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Gene Editing Technologies in Drug Development for Neurodegenerative Diseases: From Precision Medicine to Genetic Cures

1.Mubasshera Sabir Khan, 2.Dr.Syed Ayaz Ali, 3.Awais Badruddoja, 4.Saniya Mohd Rafeeque, 5. Momin Mashkoora Jabeen

[1] *Department of pharmacology -YB Chavan College of Pharmacy , Dr. RafirZakaria Campus, Aurangabad 431001,Maharashtra,India.*
[2] *Department of pharmacology -YB Chavan College of Pharmacy , Dr. RafirZakaria Campus, Aurangabad 431001,Maharashtra,India.*
[3] *Royal college of pharmaceutical edication and research, Malegaon 423203,Maharashtra,India.*
[4] *Royal college of pharmaceutical edication and research, Malegaon 423203,Maharashtra,India.*
[5] *Royal college of pharmaceutical edication and research, Malegaon 423203,Maharashtra,India.*

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ABSTRACT
Gene editing technologies, such as CRISPR/Cas9, Zinc Finger Nucleases (ZFNs), and transcription activator-like effector nuclease (TALENs), have emerged as a promising tool for developing therapies for neurodegenerative diseases. This review article aims to provide a comprehensive overview of gene editing technologies in drug development for neurological diseases, from precision medicine to genetic cures. We will explore recent advances in gene editing technology and their potential applications in neurology. Furthermore, we will discuss the challenges and considerations associated with the use of gene cutting technologies for developing effective therapies for these complex diseases, including interdisciplinary collaborations and funding priorities.

KEY WORDS: Gene Editing, CRISPR/Cas9, Neurodegenerative Diseases ,Precision Medicine, Genetic Cures ,Alzheimer's Disease ,Parkinson's Disease ,Huntington's Disease, Zinc Finger Nucleases (ZFNs) ,Transcription Activator-Like Effector Nucleases (TALENs) ,Therapeutic Approaches ,Ethical Challenges.

I. INTRODUCTION

Neurodegenerative diseases, such as Alzheimer's, Parkinson's, and Huntington's, are debilitating conditions that affect millions of people worldwide. These diseases typically worsen over time, with symptoms ranging from cognitive decline to motor impairment and behavioral changes. The prevalence of neurodegenerative diseases is increasing due to the aging population, highlighting the need for new and innovative treatments.

In recent years, gene editing technologies have emerged as a promising tool for developing therapies for neurodegenerative diseases. Gene editing technologies such as CRISPR/Cas9, Zinc Finger Nucleases (ZFNs), and Transcription Activator-Like Effector Nucleases (TALENs), enable precise modifications of genes, providing researchers with the ability to target specific genes and correct the underlying genetic defects that cause neurodegenerative diseases.

This review article aims to provide a comprehensive overview of gene editing technologies in drug development for neurodegenerative diseases, from precision medicine to genetic cures. We will explore recent advances in gene editing technologies and their potential applications in neurology. Furthermore, we will discuss the challenges and considerations associated with the use of gene editing technologies for developing effective therapies for these complex diseases. [1-30]

II. BACKGROUND

Neurodegenerative Diseases

Neurodegenerative diseases encompass a diverse range of conditions marked by the gradual breakdown of the central or peripheral nervous system. Some well-known examples include Alzheimer’s and Parkinson’s disease [23]

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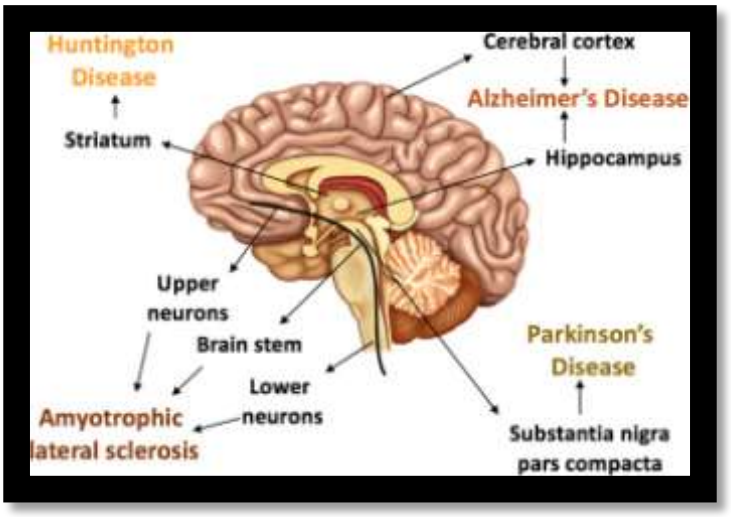

Fig1.Neurodegenerative diseases

Neurodegenerative Disorders Share A Common Trait:

