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A Review on CRISPR Techniques used in Alzheimer’s Disease.

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ABSTRACT:
Amyloid-beta (Ab) plaques and neurofibrillary tangles, which are composed of hyperphosphorylated tau, are two features of the chronic, irreversible neurodegenerative disorder known as Alzheimer's disease (AD). At least 50 million individuals are believed to be living with AD at this time, and the number of AD patients is rapidly rising. It is known that the pathophysiology of AD is correlated with mutations or changes in the genes encoding the amyloid-b precursor protein (APP), presenilin-1 (PSEN1), or presenilin-2 (PSEN2) proteins. A cure for AD remain unclear, and numerous clinical trials with gene-targeted therapies have fallen short of the expected levels of efficacy. The genome-editing technique CRISPR-Cas9, which is currently widely used in the study of AD, has been developing as a potent tool to fix abnormal genetic functioning. This straight forward yet effective method of gene editing demonstrated tremendous potential for reversing undesirable mutations in genes linked to AD, including APP, PSEN1, and PSEN2. The complexity of the neurological system may now be studied using a variety of cell types (in vitro) and animals, opening up new avenues for the creation of empirical AD models, diagnostic techniques, and treatment lines (in vivo). To better understand the associated mechanisms and potential uses of CRISPR-Cas9 as a potent therapeutic tool for treating AD, a review of the subject was conducted.While viral vectors are effective at delivering CRISPR/Cas9, they may cause catastrophic side effects and immune responses. Non-viral vectors may be promising for in vivo delivery of CRISPR/Cas9 therapies due to their improved safety profile, cost-effectiveness, and adaptability. Other prospective non-viral vectors for genome editing in Alzheimer's disease, such as nanoparticles, nanoclews, and microvesicles, are also addressed.

Keywords: CRISPR-Cas9; Clinical trial; Alzheimer’s disease (AD); Genome editing; Mutations.

Key Summery Points:
Globally, there are currently more than 50 million people living with dementia; by 2050, this number will rise to 131 million, with a cost of over US$818 billion. The pathophysiology of AD is known to be influenced by mutations or changes in the genes for presenilin-1 (PSEN1), presenilin-2 (PSEN2), or the amyloid-b precursor protein (APP). However, there are currently no safe and effective therapy approaches for AD, and clinical trial failure rates for AD are the highest of any other condition (99.5%). CRISPR-Cas9, a method for editing the human genome, is now widely used in the research of AD. It has been developing as a potent technology to fix abnormal genetic functioning. Gene transport to the target areas of cells may be ineffective because off-target mutations are one of the main obstacles that might compromise the functionality of altered cells. Non-viral vectors (nanocomplexes, nanoclews, and gold nanoparticles) outperform viral vectors in terms of efficacy and safety.

INTRODUCTION:

As an adaptive defensive mechanism that gives resistance to foreign genetic material, the CRISPR (Clustered regularly interspaced short palindromic repeats) system was first discovered in archaea. Later, the CRISPR-Cas9 system was developed into a flexible gene-editing tool that allows manipulation of downstream DNA bearing the protospacer adjacent motif (PAM). With the development of CRISPR-Cas9, genome editing is now possible in almost any organism, including human cells, rodents, mice, zebrafish, bacteria, fruit flies, yeast, nematodes, and more.

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In the Escherichia coli genome, a group of 29nt repeats separated by five 32nt non-repetitive sequences were found by Japanese researchers in 1987. The collection of interspaced repeat sequences from various bacterial and archaeal strains is rapidly growing, and in 2002, the name CRISPR (Clustered Regulatory Interspaced Short Palindromic Repeats) was used to describe interspaced repeat arrays found in microbial genomic loci. The scientific community quickly and enthusiastically embraced CRISPR-Cas9 technology over the following few years, using it to target, edit, and modify the genomes of a wide range of cells and creatures while clarifying and perfecting the mechanism of CRISPR-Cas9 library genome editing.

One of the most important discoveries in the history of biology, CRISPR-Cas9 (enzymes from bacteria that control microbial immunity) was initially proposed to be utilised for programmable genome editing in 2012 by Doudna and Emmanuelle Charpentier. Since then, Doudna has played a key role in the "CRISPR revolution" as a result of her ground breaking work and development of CRISPR-mediated genome editing.

