CRISPR-Cas9 Gene Editing in Psychiatric and Neurological Disorders Advances, Challenges, And Future Perspectives

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***Abstract*—Neurodegenerative and psychiatric disorders, including Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), and amyotrophic lateral sclerosis (ALS), are severe conditions characterized by the progressive loss of neurons and cognitive/motor function. This review aims to evaluate the transformative potential of CRISPR/Cas9 gene-editing technology in modeling and treating these disorders. Traditional therapies are largely palliative. However, CRISPR/Cas9 allows for precise DNA modification. It has demonstrated remarkable preclinical efficacy in correcting pathogenic mutations (e.g., APP and PSEN1/2 in AD, SNCA in PD, HTT in HD, and C9orf72 in ALS). Using mechanisms like non-homologous end joining (NHEJ) and homology-directed repair (HDR), CRISPR effectively targets toxic protein accumulation and neuronal death. While off-target effects and central nervous system (CNS) delivery hurdles remain, emerging vectors like extracellular vehicles (EVs) and advances in base editing offer a promising trajectory toward personalized, disease-modifying therapies.**

***Index Terms*—CRISPR-Cas9, Gene editing, Psychiatric genetics, Neurodegenerative disorders, Gene therapy.**

I. INTRODUCTION

CRISPR is a fundamental part of a microbial adaptive immune system, which recognizes foreign nucleic acids based on their sequence and eliminates them by Cas9. The first description of CRISPR loci appeared in 1987 when repeats of 29 nucleotides were found downstream of the iap gene of Escherichia coli; these sequences were interspaced by non-repetitive ones of 32 nucleotides.

In 2002, it was reported that the CRISPR loci are transcribed into small noncoding RNAs (crRNA) containing one spacer sequence used as a guide, which serves to identify foreign nucleic acids and guide Cas9 to cleave them. Subsequently, Haft and colleagues identified CRISPR-associated (Cas) genes as a basis for the eventual classification of three different types of CRISPR systems.

II. GENE EDITING TECHNOLOGY

CRISPR-Cas9 system: This tool was initially identified by Ishino in 1987.CRISPR/Cas9 has two main components: the Cas9 enzyme and single-guide RNA (sgRNA).

The target DNA sequence is recognized by the sgRNA, wherein various parameters are considered in the design process to improve specificity. Class 1 (type I, type III, and type IV) and Class 2 (type II, type V, and type VI).

Class 1 contains a variety of Cas proteins that work together, whereas Class 2 utilizes a single Cas protein, which makes it simple and desirable for genome editing.

Class 2, the type II CRISPR/Cas9 system is one of the most researched and utilized systems in pharmaceutical development. The Cas9 protein generates a double standard break after recognizing the target gene sequence.

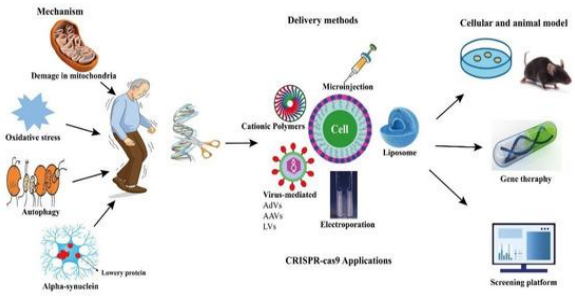

Fig: Potential applications of CRISPR-Cas9 in PD.

III. GENE SILENCING

Gene therapy refers to the introduction of defined genetic material to specific target cells or tissues of a patient for the final purpose of curing or altering particular disease symptoms. Gene therapy can be classified into two categories: gene silencing and gene editing. Gene silencing is a general term used to describe the suppression of gene expression. Double-stranded RNA (dsRNA) is a key molecule in gene silencing; dsRNAs are processed into small interfering RNAs (siRNAs) by the endonuclease Dicer, and these siRNAs are loaded into the RNA-induced silencing complex that pairs with the messenger RNA (mRNA) through base-pairing, causing the mRNA to be subsequently degraded.