A gradual decline in neurons and synaptic connections, usually manifesting later in life. Each disease is defined by specific symptoms, determined by where in the brain neuronal loss occurs. For instance, in Alzheimer's disease, neuronal loss starts in the hippocampus, impacting declarative episodic memory. In Parkinson's disease, symptoms like tremors, bradykinesia, and postural instability arise when around 70–80% of dopaminergic neurons in the substantia nigra are lost.[24]

Amyotrophic Lateral Sclerosis (ALS):

Amyotrophic lateral sclerosis (ALS) the leading cause of neurologic death in adults, stands as the primary example of degenerative motor neuron disorders. With a prevalence of 6 to 8 per 100,000 individuals, its incidence shows a gradual rise of 1.5 to 2 cases annually. Typically striking at around 74 years of age, ALS affects both genders equally. Following diagnosis, the average life expectancy is approximately 3 years, with 20% surviving 5 years and an additional 10% reaching the 10-year mark.[25]

Alzheimer's Disease:

Alzheimer's disease (AD) a prevalent neurodegenerative condition, typically impacts individuals aged 65 and older, leading to a decline in cognitive functions such as language, memory, comprehension, attention, judgment, and reasoning.

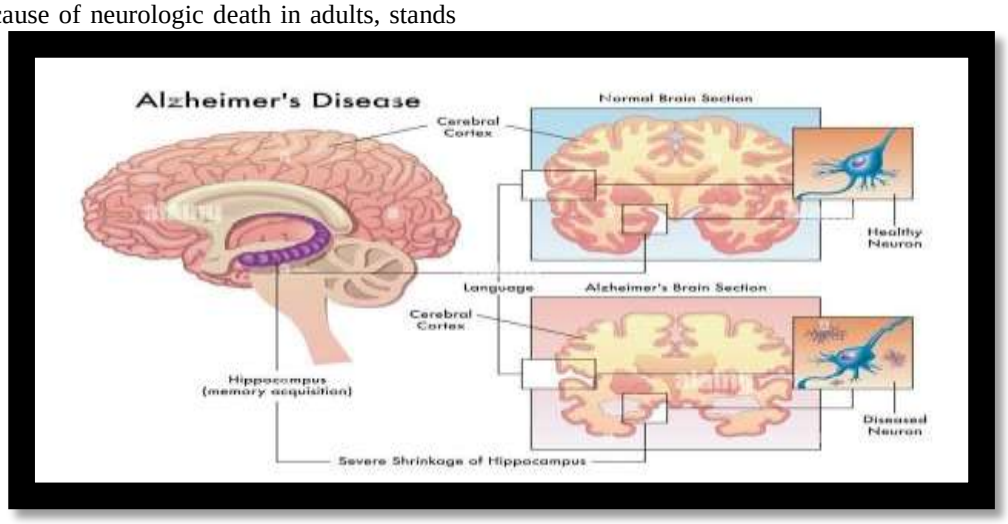

Fig2.Alzheimer's disease

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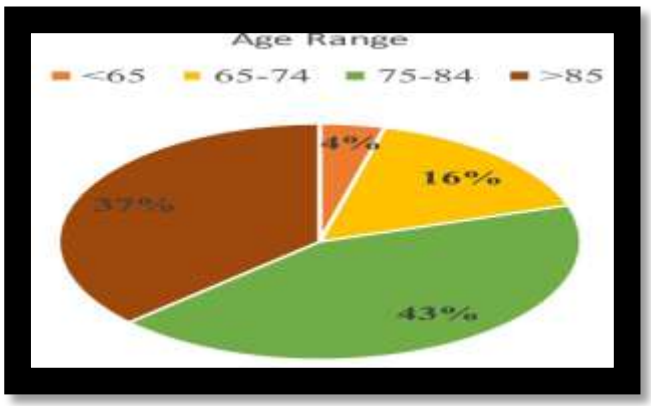
Dementia encompasses a decline in cognitive abilities impacting daily life, with Alzheimer's disease being its predominant form, affecting around two-thirds of those aged 65 and older. Alzheimer's is a neurodegenerative condition marked by a gradual onset and progressive decline in cognitive functions like memory, language, and judgment. It ranks as the sixth leading cause of death in the U.S. While early onset, occurring before 65, is rare, treatments exist to alleviate certain symptoms, though no cure currently exists, although there are treatments available that may improve some symptoms.



Alzheimer's disease progresses through distinct stages, each marked by specific cognitive and behavioral symptoms. It typically begins with episodic short-term memory loss, often accompanied by problems in problem-solving, judgment, and executive functioning. As the disease advances, language difficulties, visuospatial impairment, and neuropsychiatric symptoms such as apathy and agitation become more prominent. Late-stage symptoms include motor difficulties, olfactory dysfunction and Parkinson's disease symptoms occurs lately in disease and incontinence, ultimately leading to total dependence on caregivers.[26].