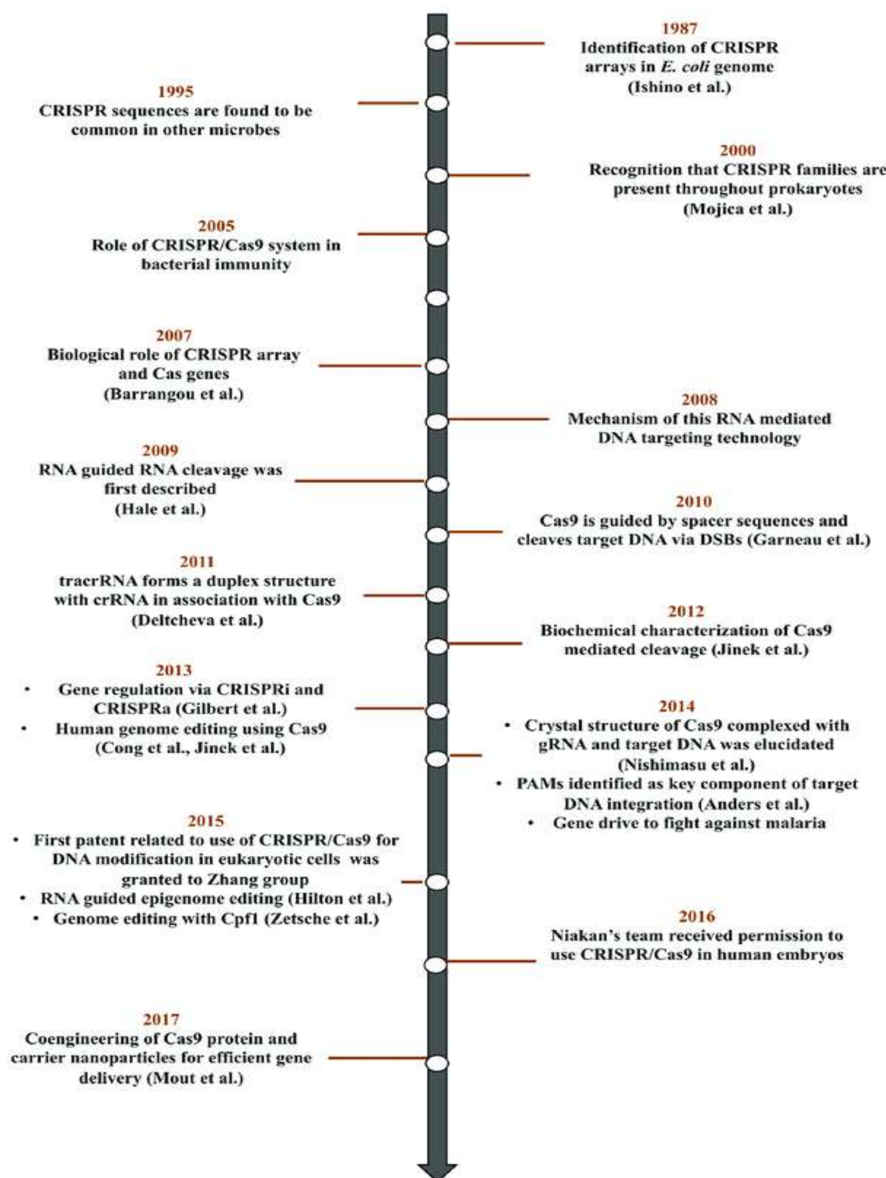

Figure 1. Timeline of CRISPR/Cas9.

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The most frequent cause of dementia is Alzheimer's disease (AD), a progressive neurodegenerative condition that typically affects adults in society. In the USA, it is the sixth most common cause of death[1,2]. Families of AD patients frequently have a heavy financial burden since managing patients with AD necessitates additional care and attention[2]. According to reports, the financial crisis among the impacted communities is expected to worsen significantly on a national and worldwide level in the future[3]. By 2050, there will be over 131 million people worldwide with dementia, costing an estimated US$818 billion[4]. Currently, there are over 46 million people who suffer from dementia. Similar findings have been observed in Bangladesh, where there are more than 450,000 dementia sufferers[5,6]. Cognitive impairment, memory loss, and behavioral abnormalities including language, emotion, mobility, and physiological malfunction are among the symptoms of AD that patients suffer. Extracellular senile plaques made primarily of amyloid-beta peptides and intracellular hyperphosphorylated neurofibrillary tangles rich in tau protein are the pathological hallmarks of AD[7,8]. The most prevalent risk factor for AD is ageing. According to age, AD cases are typically divided into two groups: early-onset AD (EOAD), which typically begins in a person's life before the age of65, and late-onset AD (LOAD), also known as sporadic AD (SAD), which typically begins after the age of 65[10]. The disease is also brought on by a number of non-genetic variables, such as oxidative stress, inflammation, lipid metabolism, and gene-environment interactions[11]. Most cases of EOAD are genetically brought on by mutations in the genes for presenilin-1 (PSEN1), presenilin-2 (PSEN2), and amyloid-beta precursor protein (APP). Worldwide, more than 400 mutations in the APP, PSEN1, and PSEN2 genes have been documented, changing the level of Amyloid beta[Ab] production (alzforum.org/mutations) [11]. The pieces of evidence that suggested the pathogenesis behind AD may be higher levels of Ab42 or changed Ab42/40 ratios caused by mutations in the APP, PSEN1, and PSEN2 genes[12]. Even though the pathogenesis of sporadic LOAD is difficult to explain and its primary genetic cause is still unclear, some genes have been shown to be connected to the disease's emergence. Genome-wide association studies have discovered more than 20 genetic loci, including apolipoprotein E (APOE), that raise the risk of LOAD by promoting excessive Amyloid beta synthesis and clearance[13]. An extended amount of Ab42 synthesis in plasma from several cell lines was found in a site-directed mutagenesis in vitro investigation, which can be associated with in vivo results. The APP gene's well-known model mutations include KM670/671NL (also known as the "Swedish APP" for HEK293) and V717I, which showed an enhanced Ab42/40 ratio[14,15]. The clinical symptoms are what determine the AD diagnosis, which is currently unclear. According to current knowledge, biomarkers play a crucial part in identifying the pathogenic development of AD through clinical testing of blood- and cerebrospinal fluid (CSF)-based biomarkers or molecular imaging technologies, which are also used to infer the disease's etiology[16]. Depending on the stage of the disease and the emergence of pathological variants of AD, biomarkers may have a slightly different diagnostic function during the preclinical, moderate cognitive impairment (MCI), and dementia stages[17]. Drug discovery for the treatment of AD followed a similar pattern. However, there are currently no consistent or viable therapeutic approaches for AD, and the failure rate of clinical trials (99.5%) for AD is higher than that of any other disease from 2002 to 2012[18]. Over 200 research projects have been dropped or proved to be fruitless in the last ten years. The most likely causes of disease-modifying therapies (DMTs) for AD failure could be late treatment initiation during the course of AD progression, inappropriate drug dosages, incorrect target selection, and primarily a lack of understanding of the intricate pathophysiology of AD, which may call for specialised and combined therapies[19].