Gene Editing

Genome editing inserts, deletes or replaces target DNA sequences in the genome using engineered nucleases such as zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and Cas9.There are three common requirements for any effective gene therapy modality: 1) the identification of the target gene that is mutated in the disease; 2) a delivery system for the genes or materials; and 3), an approach for regulating the expression of the target gene. Unfortunately, nonviral delivery tools are not sufficient for the treatment of chronic neurodegenerative conditions because they create only transient modification in gene.

Distinct from these site-specific nucleases, CRISPR/Cas9 is an RNA-guided engineered nuclease (RGEN) system, in which a synthetic guide RNA (gRNA) introduces a DSB at a specific location in the target genome. A ZFN consists of a Fok1 cleavage domain and a zinc-finger binding domain. ZFNs recognize specific target DNA through protein-DNA interactions.

Because the cleavage of DNA strands occurs after Fok1 dimerization, zinc-finger proteins need to be designed to recognize unique left and right halfsites. ZFN target sites consist of two zinc-finger binding sites separated by a spacer sequence. Although theoretically ZFNs can recognize specific 9-bp sequences, the recognition efficiency can be decreased because of interference between recognition modules. Similar to ZFNs, TALENs are chimeric proteins comprised of a Fok1 cleavage domain and a DNA binding domain from the transcription factor of Xanthomonas.

IV. REQUIREMENTS FOR EFFECTIVE GENE THERAPY

The RNA-guided clustered regularly interspaced short palindromic repeats/CRISPR associated nuclease 9 (CRISPR/Cas9) system is a revolutionary genome editing tool derived from the bacterial Type II CRISPR adaptive immune system. The CRISPR/Cas9 system was found to target and cut specific DNA sequences using only a nuclease and RNAs to target specific DNA sequence. Its primary function in bacteria is the endonucleolytic destruction of invading plasmid or phage DNA. The CRISPR/Cas9 system has advantages over other gene editing tools (zinc finger nucleases or transcription activator like effector nucleases) including ease of use, low cost, and its ability to generate gene knockouts, knockins, or smaller mutations.

Gene Editing Technologies:

The Cas9 (CRISPR-associated) protein is a component of the clustered regularly interspersed palindromic repeat (CRISPR) bacterial adaptive immune system. In terms of genome editing, the most widely used Cas9 protein is from Streptococcus pyogenes (Sp), but other Cas9 orthologs have also been investigated. SpCas9 is functionally a monomer, with two distinct and unrelated active nuclease sites (RuvC and HNH) generating a blunt-end DSB in the target substrate. Profiling of wild-type FokI relative to the obligate heterodimers revealed less cellular toxicity and lower levels of off-target cleavage at selected sites. With regard to Cas9, higher fidelity versions of Cas9 (eSpCas9, SpCas9-HF1, and HypaCas9) show substantial reductions in off-target cleavage. An alternative strategy to reducing off-specific cleavage took advantage of the fact that Cas9 has two nuclease active sites, and mutational inactivation of either the RuvC or HNH sites created a Cas9 nickase.

The use of nickase versions rather than nucleases that introduce DSBs had previously been shown for meganucleases, ZF nickases and TALE nickases to reduce toxicity while still promoting genome editing events. The dCas9-FokI enzymes require the expression of two guide RNAs to appropriately

position the FokI nuclease domains for cleavage. To avoid the dimeric requirement of dCas9-FokI fusions, Cas9-ZF fusions were created.

Overview of CRISPR/Cas System:

NDDs are defined as any disease that causes the progressive deterioration of nerve cells in the central or peripheral nervous system, a category which naturally encompasses a variety of conditions. Phenotypically, AD is characterized by the formation of extracellular plaques of β-amyloid protein (Aβ) and intracellular tangles consisting of the tau protein. To date, the only gene consistently found to be associated with the common, sporadic form of AD (late-onset AD; LOAD) is apolipoprotein E. The presence of alphasynuclein (α-syn) aggregates defines a spectrum of disorders collectively termed synucleinopathies, of which PD is arguably the most well-characterized. Aggregated α-syn is the primary component of Lewy bodies, the defining pathological feature of PD, and point mutations or multiplications in the SNCA gene (which expresses α-syn) result in familial PD. The tight link between α-syn expression and PD has led to the hypothesis that α-syn accumulation may produce toxicity through a gain of-function mechanism. Indeed, misfolding of α-syn leads to the formation of toxic oligomers and beta-pleated sheets, which are thought to impair the proper function of the mitochondria, proteasome, and lysosome-dependent degradation pathways.

