693del (Osaka) APP mutation has been associated with A synthesis and buildup, which leads to dementia. In stem cells, APP E693del mutation has been shown to enhance A oligomerization and ER stress [79], [80]. However, because FAD-linked APP mutations are found in a relatively small percentage of AD patients, this relationship does not apply to the vast majority of AD patients.

AD: Roles for peripheral nerve injury express the APOE e4 gene

In the peripheral nervous system, apoE4 is present in the glia surrounding sensory and motor neurons. It is also present in non-myelinating Schwann cells but not in myelinating Schwann cells. Macrophages are responsible for apoE4 synthesis and secretion in injured peripheral nerves. Resident macrophages and monocyte-derived macrophages recruited to the site of injury produce large quantities of apoE4 that accumulates in the extracellular matrix of the degenerating stump and the regenerating nerve [81], [82], [83]. ApoE4 is produced in abundance in the brain and serves as the principal lipid transport vehicle in cerebrospinal fluid. It is induced at a high concentration in peripheral nerve injury and appears to play a key role in repair by redistributing lipids to regenerating axons and to Schwann cells during remyelination.

AD: Roles for antioxidant to decrease the potential of ER stress

The buildup of unfolded protein in the ER lumen is sufficient to produce ROS, and both ROS and unfolded protein must be present for the UPR and apoptosis to be activated. The antioxidative stress response's ability to limit ROS accumulation and protein misfolding may be especially important for cell function and survival in cells with a high-protein-folding load and/or are susceptible to oxidative stress, such as B lymphocytes or pancreatic cells, or in cells exposed to a variety of environmental insults [84], [85]. Chop/cells and mice are protected against ER stress-induced apoptosis, implying that the ER stress-induced apoptotic cell death pathway is mediated in part by CHOP [86], [87]. Furthermore, the changes in gene expression produced by ER stress that was seen after Chop deletion reflected the changes in gene expression generated by antioxidant therapy. The data show that antioxidants and Chop deletion may work together to promote ER function. There is evidence to suggest that antioxidants and dietary changes can reduce oxidative stress and be beneficial in a variety of disease states [88], [89], [90].

CRISPR/Cas 9: A promising gene-editing tool

CRISPR/Cas9 is a newly discovered and promising breakthrough method for genome editing that enables the treatment of diseases that have few or no therapy alternatives (Figure 2). Ishino was the first to discover this tool in 1987. Since then, multiple studies have found that the CRISPR/Cas9 system is an important component of a bacterium's defense system, protecting it from the unwanted integration of mobile genetic components such as plasmids and viruses [92].

Furthermore, Doudna and Charpentier's pioneering efforts introduced CRISPR/Cas9 to the

laboratory to be studied (Figure 3). The scientific community is using CRISPR/Cas9 for various biotechnological and medical purposes. One of its most important uses is developing potential therapeutic strategies against diseases. CRISPR/Cas9-based approaches have been increasingly applied to the treatment of human diseases such as cancer, genetic, immunological, and neurological disorders, and viral diseases. These strategies using CRISPR/Cas9 are not only therapy oriented but can also be used for disease modeling as well, which, in turn, can lead to the improved understanding of the mechanisms of various infectious and genetic diseases (Figure 4). In addition, the CRISPR/Cas9 system can also be used as programmable antibiotics to kill the bacteria sequence specifically and, therefore, can bypass multidrug resistance. Furthermore, CRISPR/Cas9-based gene drive may also hold the potential to limit the spread of vector-borne diseases [58], [91].

However, several studies have revealed that Cas9 binds to unintended genomic sites for cleavage, termed as off-target effects (Table 1). The target efficiency of CRISPR/Cas9 was determined through 20 nucleotide sequences of gRNA and PAM sites adjacent to target loci. More than 3 mismatches between target sequences and 20 nucleotides of gRNA can result in off-target effects. It has demonstrated that four mismatches in the PAM-distal end induce off-target effects. Researchers have proposed two types of off-target effects, the first types of off-target effects are likely to occur due to the sequence homology of the target loci and the next types of off-target sites occur in the genome other than the target site [93]. Fortunately, significant editing efficiency and off-target effects have been achieved due to CRISPR/Cas9 becoming the subject of substantial research in recent years [59].