Through in vitro study, insightful observation of AD and its associated pathways may be examined, and the results may help to identify possible in vivo diagnostic and treatment techniques. A newly created gene editing technology called Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-Cas9 is showing great promise for use in the investigation of mutagenesis and neurodegenerative diseases, therefore there is reason for optimism. Because CRISPR-Cas9 has a stronger ability to fix a mutation in the genome of brain cells, it is currently a potential gene editing method that might theoretically be used as a therapeutic mediator of AD[20,21]. The CRISPR-Cas9 method is steadily demonstrating AD model generation's suitability for examining disease pathophysiology,

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phenotypes, and treatments. Models of the APP and PSEN1 mutations were created using CRISPR-Cas9, which also demonstrated a precise method of genome editing via gRNA synthesis[22]. A new direction for AD research is now possible thanks to CRISPR-Cas9, which may target any gene in various cell lines, tissues, or animal models for change. We have gathered data on the possible uses of CRISPR-Cas9 to specifically modify the genomes for AD in vitro or in vivo, and we have summarised the mechanism of this technology in this review. There are no investigations by any of the writers using human or animal subjects in this article; instead, it is based on already completed research.

Objectives of CRISPR/Cas9 Technology:

- **Disease Prevention:**

In the first research on human embryonic genome editing, zygotes were given CRISPR/Cas9 treatment to correct an HBB mutation that causes the autosomal recessive illness -thalassemia. This repair method is known as HDR (Homology Directed Repair). CRISPR/Cas9 embryonic genome editing seems like a potential application for preventing such monogenic illnesses before childbirth. A few instances of HDR-mediated gene change in mammalian zygotes provide support to this hypothesis. Neonatal genetic alteration is typically ineffective (10%); however, in some instances, Cas9 has demonstrated better efficacy (Crb1: 27%, Asip: 18.2%). Notably, cataract development in the progeny of mice zygotes with a dominant mutation in Crygc was prevented by microinjecting Cas9 together with a repair DNA. The ability to fix mutations in human embryos through genome editing may soon be available in clinical settings thanks to the quick development of this technology.

- **Genetic Enhancement:**

When people select a sperm donor, there may be a demand that points to the possible application of genome editing for genetic enhancement. In a recent study, 1597 women who used donor spermatozoa to start their babies said that, in addition to the donor's health, 50.0% of them thought the donor's IQ was significant. They also said that 42.7% and 40.7% of them thought the donor's height and ethnicity were relevant. By modifying the embryo, it would be tough to increase the child's IQ. Particularly in nations with ethnically varied populations, outwardly apparent features like eye, hair, and skin colour may be thought of as a target phenotype in terms of ethnicity. In a recent study using rat zygotes, Cas9 treatment corrected Tyrc, Asipa, Kith via HDR and subsequently led to recovery from albinism via an SNP exchange. In the non-agouti and hooded phenotypes, this was accomplished by integrating a 19 bp DNA sequence and removing a 7098 bp insertional DNA fragment, respectively. The successful rat coat colour modifications raise the possibility that an already existing variant may be replicated and used to alter human pigmentation through embryonic editing. Some mutations linked to hair and eye colour in European people were discovered by a genome-wide association study (GWAS). It's important to note that the OCA2 mutation rs1667394 A is linked to blond hair and blue eyes. It's possible that some would-be parents will want to use HDR to introduce the OCR2 variant into their embryos, but it's unlikely that they'll be able to get the iris and hair phenotypes they want in such a straightforward way. Given that at least 16 genes control the colour of the human iris, the genetic backgrounds of the parents would have a significant influence on the appearance of the progeny. A child with an OCA2, TYR, TYRP1 or SLC45A2 variation could have pink eyes, a characteristic of albinism, but they would also likely have vision problems and be more susceptible to skin cancer and sunburn. Therefore, by introducing a naturally occurring variety via HDR, it is possible to influence features that are observable from the outside.