This in turn leads to dopamine deficiency in the striatum, which is responsible for the overt symptoms of PD. As elevated levels of α-syn have been implicated in the pathogenesis of PD, targeting SNCA expression levels is an attractive neuroprotective strategy, and manipulations of SNCA expression have demonstrated beneficial effects. Several studies have attempted to reduce the expression of α-syn and rescue PD-related phenotypes by directly targeting SNCA mRNA. showed that a lentivirus expressing a short hairpin RNA (shRNA) targeting SNCA was capable of rescuing multiple phenotypic abnormalities in SNCA-Tri (triplicated) human neuroprogenitor cells (NPCs), including viability, growth, energy metabolism, and stress resistance. While most cases of PD and AD are sporadic, a small subset of both AD and PD cases result from single, causative mutations, which are inherited in a classic Mendelian fashion. Specifically, early-onset AD is caused mostly by mutations in APP, PSEN1, and PSEN2.Mutations in any of these three genes result in increased AB42/AB40 ratios, and the increase in aggregation-prone AB42 leads to early plaque formation and symptom onset. previously mentioned mutations/multiplications in the SNCA gene, autosomal-dominant forms of PD are caused by mutations in leucine repeat kinase 2 (LRRK2), and autosomal-recessive PD is caused by mutations in parkin, PTEN-induced putative kinase 1 (PINK1), and Daisuke-Junko-1 (DJ-1, Scott et al., 2017). These and other genes involved in the etiology of PD, including FBX07, ATP13A2, DNAJC1, PLA2G634, SYNJ1, VPS35, eiF4G1, and CHCHD2, are reviewed elsewhere.

Two years earlier, the same lab validated the base-editing system in vitro by converting APOEε4 into APOEε3 in immortalized mouse astrocytes, in which the endogenous APOE gene was replaced by human APOEε4.Indeed, the generation of APOEε3/4 iPSC lines via base-pair editing have become a routine task for many labs and is now offered as a service from biotech companies. CRISPR'' strategy (i.e., creation of disruptive indels by nucleaseactive Cas9) to successfully alleviate behavioral deficits in two mouse models of familial AD.

These constructs were delivered not by a viral vector, but via nano complexes composed of a synthetic, amphiphilic peptide.

V. STRUCTURE OF CRISPR/CAS9:

According to the different CRISPR/Cas sites, CRISPR systems can be divided into six different types, and the commonly used CRISPR/Cas9 genome editing system is the Type II CRISPR system. with three functional regions on the CRISPR site: The trans activating CRISPR RNA (trancrRNA) region, the different Cas genes region, and the CRISPR array region, three functional regions are linearly arranged on a single DNA strand.

The Cas gene region is used to guide the formation of nucleases that cut DNA sequences, while the CRISPR array region contains repeat sequences and spacer sequences, and the spacer sequences will be inserted for transcription when foreign genes invade.

Mechanism of CRISPR/Cas9:

In the CRISPR/Cas9 system, matured CRISPR RNA (crRNA) combines with trans activating crRNA track rRNA) to form sgRNA. The sgRNA forms a complex with the Cas9 protein. Cas9 protein is guided with sgRNA through base complementary pairing to bind to the specific locus of the genome. Subsequently, the Cas9 protein recognizes the protospacer adjacent motif (PAM) of the target gene and starts to cut the target DNA at the third base upstream of the PAM, producing double strand break (DSB.[11].

Type I CRISPR-Cas: immunity is mediated by the Cascade complex and the Cas3 nuclease. Selective subunits of Cascade, known as Cas6, cleave the precursor crRNA producing short crRNAs that remain associated with Cascade and are used to locate a complementary sequence in the target DNA known as the protospacer. Another subunit of Cascade, Cas8 (also known as CasA or Cse1), recognizes a short sequence motif located immediately upstream of the target sequence identified by the crRNA, named protospacer adjacent motif PAM. PAM promotes the binding of Cascade to the target and the formation of the R-loop between the crRNA spacer sequences and the dsDNA. The first eight base pairs at the 5′ end of the crRNA-DNA duplex is critical for immunity and define a trigger sequence within the target. Type III CRISPR-Cas: the precursor crRNA is cleaved at its 50 by a repeat-specific endoribonuclease, Cas6. The small crRNAs generated after Cas6 cleavage become part of the Cas10–Csm or Cas10–Cmr complex for type III-A or III-B systems, respectively. Within these complexes, the crNRAs undergo further processing by cleavage of their 30. Type III CRISPR-Cas does not require a PAM sequence.