CRISPR/Cas9 potential in AD: A promising role of CRISPR/cas9 in APOE e4, APP gene, and PSEN-1

The most powerful genetic risk factor for SAD is the APOE4 isoform [94]. As previously stated, APOE is mostly expressed by astrocytes in the central nervous system. However, the presence of APOE in neurons indicates the occurrence of these events, which include age-related cognitive decline, neurological damage, and neurodegeneration [95]. Some research found that when the E4 allele was corrected to E3/E3 genotype in iPSCs from two individuals with AD using the CRISPR/Cas9 technique, E3 neurons were less vulnerable to ionomycin-induced cytotoxicity and demonstrated a reduction in tau phosphorylation. Furthermore, it was found that APOE4 function utilizing hiPSC and CRISPR/Cas9 technology; their findings revealed that APOE4 had a cell-type-specific influence on A metabolism in a variety of ways. More promising findings revealed that isogenic conversion of APOE4 to APOE3 may reduce a variety of AD-related diseases. These data suggest that APOE4 is a viable target for the therapy of AD [96].

As a consequence of enhanced β-secretase cleavage of the Aβ precursor protein, a mutation in the APP gene causes dominantly inherited AD. The KM670/671NL APP mutation, which is unique to Sweden (APPsw for the mutation and APPsw for the mutant allele), causes an increase in enzymatic cleavage by β-secretase, resulting in higher Aβ protein levels [57]. It was discovered that when APP alleles are knocked out using CRISPR/Cas9 technology, the production of Aβ protein reduces. As a result, the CRISPR/Cas9 system may give gene therapy methods for AD patients with APP mutations. Furthermore, discovered potentially protective deletion mutations in the APP gene's 3′-UTR in mice. They discovered a significant decrease in Aβ accumulation when they removed 700 bp of the 891 bp APP 3′-UTR in mouse model zygotes utilizing CRISPR/Cas9 technology [58]. Surprisingly, the A673T mutation is responsible for an Icelandic population that does not display indications of AD even at a young age. This mutation has been shown to lower β-secretase cleavage by 40% [97]. As a result of the previous research, inserting the A673T mutation into patient's neurons might be an effective and long-term the strategy of reducing the risk factor for patients with Alzheimer's disease [91]. To that goal, they used a CRISPR/Cas9-based method to change the APP gene in HEK293T and SH-SY5Y cells by transforming the alanine codon to a threonine (containing the APP gene with deaminated cytosine1 and cytosine2 positions). The buildup of Aβ peptide has been decreased further as a result of the effective insertion of the A673T mutation in 53% of HEK293T cells with a novel mutation (E674K). Similarly, Sun's team selectively altered endogenous APP at the extreme C-terminus in cell and animal models using a CRISPR/Cas9-based method, and reciprocally modified the amyloid pathway. As a result, A production has been lowered by inhibiting APP-β-cleavage while increasing neuroprotective APP-α-cleavage [68].

For the 1st time, the CRISPR-Cas9 system was utilized to simulate AD mutations in 2016 (cotransfection of APP Swedish mutant and PSEN1 M146V mutation) [99]. The authors examined Aβ42 levels and the Aβ42/40 ratio in human APPSwe and M146V knock-in cell lines using iPSCs, neural precursor cells, and neurons. M146V was studied in both homozygous and heterozygous forms, and the results were compared to those of controls [99]. Sun *et al*. recently modeled 138 PSEN1 mutations from neuroblastoma (N2a) cell lines and utilized the CRISPR-Cas9 system to create mutant cells. This study looked at the effects of mutations on the amyloid formation and the Aβ42/40 ratio on gain or loss of function. Several of these mutations were linked to an increased Aβ42/40 ratio; however, there was no significant relationship between amyloid levels or ratio and age at the start [100].