Mechanism of CRISPR/Cas9:

The CRISPR-Cas9 system was first identified in the prokaryotic adaptive immune system, namely in bacteria and archaea[23]. Three essential elements make up the often utilized Type II CRISPR-Cas9 system: the endonuclease Cas9, CRISPR RNA (crRNA), and trans-activating crRNA (tracrRNA)[24]. Among these three parts, Cas9 is an enzyme that breaks down the target DNA. It has six domains, including REC I, REC II, bridge helix, protospacer adjacent motif (PAM)-interacting domain, HNH nuclease domain, and RuvC[25].

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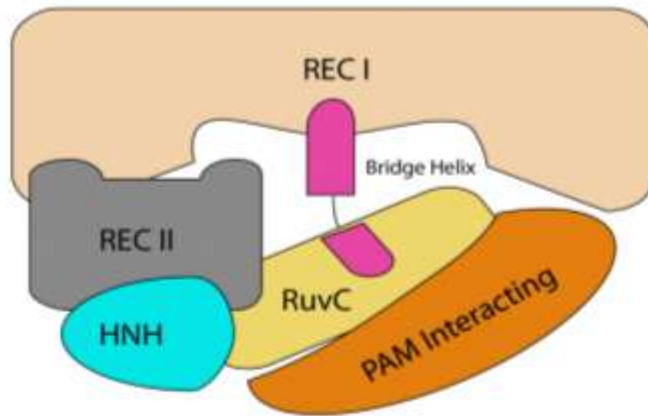

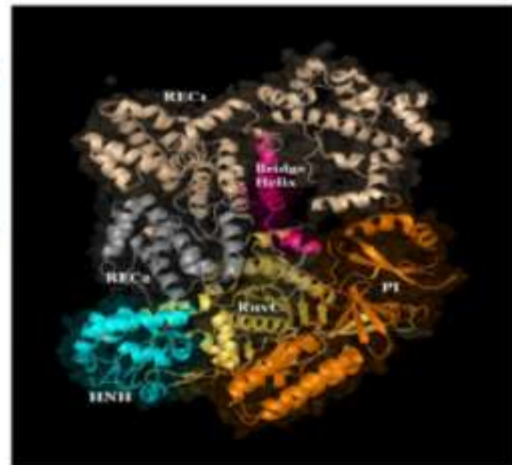

Figure 2. Cas9 Enzyme.

The guide RNA can bind to the target sequence with the aid of the REC I domain. When target DNA is bound, the bridge helix is essential for starting cleavage activity, and the PAM-interacting domain is then in charge of starting the interaction with target DNA. For the effective advancement of genome engineering, the crRNA-tracrRNAduplex can be joined to create a chimeric single guide RNA (sgRNA) for a complementary 20-nucleotide guide sequence makes up the sgRNA's target location[24.25].

The Cas9 enzyme is directed to cleave the DNA strand and create a double strand break (DSB) at the target spot when the sgRNA detects the target sequence. The target DNA is cut by the nuclease domains of RuvC and HNH. Two major repair mechanisms are mainly responsible for repairing the breaks: non-homologous end joining (NHEJ) and homology-directed repair (HDR) as shown in fig no: 03[26].

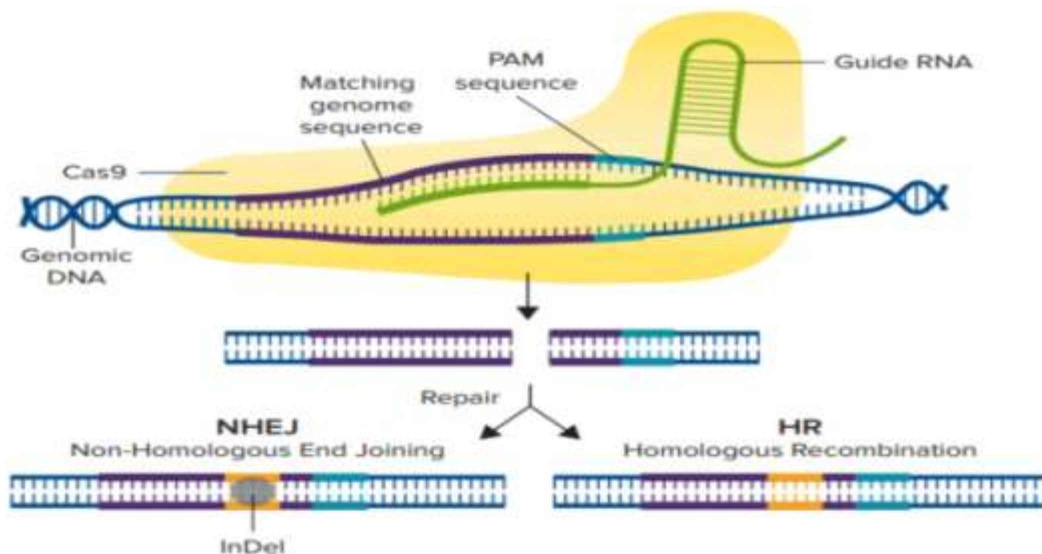

Figure 3.Genome editing mechanism of CRISPR/Cas9.

While the NHEJ repair mechanism typically causes genomic insertions or deletions (indels) for gene disruption with higher efficiency, the HDR repair mechanism uses a donor DNA template to accurately repair DSBs for gene alteration with low efficiency. The NHEJ typically introduces random insertions or deletions (indels) at the DSB site in addition to being error-prone and capable of directly joining the break sequences. By combining the production of sgRNA and Cas9, high-efficiency cleavage of any target sequence can be accomplished with ease[27,28].