Catalytic Domains of Cas9:

Previous studies have shown that two catalytic domains in Cas9, RuvC and HNH, are responsible for the cleavage of one DNA strand, respectively. By inactivating one of the domains, Cas9 nickase (nCas9) that catalyzes single strand breaks can be generated.[11]

Modified CAS9 Variants:

By inactivating one of the domains, Cas9 nickase (nCas9) that catalyzes single strand breaks can be generated. Yin et al. (Yin et al., 2022a) fused SpCas9 with the optimized human recombinant protein TREX2 to generate a new gene editing enzyme, Cas9TX, which can inhibit structural abnormalities such as chromosomal translocations during gene editing and significantly improve the safety of gene editing. Two mutations were introduced to obtain a catalytically inactive Cas9 protein, also known as “dead Cas9”. The tool simultaneously binds two dCas9-FokI monomers at the target site to trigger DSBs, and the probability of two adjacent dCas9-FokI fusion molecules to bind to the off-target site nonspecifically is significantly lower than that of single molecule binding.

VI. EPIGENETIC & TRANSCRIPTIONAL REGULATION USING CRISPR:

a fusion of dCas9 and the catalytic domain of the p300 histone acetyltransferase caused robust, target specific histone acetylation and gene activation. Conversely, a dCas9-histone deacetylase 3 (HDAC3) fusion protein reliably produced target-specific histone deacetylation, although this effect curiously led to opposing transcriptional effects in two different cells lines. variety of histone methyltransferase domains have also been fused to dCas. Interestingly, direct methylation of H3K4 by a dCas-PRDM9 fusion was sufficient to cause reactivation of silenced genes. but direct methylation of H3K27 (by one of three methyltransferase fusion constructs) was not sufficient for de novo gene silencing. They found that dCas9-LSD1 is capable of causing targeted loss of H3K4 methylation.

VII. IN-VIVO DELIVERY CHALLENGES:

Efficient delivery in vivo is a significantly more difficult problem. A large or multi-component transgene is physically split into two pieces, which are packaged into separate AAV vectors. The resulting AAVs are then codelivered, and the complete protein is reassembled in situ by a split intein a pair of domains which ‘‘splice themselves out,’’ thus joining two peptide chains end-to-end. Size restrictions are also critical when working with dCas9-effector complexes, although to a more flexible extent. One common solution when using CRISPR in mice is to simply use a transgenic line stably expressing dCas9 fused to a domain from one of several protein-protein or RNA-

protein interaction systems. The complementary domain can then be fused to an epigenetic effector of choice and delivered along with the targeting sgRNA, all of which will fit in a single AAV.

VIII. APPLICATIONS

In Neurodegenerative Diseases Alzhimer:
Studies on the AD brain revealed neuropathological changes that represent the hall marks of the condition, such as intracellular neurofibrillary tangles (NFTs) comprising hyperphosphorylated Tau and accumulation of extracellular Ab plaques. An increase in b-secretase 1 (BACE1) activity is responsible for AD owing to aggregation of Ab monomers into oligomers, and consequently the generation and deposition of Ab plaques.