Genetic Component Of Alzheimer's Disease:

Alzheimer's disease has a genetic component, with specific genes like APOE being implicated. The APOE e4 allele, in particular, is associated with an increased risk of late-onset Alzheimer's. Studies indicate that possessing one copy of this allele raises the risk, and having two copies intensifies it further.[71].

Parkinson's Disease:

Parkinson's disease (PD) is a prevalent neurodegenerative condition characterized by a range of non-motor symptoms, though its hallmark features include bradykinesia, resting tremor, and rigidity, with postural instability often emerging later in the progression of the disease. While the exact cause of PD remains elusive, researchers have identified various genetic risk factors and rare familial genetic mutations associated with the condition.

Various environmental factors, such as smoking, caffeine intake, and exposure to pesticides, have been suggested to potentially influence the risk of developing Parkinson's disease (PD). PD is characterized by the loss of dopaminergic neurons in the substantia nigra pars compacta, accompanied by the presence of intracellular aggregates of α-synuclein, known as Lewy bodies and Lewy neurites. While mitochondrial dysfunction, impaired protein clearance, and neuroinflammation are among the processes implicated in PD, the precise interplay between these factors is not fully elucidated.

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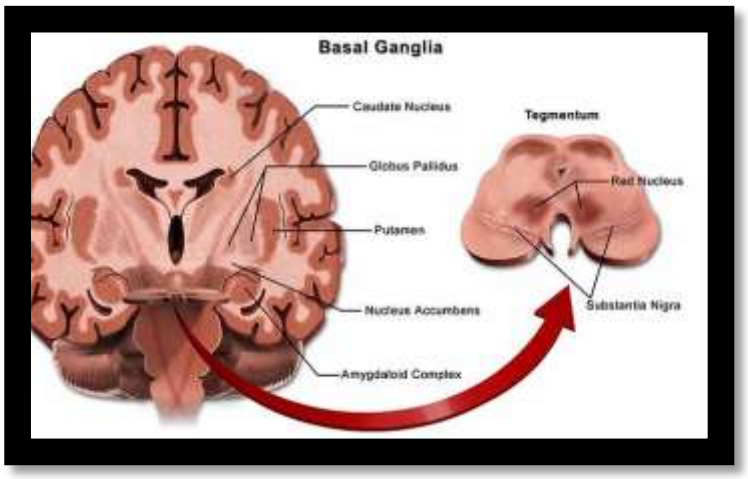

Fig3.Parkinson's disease

Parkinson's disease (PD) ranks as the second most prevalent neurodegenerative condition following Alzheimer's disease, especially affecting individuals aged 65 and above, where its prevalence ranges from 0.5% to 1%. Among those 80 years and older, this prevalence rises to 1% to 3%. As our population ages, PD's prevalence and incidence are projected to surge by over 30% by 2030. This anticipated increase will impose significant direct and indirect burdens on society and the economy at large.

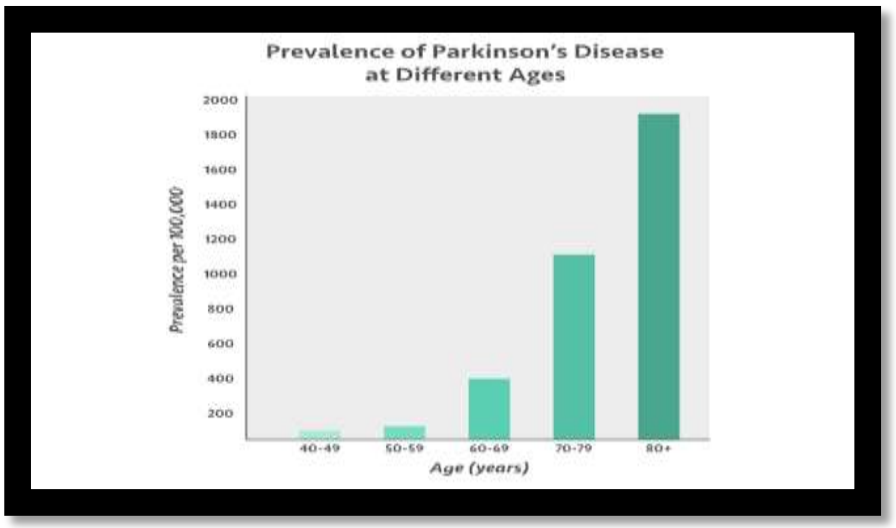

Fig4.Parkinson's disease

Parkinson's disease (PD) is marked by a significant loss of dopamine in the nigrostriatal pathway, yet its impact extends beyond these specific neurons, affecting various regions of the brain's network. This complexity contributes to the heterogeneous nature of PD, presenting challenges in developing a definitive diagnostic test. At present, diagnosing Parkinson's disease relies on observing clinical symptoms. The diagnosis typically necessitates the presence of at least two of the following: resting tremor, bradykinesia, rigidity, or postural instability. However, based on clinical criteria alone, only a probable diagnosis can be made. To confirm PD definitively, histopathological examination is required, specifically identifying Lewy bodies (LBs) or Lewy neurites containing α-synuclein.[27]

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Genetic Component Of Parkinson's Disease:

Indeed, Parkinson's disease is influenced by genetics. Researchers have identified specific genes like SNCA, responsible for producing alpha-synuclein protein, mutations in which can cause abnormal buildup in the brain, a key feature of Parkinson's. Similarly, genes like LRRK2 and PARK2 have also been linked to the disease's development. These genetic factors contribute to our understanding of Parkinson's pathology. (93).