In Fig no: 03 Genome editing mechanism of CRISAR/Cas9, when Cas9 locates its DNA-binding sites, a single guide RNA (sgRNA) forms an RNA-DNA complex with a piece of complementary genomic DNA. A double-strand break caused by Cas9 endonucleases leads to DNA mutagenesis by either the error-prone NHEJ mechanism or the HDR process. NHEJ repair results from insertion- or deletion-related (indel) alterations that can result in a frameshift mutation. In the event that a homologous DNA template is present, the HDR pathway can also be utilised to make precise genetic alterations[29].

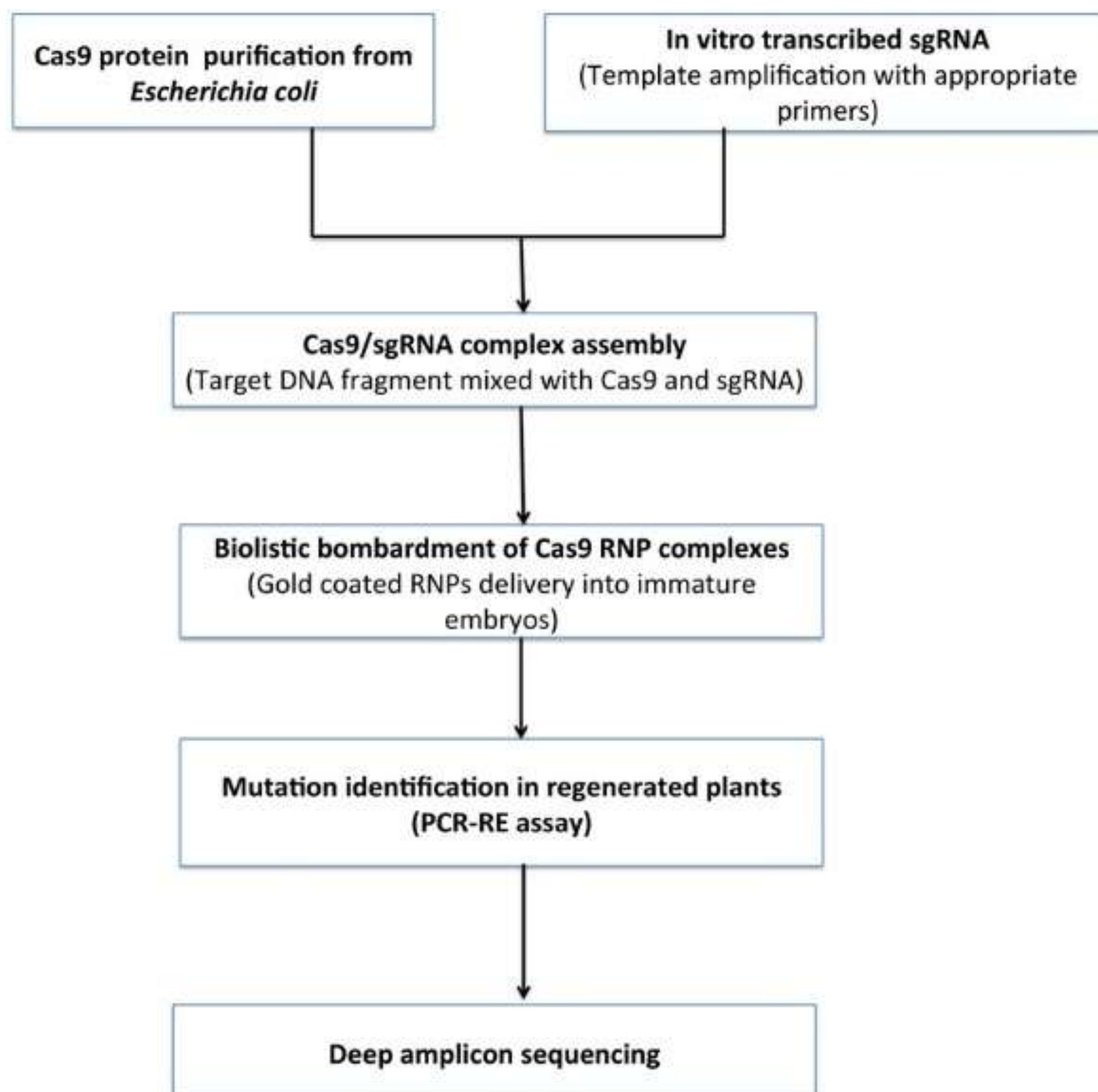

Figure 4.Basic flow diagram of target cell's genome being modified by CRISPR-Cas9 technology.