Three genes, presenilin 1 (PSEN1), presenilin 2 (PSEN2), and amyloid precursor protein (APP), have known pathogenic mutations that are strongly associated with the development of early-onset AD; although mutations in additional genes may be involved in the pathology of the disease as well. The recent development of a mitochondria-targeted CRISPR-Cas9 known as “mitoCas9” could be applied to edit mtDNA and remove or revert mutations that accumulate with age, impairing oxidative phosphorylation and increasing ROS production. The CRISPR-Cas9 system using the sgRNAs was also successfully applied to correct A79V and L150P point mutations in the PSEN1 gene of human-induced pluripotent stem cells (hiPSCs) derived from familial AD patients. Another example includes the correction of the PSEN2 N141I mutation in an iPSC-derived neuronal model where the amyloid-β 42/40 ratio was normalized after gene editing treatment.
The CRISPR/Cas9 system has been tested to rectify the mutations in the PSEN1 gene, APP gene, and the APoE4 gene. Autosomal dominant mutations in genes such as PSEN1 can be corrected with the use of this technology, according to recent studies. A recent study utilized the CRISPR/Cas9 technique to specifically interfere with the PSEN1M146L allele. This intervention may also have the ability to partially restore the imbalanced Aβ42/40 ratio. Furthermore, another study introduced a novel mutation (E674K) via a CRISPR/Cas9-mediated system to alter the APP gene. They also demonstrated a decrease in the accumulation of Aβpeptide as a result of the successful introduction of the A673T mutation in 53% of HEK293T cells. The researchers employed the CRISPR/Cas9 technique to rectify the E4 allele to the E3/E3 genotype in induced pluripotent stem cells of AD patients. Following the research, it was observedthatE3neuronsexhibited greater resistance to ionomycin-induced cytotoxicity and dis played a decrease in tau phosphorylation in comparison to E4 neurons.[5]

CRISPR/Cas9-correctable mutation in AD for PSEN2 N141I neurons derived from induced pluripotent stem cells (iPSCs). CRISPR/Cas9-mediated disruption of the APPSW (Sweden) allele in the APP gene in early-onset AD revealed that CRISPR/Cas9 selectively disrupted the APPSW allele both ex vivo and in vivo showing reduction of presented pathogenic Aβ. CRISPR/Cas9-mediated glia maturation factor (GMF) led to reduced microglial activation by gene regulation. In vivo activation of Adam10, a glycosylated type I membrane protein in the mouse brain, with the Cas9 activator nanocomplex ameliorated cognitive deficits in a mouse model of AD is an example of the activator nanocomplexes. Another approach, the use of various CRISPR associated proteins such as Cas9 and Cas12a in AD, allows for precise changes in the genome.

Parkinson;

- For instance, the CRISPR/Cas system was used to eliminate PARKIN (PRKN), DJ-1, and ATP13A2 (PARK9) genes from dopaminergic neurons in a study."
- "A recent study demonstrated that the utilization of the CRISPR/Cas9 system for the purpose of eliminating the A53T-SNCA gene mutation resulted in notable improvements in various aspects associated with PD."
- "These improvements included the reduction of α-synuclein overproduction, prevention of dopaminergic neurodegeneration, mitigation of reactive micro gliosis, and alleviation of motor symptoms."
- "Kantor and colleges have developed a novel all-in-one lentiviral vector that employs CRISPR-Cas9 technology to specifically downregulation of SNCA mRNA and protein expression led to the reversal of disease-related phenotypic perturbations."

The effect of DNA methylation on the level of SNCA expression was tested as the gRNA-deactivated Cas9-DNA methyltransferase 3A (gRNA-dCas9--DNMT3A) system was introduced into hiPSC-derived neurons of a PD patient. This effect reduced SNCA production and ameliorated disease-induced neuronal damage, and the system consists of CRISPR/dCas9 combined with the catalytic domain of DNMT3A. Inhibition of histone marks in SNCA reduces α-SYN burden in PD. RVG-CRISPR Intervention-Exo was found to perform CpG methylation at specific SNCA sites in PD model mice. This precise regulation has been shown to attenuate neuronal cell damage using induced pluripotent stem cells (iPSCs) from marmosets (a non-human primate), Vermilyea and colleagues utilized CRISPR-Cas9 to introduce the G2019S mutation in the LRRK2 gene. Guhathakurta and colleagues used a clever CRISPR-dCas9-mediated approach. Specifically, they recruited a specific histone lysine demethylase, JARID1A, to the SNCA promoter of PD-iPSCs. They reported that their system was sufficient to decrease the expression of α-synuclein.

Hutingsons:

CRISPR/Cas9 can selectively suppress mHTT expression through direct interaction with the DNA. The presence of the mHTT gene leads to sensitization of type 1 inisitol 1,4,5-triphosphate receptor (InsP3R1) and, consequently, calcium outflow from the ER and a compensatory elevation in neuronal store-operated calcium (nSOC) entry, ultimately resulting in synaptic damage of striatal MSNs in an HD animal model.