Huntington's Disease:

Huntington's disease is a rare, hereditary neurological condition characterized by the degeneration of neurons in specific areas of the brain, notably the basal ganglia and frontal cortex. This deterioration results from a faulty gene, leading to a variety of symptoms such as involuntary movements, cognitive decline, and alterations in behavior and personality. Unfortunately, there is currently no cure for Huntington's disease.(6)Each child of an individual affected by Huntington's disease has a 50% chance of inheriting the defective gene responsible for the condition.[29].

The comprehensive description of Huntington's disease in "On Chorea" by George Huntington in 1872 brought widespread recognition to the condition. The genetic mutation associated with Huntington's disease involves an unstable expansion of CAG trinucleotide repeats in exon 1 of the Huntingtin (HTT) gene, located on human chromosome 4. In individuals with Huntington's disease, there is an abnormal increase in the number of CAG repeats, with normal individuals having between 6 and 35 repeats. Individuals with 36–39

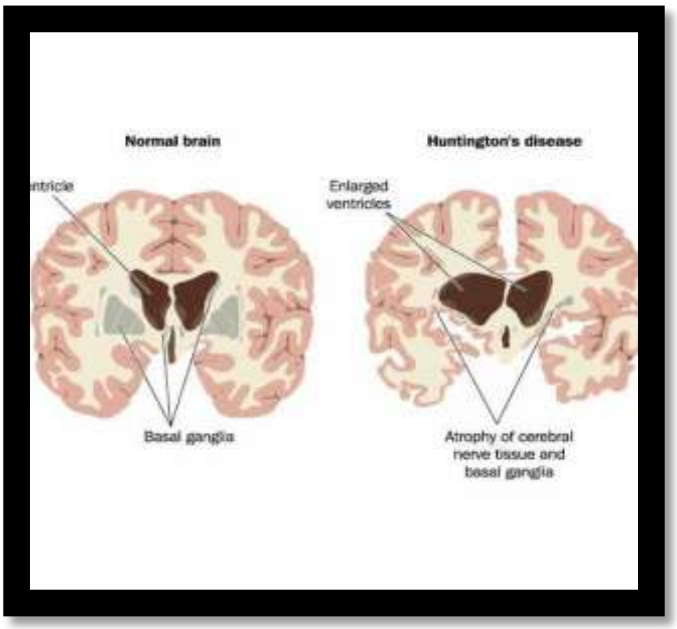

Fig5.Huntington's disease

repeats may exhibit variable and incomplete penetrance of the Huntington's disease phenotype, while repeats exceeding 39 are fully penetrant, leading to the development of the disease. Expansions of more than 57 repeats are typically linked to juvenile-onset Huntington's disease. Currently, there are no effective treatments for Huntington's disease, and individuals affected by it will eventually succumb to the condition 15–20 years after experiencing symptomatic onset.

The number of CAG repeats in Huntington's disease has been found to have an inverse correlation with the age of onset of symptoms. However, while there is variability in symptom presentation, there isn't a clear association with the number of CAG repeats. As a result, the underlying factors contributing to the variability in symptom subtypes remain unclear. Consequently, there is significant interest in researching the pathological changes occurring in the brains of individuals with Huntington's disease to better understand the heterogeneity of symptoms.

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Analysis of postmortem tissue from individuals with Huntington's disease reveals significant degeneration in both the striatum and the cerebral cortex, particularly affecting medium spiny neurons in the striatum and pyramidal neurons in the cortex. The severity of the disease is often assessed using a 5-point grading system based on the extent of striatal degeneration. Although the exact mechanism by which mutant Huntingtin causes neuron degeneration remains incompletely understood, it is believed that abnormal accumulation of Huntingtin fragments in neuronal nuclei and cytoplasm, along with the formation of protein aggregates, may initiate a cascade of pathological events ultimately leading to neuronal cell death.[30]

Genetic Component OfHuntington's Disease:

Huntington's disease arises from a mutation in the HTT gene, resulting in the production of an altered huntingtin protein. This abnormal protein then harms nerve cells within the brain.[95]

Gene Editing Technology:

Targeted nucleases have revolutionized genomic manipulation, granting researchers the capacity to modify nearly any genetic sequence. This breakthrough facilitates the creation of isogenic cell lines and animal models to investigate human diseases, while also opening promising avenues for human gene therapy.