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Study in early onset AD (EOAD) models:

Generally speaking, point mutations or deletions in the genes encoding the amyloid precursor protein APP, PSEN1, and PSEN2 can be the cause of early-onset dominant familial variants of Alzheimer's disease (AD)[29]. The bulk of cases or triggers for AD are still unknown. Although more than 300 mutations in the PSEN1 gene have been found, they support a common cause of early onset disease, whereas a missense mutation in PSEN2 is an uncommon cause of early-onset Alzheimer's disease[30]. PSEN1 and PSEN2, two c-secretase subunits that increase beta amyloid peptide synthesis, are both involved in the process. It has also been reported that mutations to these two genes increase the synthesis of beta-amyloid, possibly by changing the location of the cleavage site in the APP. Potentially, these kinds of mutations can be fixed through CRISPR-cas9. Induced pluripotent stem cell (iPSC)-derived neurons made from basal forebrain cholinergic iPSC neurons of an individual with the PSEN2N141I mutation were modified using CRISPR-Cas9 to fix a PSEN2 dominant mutation[32]. (Fig No: 04)

By using the CRISPR-Cas9 system, which allows for the selective disruption of the mutant allele while maintaining the wild-type alleles, which prevent Ab production, it is also possible to target the APP^{Swe} mutation in individuals with AD. The Swedish mutation in the APP is actually a double mutation caused by the change of two amino acids, lysine and methionine, to asparagine and leucine, respectively[29,33]. It is located just next to the b-secretase site. György et al. created an AAV carrier carrying a vector containing the coding sequences for the APP^{Swe} specific guide RNA and Cas9 and injected it into the hippocampus of transgenic mice. The disruption of the APP Swedish gene, primarily in the form of single base pair insertions, was demonstrated by those authors, leading to a reduction in the production of pathogenic antibodies.

After the target site is chosen, various bioinformatic tools are used to create sgRNAs, which are then loaded into particular expression vectors with enhanced Cas9. It is possible to screen and validate potential transformant cells after delivery into target cells (next gene sequencing ELISA, copy number analysis, etc.)[29]

Study in late onset/sporadic AD (LOAD) models:

SAD is a complex condition that can be caused on by a variety of rare genetic variants (70%) and environmental variables (30%), including nutrition, toxic substances, and hormone influences[34,35]. The apolipoprotein E4 (APOE4) allele and mutation in the APOE gene, which produces the apolipoprotein E protein, are the main risk factors for late-onset AD. Three main versions of the polymorphic human APOE, APOE2, APOE3, and APOE4, exist[36,37]. The most uncommon variant of APOE is E2, and having one copy appears to cut the risk of AD by up to 40%. The normal version of APOE3, does not appear to be a risk factor for AD, however APOE4, which is present in roughly 10-15% of the population, increases the risk [38.39]. One copy of E4 (E3/E4) may raise risk by two to three times, and two copies of E4 (E4/E4) may increase risk by ten to fifteen times. The majority of APOE4 side effects seem to be related to beta-amyloid; According to a recent discovery, APOE4 may promote disease pathogenesis in human iPSC-derived neurons, such as tau phosphorylation[40]. To change APOE4 into APOE2 or E3 form, the CRISPR-Cas9 system is capable. An effective strategy to promote recovery from AD in APOE4 carriers is the structural and functional modification of APOE4 to APOE3 or APOE2 using CRISPRCas9[20]. According to Wang et al research's CRISPR-Cas9 mutation of APOE4 to APOE3 reduces the disease associated with APOE4 in a model system[40]. The neuro inflammation in AD is also mediated by a number of newly discovered related genes, such as ABCA7, BIN1, CASS4, CELF1, CD33, CD2AP, CELF1, BIN1, PICALM, EPHA1, SORL1, CR1, EPHA1, HLA, IL1RAP, INPP5D, MS4A, TREM2, and TREM2L[13,41-44].

Delivery system in CRISPR/CAS9 in AD:

A promising method of genome editing for the treatment of AD is CRISPR-Cas9. To put this technology into practical applications, a safe and effective delivery method remains a significant barrier that requires updating. Either viral or non-viral methods can be used to distribute the CRISPR-Cas9 system.

Viral Vectors:

CRISPR-Cas9 is typically delivered in vitro and in vivo via viral vectors. The CRISPR-Cas9 system, which is plasmid-based, is most effectively delivered through viral vectors.

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However, they have the potential to bring about unintended alterations that have negative consequences. Due to its low immunogenicity, high infection potential, and overall inability to integrate into the human genome, adeno-associated virus (AAV) is the most often utilised viral vector[53,54]. Single-stranded DNA makes up the majority of the more than 200 variations in the AAV genome[55].

By injecting the viruses into the hippocampi of Tg2576 mice, the viruses were examined both in vitro and in vivo. In the human-derived fibroblasts, this therapy resulted in a 60% decrease in the synthesis of Amyloid beta[65]. It may be necessary to co-inject two viruses because AAV has a smaller packaging capacity of just 4.7 kb. This complicates the process because both viruses might not infect the same cell at the same time. Lentivirus is more likely to trigger immunological responses than AAV, is more difficult to purify in large amounts, and integrates into the human genome effectively[56]. Long DNA inserts (8–10 kb) can be included into lentivirus, however doing so results in less effective brain dissemination (transmission)[54]. Researchers demonstrated the potential to use lentivirus to target the APP, APOE E4, and caspase-6 genes, which are implicated in SAD and familial AD[57-59].

Non-viral Vectors:

Non-viral vectors provide more welfare, more cost-effectiveness, and flexibility in terms of the size of the transgenic portion. They are hence more suitable for applications in AD. The positively charged peptides of CRISPR-Cas9 can easily form nanocomplexes by complexing with the negatively charged nucleic acid cargo. The viral vectors are known to be more immunogenic than these ones. They would have a variety of applications because they can interact with ligands.