The CAG repeat-expansion, which underlies disease pathogenesis, has been altered by Cas9 in the pig model of HD and showed that a single injection of AAV-CRISPR/Cas9 into the brain or peripheral vessels could effectively reduce neurodegeneration and improve associated symptoms. CRISPR/Cas9-mediated genome editing prolongs lifespan and improves motor impairments in the HD mouse model. Regulation of the mHTT gene reduced neuronal accumulations by 50%, and CRISPR/Cas9 technology can be used to treat HD and other autosomal dominant neurodegenerative disorders caused by trinucleotide repeat expansion. CRISPR intervention without DNA double-strand breaks delayed the progression of HD in mice. They utilized a personalized allele-selective CRISPR/Cas9 strategy that was based on Protospacer Adjacent Motif (PAM)-altering SNPs. The goal was to selectively inactivate the mutant HTT allele for a particular diplo type in in both dividing and non-dividing cells, both in vitro and in vivo. Yang et al. demonstrated that using CRISPR/Cas9 in HD140Q-KI mice to deplete HTT in a nonallele-specific manner can effectively and permanently eliminate polyglutamine expansion-induced neuronal toxicity in the adult brain.

ALS:

ALS associated pathology was observed in mutant SOD1 motor neurons derived from iPSCs genome-edited with CRISPR/Cas9. Gene editing delivered by AAV capsids reduced mutant human SOD1 protein levels and slowed disease progression. mice treated with the split-intein CRISPR base regulator had a lower rate of muscle atrophy and reduced muscle denervation compared to the control. CRISPR/Cas9 is being used to correct a genetic defect caused by the G4C2 hexanucleotide repeat expansion in the C9orf72 gene.

Meijboom and colleagues utilized an adeno-associated viral vector system to transport CRISPR/Cas9 gene-editing machinery to remove HRE from the C9ORF72 genomic locus. As a result, there was a decrease in RNA foci, polydipeptides, and haploinsufficiency, which are major indicators of C9-ALS/FTD. Deng et al. conducted a study where they used CRISPR/Cas9-mediated editing on transgenes (hSOD1-G93A) associated with ALS in two transgenic models. They showed that this in vivo gene-editing strategy is effective in targeting hSOD1, resulting in a disease-free state in two different hSOD1-G93A transgenic mouse models (G1H and G1L) of ALS. Standard CRISPR-Cas9 has been used to introduce various mutations into the SOD1 gene of iPSCs. In this case, however, the authors employed two different gRNAs to reduce off-target effects. The results were exciting as the CRISPR-Cas9-mediated correction reversed some key aspects of the ALS-like phenotype caused by mutant C9orf72.

Spinocerebellar ataxia:

The spinocerebellar ataxias (SCAs) are a group of progressive neurodegenerative disorders primarily affecting the cerebellum that are inherited in an autosomal dominant manner. SCA1, SCA2, SCA3, and SCA6 are prevalent and clearly defined subtypes,

comprising more than 50 % of cases. the SCAs can be classified into two main categories: those that are a result of dynamic repeat expansion mutations and those caused by non-repeat mutations. There are at least 12 SCAs that are caused by repeat expansion mutation. In a study that employed CRISPR/Cas9 genome-editing therapy of SCA3, the primary objective of suppressing mATXN3 was effectively accomplished by eliminating the mutated CAG repeats in the ATXN3 gene through the CRISPR/Cas9 system, specifically by knocking out the mutant CAG expansions in exon 10. , He et al. demonstrated that the use of paired sgRNAs/Cas9n and HR strategy can effectively repair the 74 CAG expansions in exon 10 of ATXN3 in SCA3-iPSCs (spinocerebellar ataxia type 3 patient-derived induced pluripotent stem cells), leading to a specific and efficient suppression of mutant ataxin-3 protein expression[8].

IX. ADVANTAGES OF CRISPR:

The CRISPR/Cas system offers notable advantages over earlier genome-editing technologies, the two most prevalent of which are zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs).

The CRISPR/Cas9 system has advantages over other gene editing tools (zinc finger nucleases or transcription activator like effector nucleases) including ease of use, low cost, and its ability to generate gene knockouts, knockins, or smaller mutations.