Ethical concerns with CRISPR technology

CRISPR/Cas9 genome-editing technology is quickly altering the field of molecular biology, allowing

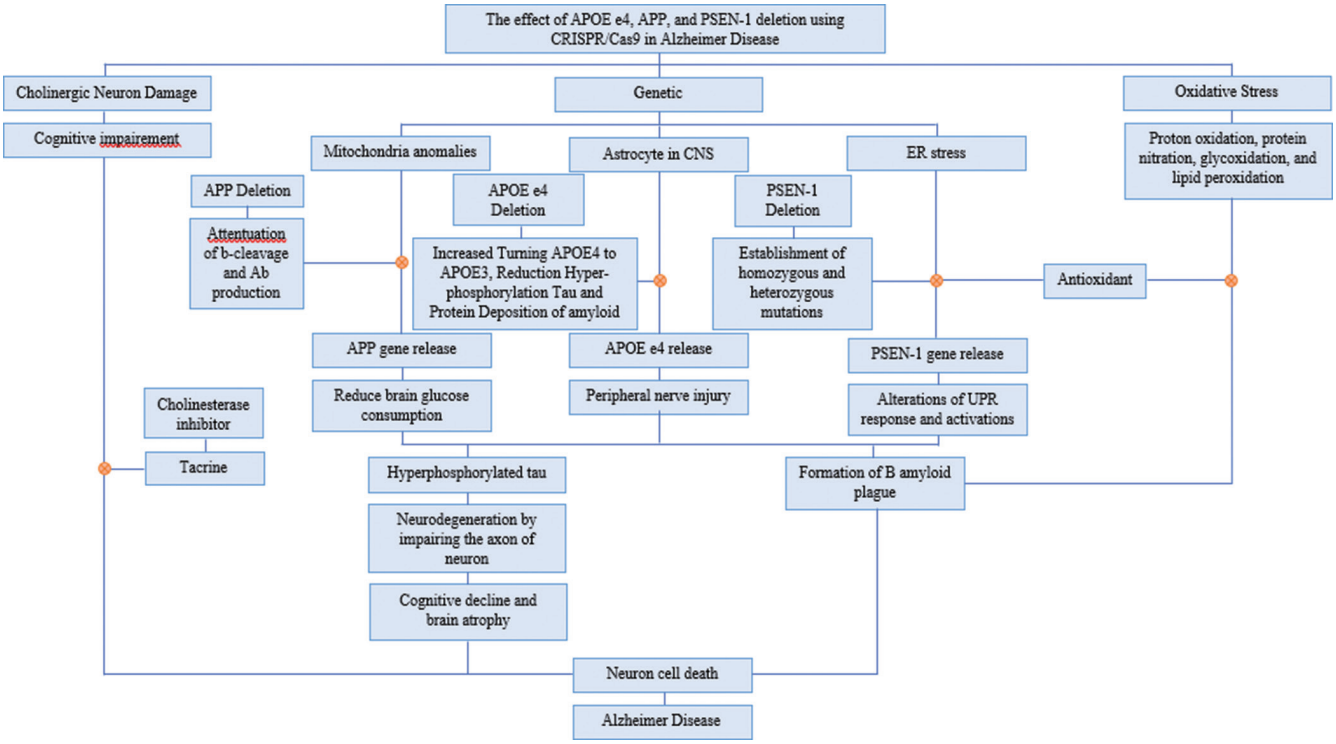


Figure 5: The framework of the deletion APOE e4, APP, and PSEN-1 in preventing the neuron death cell

scientists to create desired alterations in DNA in a range of animals. It was immediately accepted after its introduction in 2012 due to its ease of use and simplicity. It is being studied for a wide range of uses, including agricultural and medicinal medicines, as well as human germline changes to rectify genetic problems [101]. Although the ethical controversy surrounding human genetic modification is not new (as previously stated), CRISPR/Cas9-mediated genome editing has given it a new edge. Because of the unforeseen and far-reaching repercussions of appealing uses of this technology, a detailed discussion of its ethical and societal ramifications is required.

The current paper analyzes the ethical considerations associated with this new technology, with a focus on its potential and concerns about human germline modification. It emphasizes the importance of wide communication among scientists, ethicists, industrialists, and policymakers. The views of many sectors of society, including the general public and religious intellectuals, are critical.