However, it is difficult to deliver nanocomplexes to the brain because they are actively eliminated from the blood circulation by the reticuloendothelial system and are unable to pass the blood-brain barrier (BBB) via the systemic route (RES). Injections into the intrathecal and intracerebroventricular regions are therefore frequently employed. Direct injection techniques, however, are limited in their application because it takes several injections to obtain a correct distribution throughout the brain. R7L10 peptide-Cas9-sgRNA ribonucleoprotein nanocomplexes were created by Park et al. to specifically target the gene BACE1[66]. They claim that the BACE1 gene was successfully targeted by the nanocomplexes, which reduced expression without significantly increasing the rate of off-target mutations in vivo.

Applications in AD may be successful using the following delivery mechanisms. A potential methodology for delivering the Cas9-sgRNA combination is DNA nanoparticles (nanoclews). The laborious and time-consuming process of base-pairing is the foundation for how DNA nanostructures are typically put together. DNA nanoclews, which were first described by Sun et al. , are confined DNA molecules that are microscopic in size and include polyethylenimine to provide a positive charge for improved endosomal escape and cell uptake [60]. After being locally injected into the tumours of mice with EGFP tumours, nanoclews carrying sgRNA-Cas9 complexes targeting EGFP showed around a 25% lower expression 10 days after therapy. Despite their benefits, nanooclews may cause immunogenic reactions that still need to be studied.

Additionally, polymeric and lipid nanoparticles may be used as CRISPR-Cas9 delivery vehicles. They have been extensively used in cancer, hepatitis, and other viral conditions before delivering gene editing cargos. However, more research has to be done on how they could be used in AD management[61-63].

Similarly, Wang et al study's made use of gold nanoparticles. In a variety of human cell types, CRISPR-Gold targeting the CXCR4 gene achieved 3-4% HDR efficiency. When CRISPR-Gold was administered locally to the gastrocnemius and tibialis frontal muscles of mdx mice, the dystrophin gene mutation that causes inborn Duchenne muscular dystrophy could be fixed[63].

Futhermore, the inflammatory cytokine profile remained mostly unchanged following CRISPR-Gold injection, demonstrating its tolerance and low toxicity.

The delivery of CRISPR-Cas9 therapies using microvesicles has come to light recently. Typically, sgRNA, Cas9 protein, and a microvesicle-prompting protein (RAB proteins) are transfected into a "producer" cell line[64]. The Cas9-sgRNA complex is produced by the cells as microvesicles. Microvesicles shed into the medium, which is then cleaned up and employed again to deliver their gene-altering burden to the target cells.

Application:

In both early-onset and sporadic AD models, the CRISPR-Cas9 method can be applied for therapeutic objectives. Any specific gene sequence can be effectively targeted with CRISPR

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to fix mutations and introduce genetic elements to the target regions of DNA in cells or tissues.

Table 1.Alzheimer's illness CRISPR-Cas9 Clinical trials:

Targeted genes for CRISPR-Cas9	Mutations that can be corrected with CRISPR-Cas9	Consequences	References
APP	Deletion of Swedish mutation	Reduced pathogenic Ab production ex vivo and in vivo	[29]
APP	Several mutations (T48P, L52P, and K53N)	Made a model for the impact of APP mutations in c-secretase cleavage and notch processing	[48]
APOE	APOE E4 allele to E3 allele	Conversion of Arg158 to Cys158 in 58–75%	[49]
PSEN1	Met146Val	More efficient introduction of specific homozygous and heterozygous mutations	[14]
PSEN2	N141I	Increased Ab42/40 was normalized through CRISPR-Cas to correct the mutation of PSEN2N141I	[32]
APP	Reciprocally manipulate the amyloid pathway	Attenuating b-cleavage and Ab production	[47]
APPS	Homology-directed repair (HDR)-mediated mutation	Disease models generated by CRISPR	[22]
MAPT	Non-homologous end joining (NHEJ)-mediated exon removal	Generation of a new Tau knockout (tauDex1) line in mice	[50]
Bace1	Manipulation amyloid-b (Ab)-associated pathologies	Significant reduction of Ab42 plaque accumulation in mice	[46]
PSEN2	$PSEN2^{N141I}$ mutation	Reduction in the Ab42/40 ratio	[51]

This technology is demonstrating its effectiveness in producing improved cellular and molecular copies, knocking out function, exploring fatal neuronal damage, simulating the illness model, and inserting the guide gene sequence into the genome[75,76]. In order to fully screen for connections between risk variants and cellular pathways, pathogenesis-related specific pathways, and phenotypic variations, CRISPR-Cas9 is able to carry out the entire screening process[77]. CRISPR-Cas9 has been used to change specific gene sequences and has shown important effects on AD (Table 1). Both in vitro and in vivo trials have mostly been conducted on it. With CRISPR-Cas9 components that transfected successfully and produced reliable results, knock-in mouse models were created[78,79]. Numerous studies have demonstrated that the CRISPR-Cas9 system can restore and regulate the physiological consequences of Amyloid beta and the irregular production of misfolded Amyloid beta proteins[80,81]. This technique has been used to alter a gene in post-mitotic neurons of the adult brain, correct endogenous APP at the extreme C-terminus that reciprocally manipulates the amyloid pathway, and enhance astrocyte capacity to clear the accumulated

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Amyloid beta by knocking down calpain[83]. It may also be involved in epigenetic modifications. These findings show that CRISPR-Cas9 is effective in treating AD. To enable the reversal of neurodegeneration in AD, an alternative cell-based therapy is currently undergoing testing.