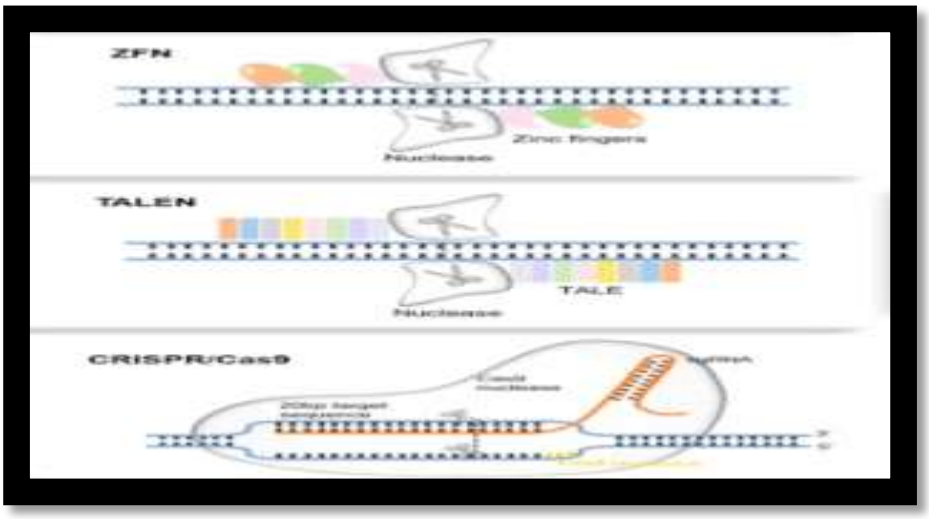

Fig6.Gene editing

Three fundamental technologies form the bedrock of this advancement:-

1) Clustered regularly interspaced short palindromic repeats (CRISPR)-CRISPR-associated protein 9 (Cas9)
2) Transcription activator-like effector nucleases (TALENs)
3) Zinc-finger nucleases (ZFNs)

The versatility of these genome-modifying enzymes stems from their efficient induction of targeted DNA double-strand breaks (DSBs). These breaks initiate cellular DNA repair pathways, enabling the introduction of specific genomic modifications. Typically, this process leads to gene knockout through random base insertions and/or deletions via nonhomologous end joining (NHEJ). Alternatively, with a donor template homologous to the targeted chromosomal site, gene integration or base correction via homology-directed repair (HDR) can occur. These enzymes also underpin artificial transcription factors, enabling the modulation of gene expression across diverse genomic contexts.[53]

1) Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-CRISPR-Associated Protein 9 (Cas9) :

Targeted nucleases are potent tools for precise genomic alterations in living cells, enabling control over functional genes with remarkable accuracy. The CRISPR-Cas9 system, inspired by bacterial immune mechanisms, has emerged as a pivotal tool in genetic engineering. Its versatility has led to substantial advancements, facilitating gene knock-ins, knock-outs, and precise point mutations.

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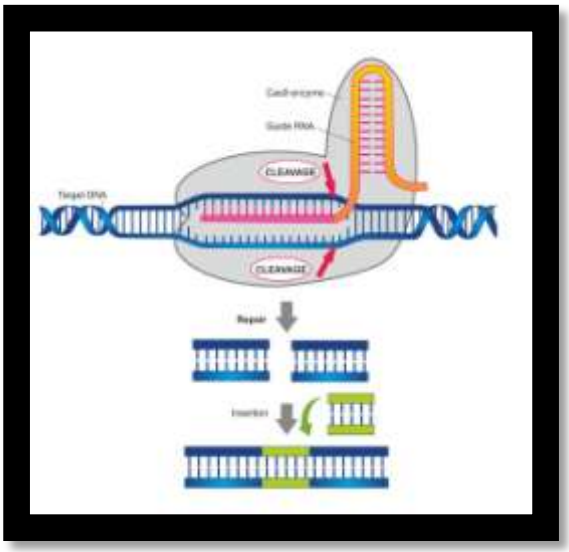

Fig 7.(CRISPR)-CRISPR-associated protein 9 (Cas9)

In 1987, Ishino et al. of Osaka University (Japan) made the groundbreaking discovery of CRISPR sequences within Escherichia coli DNA. However, at that time, the biological significance and potential applications of CRISPR remained unknown. (32,33).

Based on effector proteins, researchers have categorized the CRISPR system into two primary classes with six subtypes.[34,35]. The Type 2 CRISPR-Cas9 system stands out as the predominant choice in genome editing. It comprises three essential components:
(i)CRISPR RNA (cRNA)
(ii) The endonuclease Cas9
(iii) Transactivating crRNA (tracrRNA).(36).