The efficiency with which CRISPR/Cas9 may generate genetic modifications makes it similarly difficult to recognize the respective modified creature outside of the laboratory, raising worries about their management. If CRISPR/Cas9 continues to spread over the world, we may expect a larger market for genetically modified species, raising concerns about their control. Furthermore, the patenting difficulties must be settled. There is a schism within scientific groups about the patenting of genetically modified organisms for medicinal purposes. Many commercial interests focus on CRISPR/Cas9. It is believed that patenting CRISPR/Cas9 methods will grant the relevant corporations considerable influence [102], [103].

The reason using CRISPR/Cas9 as an effective therapy for AD

Biomarker evidence of Aβ and tau pathology is employed to identify AD, while clinical manifestations are utilized to grade severity (Table 2). The term “AD” is used in this framework anytime there is evidence of A-β and tau pathology, regardless of the clinical signs [104]. Other age-related, protective, and disease-promoting variables, such as vascular dysfunction, oxidative stress, proteinopathy, metal ions, neuroinflammation, mitochondrial dysfunction, and the microbiota-gut-brain axis, are also strongly linked to the progression of AD. The clinical signs of AD vary widely between individuals due to the etiology’ intricacy [105].

Because of its irreversible symptoms and progressive nature, establishing more effective therapeutic strategies for AD have been difficult [113]. CRISPR/Cas9 technology has recently advanced significantly, demonstrating considerable promise in the fields of basic research and medical therapies. In addition, gene-editing technology was deemed a potential method in AD research and treatment [114], [115]. These advanced techniques can be used to treat both common neurological diseases and neurodevelopmental disorders such as Parkinson’s disease and autism spectrum disorder, as well as rare diseases such as amyotrophic lateral sclerosis, spinal muscular atrophy, lysosomal storage diseases, X-linked adrenoleukodystrophy, and oncological diseases [116].

Three main reasons are causing AD: Cholinergic neuron damage, genetic predisposition, and oxidative stress. Cognitive impairment is believed to be caused by cholinergic neuron damage. Tacrine, which is part of a cholinesterase inhibitor, can prevent this process, thus preventing cell death and Alzheimer's. On the other hand, genetic predispositions such as mitochondria anomalies, endoplasmic reticulum stress, and astrocytes in CNS can also cause Alzheimer's through their respective pathways. The first pathway is through mitochondria anomalies. These anomalies cause the release of the app gene, which will reduce brain glucose consumption. APP gene deletion is believed to prevent APP gen release through attenuation of b-cleavage and Ab production. The second pathway is ER stress. PSEN-1 gene release is believed to be caused by ER stress. The release of this gene will lead to alterations of UPR response and activations. Fortunately, PSEN-1 release is prevented through PSEN-1 deletion due to the establishment of homozygous and heterozygous mutations.

The third genetic predisposition for Alzheimer's is the occurrence of astrocytes in the CNS. Astrocyte in CNS will trigger the release of APOE e4, which occurs during peripheral nerve injury. One method proposed to prevent this release is APOE e4 deletion which will turn APOE e4 into a less harmful form (APOE e3). These three pathways will result in hyperphosphorylated tau, neurodegeneration, cognitive decline, brain atrophy, formation of amyloid tau, neuron cell death, and Alzheimer's. Last but not least, oxidative stress is believed to be the cause of proton oxidation, protein nitration, glycoxidation, and lipid peroxidation. All of these will also play a role in forming amyloid tau. Prevention of these harmful processes is believed to be achieved by antioxidant consumption.

Conclusion

AD is a neurodegenerative disorder with a reasonably high incidence and often becomes a global problem. This disease is characterized by the formation of β-amyloid plaques and NFTs from hyperphosphorylated tau. Aducanumab is still the primary therapy for patients who have Alzheimer's. The deletion of APOE e4, PSEN-1, and APP using the CRISPR/Cas9 genome-editing method can be a promising solution as a current therapy for AD. Apart from being faster, cheaper, and highly effective, this therapy is also believed to have great potential to provide therapy with a permanent effect. It is expected that further research will be carried out to determine the effectiveness of APOE e4, PSEN-1, and APP removal therapy with the CRISPR/Cas9, the method was clinically tested to determine the side effects that its use can cause. So later, this therapy can be used as the primary therapy for people with AD (Figure 5).

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