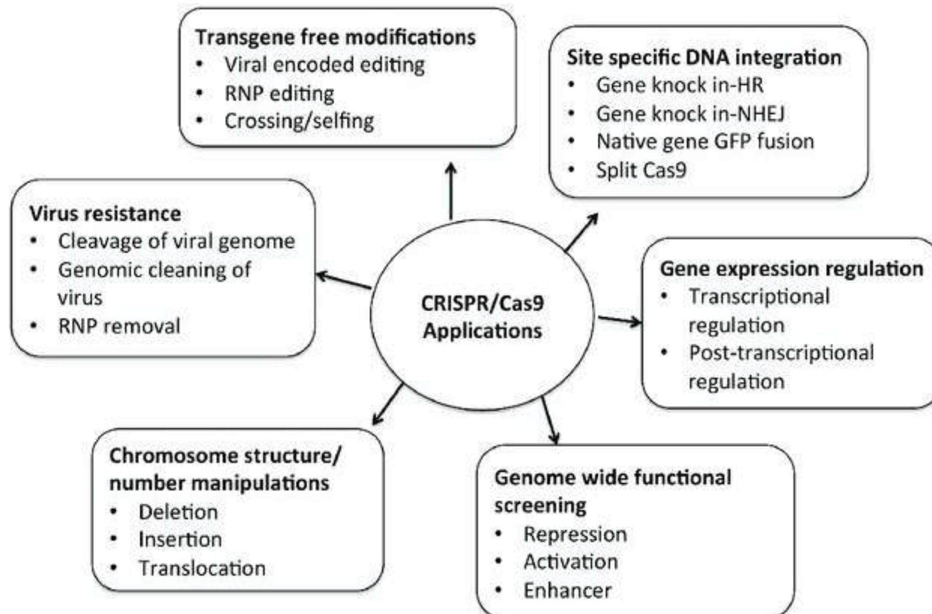

Figure 5. Brief application of CRISPR/Cas9 system

Concluding Remarks, Challenges and Future Perspective:

CRISPR/Cas9 is a promising gene-editing technique since it can fix particular gene sequences and has a lot of potential for treating AD and other human disorders [67-72]. Regardless of hereditary considerations, an altered Amyloid beta metabolism is frequently seen in SAD and EOAD. Therefore, as mutations in the APP, PSEN-1, and PSEN-2 genes are a contributing factor to EOAD, CRISPR/Cas9 technology could address excessive Amyloid beta production or mutations in these genes The effective management of AD following CRISPR/Cas9 brain delivery using non-viral vectors faces a number of difficulties. The vectors should ideally be steady and effective at transporting the weight to the target location. The vectors should be internalised when they come in contact with the targeted cells in order to prevent lysosomal breakdown and target the nucleus. For upcoming designs and applications, it is crucial to take the vast size of CRISPR/Cas9 into account. Because of its reduced size, the Cas9-sgRNA combination is chosen over plasmid-assisted delivery strategies. The components of the created formulations are also susceptible to degradation because of the circulating nucleases and proteases. PEGylation is frequently used to minimize the identification of these systems by RES, but it can produce particular PEG-antibodies and decrease cellular absorption, which can result in immunogenic reactions[73]. Non-viral vectors are favoured for in vivo applications, but real-world applications can only be expanded by increasing the varied formulation properties. Despite the problems with the stability and targetability of the delivery vector, the systemic route is extensively researched because to its in vivo viability, particularly in the case of AD patients. In order to administer medication, intracerebroventricular and intrathecal injections are typically used. In experimental animals with PD, stereotaxic microinjection surgery has been utilised to deliver genes to their brains[74]. This approach, however, can be difficult for AD due to the broad nature of Amyloid beta disease. The intranasal route is another interesting strategy because it avoids the BBB. More clinical investigations on the nasal administration of CRISPR/Cas9-based therapies are nevertheless required. Because genome editing is irreversible, more study is required to guarantee the security of CRISPR/Cas9 medicines. Additionally, there are still not enough studies examining potential off-targets and long-term effects, thus ethical considerations are required before any application in humans. The

CRISPR/Cas9tool, which is significant, affects somatic cells as opposed to germline cells. Gene editing would therefore only be seen in the patients receiving therapy and would not be passed down to future generations[52]. Despite the fact that CRISPR/Cas9 causes double-stranded DNA breaks, current advances in primary editing allow for the correction of gene variants without these breaks. Prime editing, on the other hand, uses a defective Cas9 that is attached to a reverse transcriptase and follows catalytic processes with the aid of a prime-editing guide RNA (pegRNA). guiding the system to the DNA region where the necessary correction is to be made[64]. To determine the off-target and potential of this new technology, more research is needed.

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