The system consists of two critical components:
(i) The Cas9 protein, responsible for DNA cleavage
(ii) The guide RNA, which identifies the target DNA sequence for modification.

To utilize CRISPR-Cas9, researchers first pinpoint specific sequences within the target genome. A customized guide RNA is then designed to precisely match a particular sequence of nucleotides in the DNA. This guide RNA is linked to the DNA-cutting enzyme Cas9, forming a complex that is introduced into the target cells. Once inside the cell, Cas9 homes in on the target sequence and cleaves the DNA at that precise location. This precise cut enables scientists to modify or insert new sequences into the genome, effectively employing CRISPR-Cas9 as a tool for precise DNA editing.[37,38]With CRISPR-Cas9, researchers can precisely modify any genomic sequence by utilizing a short guide RNA to target specific regions for editing. This revolutionary technology allows for exact modifications to be made to the genome, offering unprecedented precision in genetic engineering.[39].In 2013, scientists achieved a significant milestone by successfully applying the CRISPR-Cas9 system to target the human genome for the first time. This breakthrough marked a pivotal moment in genetic research, opening up new possibilities for precise manipulation of the human genetic code.[40,41,42]CRISPR-Cas9 has become a versatile tool widely employed across different scientific disciplines. It is commonly utilized for gene editing in plants, animals, and human samples, making significant contributions to fields such as medical science, therapeutics, and agriculture. Its widespread use underscores its importance in advancing research and applications in various domains.[43,44,45,46]

2] Transcription activator-like effector nucleases(TALENs):-

Nature Methods recognized precise genome editing methods, such as the TALEN system, as the method of the year in 2011.[47]The development of this system traces back to the exploration of Xanthomonas bacteria, known for their pathogenic effects on crop plants like rice, pepper, and tomato, leading to substantial

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agricultural losses. Through extensive research, it was discovered that these bacteria release effector proteins, specifically transcription activator-like effectors (TALEs), into the cytoplasm of plant cells. These effectors manipulate cellular processes, rendering the plant more vulnerable to infection. Further analysis unveiled that these proteins possess the ability to bind to DNA and induce the expression of target genes by imitating eukaryotic transcription factors.[48]TALE proteins consist of a central segment tasked with DNA binding, a nuclear localization signal, and a domain that stimulates transcription of the target gene.[49].In 2007, the ability of these proteins to attach to DNA was initially documented.[28].In 2008, two separate teams of researchers successfully decoded the mechanism by which TALE proteins recognize and bind to target DNA sequences.[51,52].The DNA-binding domain comprises monomers, each interacting with a single nucleotide in the target sequence. These monomers are repetitive units of 34 amino acids, with specific variability at positions 12 and 13, known as repeat variable diresidues (RVDs), responsible for nucleotide recognition. This code exhibits degeneracy, allowing some RVDs to bind multiple nucleotides with varying efficiencies. Additionally, a thymidine nucleotide consistently precedes the 5'-end of the bound sequence, influencing binding efficiency.[53]The final tandem repeat responsible for binding the nucleotide at the 3'-end of the recognition site is termed a half-repeat, containing only 20 amino acid residues.

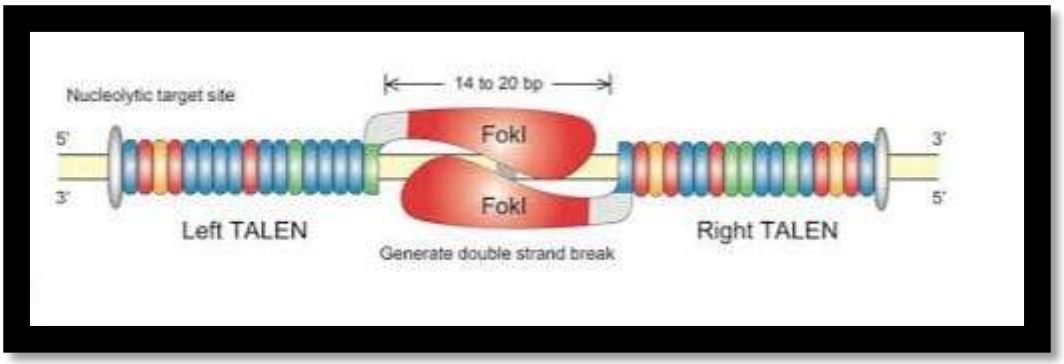

Fig8 .(TALENs)