CRISPR/Cas9 gene editing technology has obvious advantages in the treatment of AD. Its high specificity and efficiency make it capable of precisely editing AD-related genes and correcting the genetic errors that cause AD at the source.

These approaches have the major advantages of removing the confounding of having an exogenous promoter drive the expression of TDP-43, such as in the case of the generation of traditional transgenic mouse models. Furthermore, the use of smaller Cas9 allows the experimenter to pack into the viral vector more control genes (e.g., GFP) or multiple gRNAs.

X. LIMITATIONS AND CHALLENGES

While off-target effects and central nervous system (CNS) delivery hurdles remain, emerging vectors like extracellular vesicles (EVs) and advances in base editing offer a promising trajectory toward personalized, disease-modifying therapies. Efficient delivery in vivo is a significantly more difficult problem. Size restrictions are also critical when working with dCas9-effector complexes, although to a more flexible extent.

CRISPR/Cas9 technology has shortcomings when it comes to AD therapy in that off-target phenomena have not been resolved, and unintended editing of non-target genes may cause unpredictable biological effects the immune response cannot be ignored, as foreign Cas9 proteins and gRNAs may stimulate the immune system, which may interfere with the editing process and cause adverse effects, and the special physiological barriers of the CNS and the complexity of the cell types make it very difficult to successfully deliver the CRISPR/Cas9 system to patients with AD. as a therapeutic approach, the disruption of the mutant, disease-causing allele is limited to familial AD cases, as in sporadic cases, such alleles are not present.

However, this method has limitations at the gene level by ignoring the changes at histone level. Some of the critiques include the fact that experiments on rat models and cell cultures are unable to accurately reflect human disease, and the lack of efficacy and safety studies in humans has not been fully elucidated. One reason that the studies have not been done on humans might be due to the inadequate investigation of response rates to treatment at different stages of the disease and uncertainty of long-term effects limit the future potential of these methods in the early diagnosis and treatment of AD.

AAV vectors, however, have a limited carrying capacity that restricts single-particle delivery of the prototypical Cas9 nuclease from Streptococcus pyogenes (SpCas9) alongside an sgRNA expression cassette.[23]

XI. FUTURE PERSPECTIVES

Future Implications: While off-target effects and central nervous system (CNS) delivery hurdles remain, emerging vectors like extracellular vesicles (EVs) and advances in base editing offer a promising trajectory toward personalized, disease-modifying therapies.

Therefore, the codetection of Aβ42 oligomers in serum and exosomes may help to indicate a herald in new diagnosis, prevention, and timely treatment of AD

by improved clinical efficacy. Accordingly, Slb-loaded macrophage-derived exosomes as a multifunctional drug delivery system may be counted as an alternative therapy for AD in the future. Targeted in vivo GMF gene editing could result in a unique and novel AD therapy. Their results demonstrated that the potential of using stem cell–derived exosomes in the treatment of AD and may provide promising data for future clinical trials in this field. One reason that the studies have not been done on humans might be due to the inadequate investigation of response rates to treatment at different stages of the disease and uncertainty of long-term effects limit the future potential of these methods in the early diagnosis and treatment of AD. GMF-targeted drug and gene therapies are a new way to deleterious PD by regulating microglia function. Implantable exosome producing cells made with exosomal transfer into cell devices may offer new therapeutic opportunities with in situ and in vivo delivery of therapeutic.[7] The study demonstrated that the absence of SNCA leads to resistance against Lewy pathology, indicating the possibility of utilizing CRISPR/Cas9n-mediated gene editing as a potential treatment for PD .Loss-of-function mutations in DNAJC6, the gene responsible for producing HSP40 auxilin, have recently been found in individuals with early-onset PD.

Studies indicate that EV-based therapies targeting molecular mechanisms in HD and gene editing with CRISPR method may be new therapeutic treatments.

XII. CONCLUSION

- "CRISPR/Cas9 gene editing technology has obvious advantages in the treatment of AD."
- "Its high specificity and efficiency make it capable of precisely editing AD-related genes and correcting the genetic errors that cause AD at the source."
- "Overall, CRISPR method with exosomes could be helpful in discovering biomarkers used for early diagnosis of the diseases and monitoring the response to treatment."

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