Following the elucidation of the DNA recognition code by TALE proteins, which intrigued researchers worldwide due to its straightforward nature (one monomer corresponds to one nucleotide), investigations into constructing chimeric TALEN nucleases were initiated. To achieve this, the sequence encoding the DNA-binding domain of TALE was integrated into a plasmid vector previously employed for generating ZFNs.[54]This led to the development of genetic constructs expressing artificial chimeric nucleases containing both the DNA-binding domain and the catalytic domain of the restriction endonuclease FokI. This system enables the creation of artificial nucleases by combining monomers of the DNA-binding domain with different RVDs, allowing targeting of any nucleotide sequence. Commonly used RVD combinations include Asn and Ile (NI) for A, Asn and Gly (NG) for T, two Asn (NN) for G, and His and Asp (HD) for C. However, due to the NN RVD's ability to bind both G and A, efforts have been made to enhance specificity. Research has demonstrated that using NH or NK monomers for more precise guanine binding reduces the risk of off-target effects.[55,56] The initial amino acid residue in the RVD (H and N) was discovered to play a role in stabilizing the spatial conformation rather than directly participating in nucleotide binding. In contrast, the second amino acid residue interacts with the nucleotide, but the nature of this interaction varies: D and N form hydrogen bonds with nitrogenous bases, while I and G bind target nucleotides via van der Waals forces.[57]

An artificial DNA-binding domain is incorporated into a genetic construct containing a nuclear localization signal, half-repeat, N-terminal domain, and the FokI catalytic domain. TALENs operate in pairs, with their binding sites strategically selected to reside on opposing DNA strands and separated by a short spacer sequence (12–25 bp). Upon entering the nucleus, the artificial nucleases attach to their target sites, where the FokI domains positioned at the C-termini of the

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chimeric protein dimerize, inducing a double-strand break within the spacer sequence.

Theoretically, artificial TALEN nucleases can induce double-strand breaks in any genomic region with known recognition sites of the DNA-binding domains. The primary limitation in site selection is the requirement for a T nucleotide before the 5'-end of the target sequence. However, in many instances, site selection flexibility can be achieved by adjusting the length of the spacer sequence. Additionally, research has shown that the W232 residue in the N-terminal region of the DNA-binding domain interacts with the 5'-T, influencing the efficiency of TALEN binding to the target site.[36]Nonetheless, this constraint can be addressed by opting for mutant variants of the TALEN N-terminal domain, which possess the ability to bind to A, G, or C nucleotides.[59]

3]Zinc-finger nucleases (ZFNs):

Zinc finger nucleases (ZFNs) are gaining traction in both academic and industrial settings, utilized for a wide array of applications spanning from creating animal models to developing potential human therapies.Zinc finger nucleases (ZFNs) consist of a fusion of DNA-binding domains derived from zinc finger-containing transcription factors, coupled with the endonuclease domain of the bacterial FokI restriction enzyme. Each zinc finger domain targets a specific 3- to 4-bp DNA sequence, and when combined in tandem, they can recognize longer, unique nucleotide sequences within a cell's genome, typically ranging from 9 to 18 bp in length.

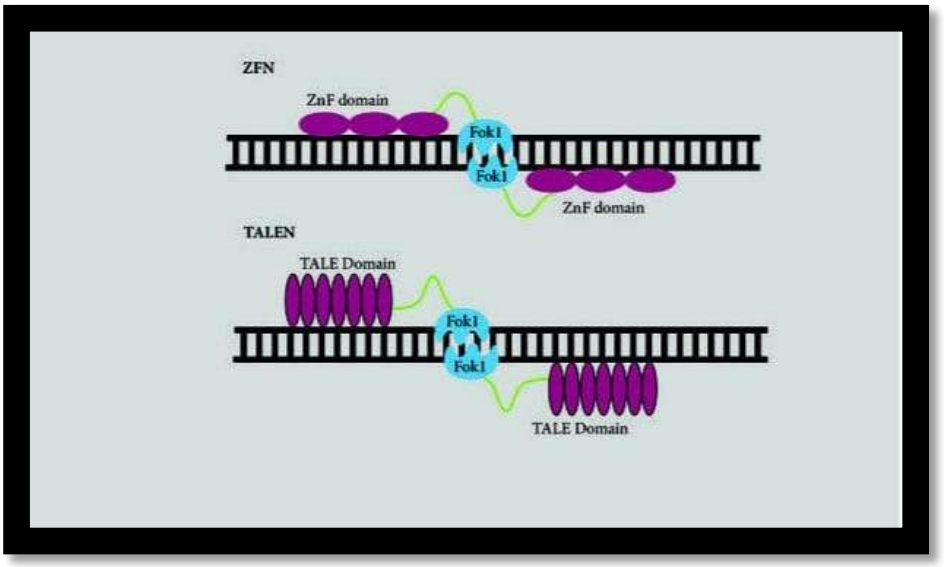

Fig9.(ZFNs)

For targeting a precise location in the genome, ZFNs are engineered in pairs that recognize two adjacent sequences, one on the forward strand and the other on the reverse strand, flanking the target site. When these ZFN pairs bind on either side of the target site, the FokI domains within each pair form dimers, initiating the cleavage of the DNA at the site, resulting in a double-strand break (DSB) with 5′ overhangs.[60].Cells employ two main mechanisms to repair double-strand breaks (DSBs) in DNA:

(a) nonhomologous end joining (NHEJ), which can take place at any stage of the cell cycle and may lead to error-prone repair, and (b) homology-directed repair (HDR), which predominantly occurs during late S phase or G2 phase when a sister chromatid is accessible to act as a repair template.The error-prone characteristic of NHEJ can be harnessed to induce frameshift mutations in a gene's coding sequence, potentially leading to gene knockout through two mechanisms: premature protein truncation and potential degradation of mRNA transcripts via nonsense-mediated decay, although this degradation isn't always highly efficient. On the other hand, HDR offers the ability to introduce specific mutations by incorporating a repair template containing the desired mutation flanked by homology arms. When a DSB occurs, HDR relies on a closely matching

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DNA sequence to facilitate repair. Mechanistically, HDR can mirror traditional homologous recombination, utilizing an exogenous double-stranded DNA vector as a repair template.[61].Using an exogenous single-stranded DNA oligonucleotide (ssODN) as a repair template is indeed a versatile approach. With homology arms as short as 20 base pairs, it allows for precise introduction of mutations into the genome without plagiarizing existing sequences. This method offers a powerful tool for genome editing.[62,63].In both scenarios, the efficiency can reach levels where antibiotic selection to identify accurately targeted clones becomes unnecessary. This streamlined process reduces the need for additional selection steps, enhancing the efficiency and simplicity of genetic engineering.[64,64].Antibiotic selection indeed eliminates the need for extra steps to remove the antibiotic resistance cassette from the genome, as required in traditional homologous recombination methods. Systems such as Cre-lox and Flp-FRT become unnecessary, streamlining the process of genetic modification while maintaining precision and efficiency.

Although genome editing with ZFNs offers numerous advantages, there are several potential drawbacks to consider. One significant challenge lies in the complexity of assembling zinc finger domains to effectively bind an extended stretch of nucleotides with high affinity. This process has not proven to be straightforward, hindering the widespread adoption of ZFNs for genetic engineering purposes.[44].The complexity of engineering ZFNs has indeed posed a challenge for nonspecialists. To address this issue, an academic consortium has developed an open-source library of zinc finger components and protocols. These resources enable researchers to perform screens aimed at identifying ZFNs that bind with high affinity to a desired sequence, democratizing access to this technology and facilitating its routine use in genetic engineering applications.[67.68].Despite their potential, obtaining optimized ZFNs can still be time-consuming for nonspecialists, sometimes taking months. Additionally, there are limitations in target site selection with open-source ZFN components, restricting binding sites to every 200 bps in random DNA sequences. Commercial sources offer higher design densities, allowing targeting every 50 bps. While this limitation might not be an issue for gene knockout purposes, it can pose challenges for precise site targeting, such as introducing specific mutations. Alternative platforms have emerged to engineer optimized ZFNs, offering varying degrees of speed, flexibility in site selection, and success rates.[69,70]

Concerns regarding the use of proteins designed to induce double-strand breaks (DSBs) in the genome include potential off-target effects, where DSBs occur not only at the desired site but also at unintended sites. In a study involving ZFNs for genome editing in human pluripotent stem cells, researchers identified ten potential off-target genomic sites due to high-sequence similarity to the intended target site. Among the 184 clones assessed, only a single off-target mutation was found, highlighting the importance of careful assessment and mitigation strategies to minimize off-target effects in genome editing applications.[71].Two follow-up studies investigating potential off-target sites for various ZFN pairs uncovered off-target events at multiple loci within a cultured human tumor cell line. These findings emphasize the need for thorough characterization and mitigation strategies to address off-target effects in ZFN-mediated genome editing approaches.[72,73].Hence, researchers should be aware of the potential for ZFNs intended for a specific task to cause unintended off-target events, albeit at a low frequency. One approach to mitigate off-target effects is to employ a pair of ZFNs with separate FokI domains that necessitate heterodimerization. This strategy can help enhance specificity and minimize off-target cleavage events during genome editing endeavors.[74,75].This approach prevents a single ZFN from binding to two adjacent off-target sites and inducing a double-strand break (DSB). Instead, for an off-target event to occur, both ZFNs in a pair must bind adjacently, facilitating the formation of the FokI dimer. Another effective strategy shown to decrease off-target events involves delivering purified ZFN proteins directly into cells. This method can enhance specificity by limiting the exposure of cells to ZFN activity and reducing the likelihood of off-target effects compared to using DNA-based delivery methods.[76].

Relevance To Neurology:

1- Relevance Of CRISPR/Cas9 In Alzheimer's Disease:

Alzheimer's disease is principally defined by two neuropathological hallmarks: the buildup of extracellular amyloid plaques containing amyloid β-protein (Aβ) and the presence of neurofibrillary tangles (NFTs) primarily composed of hyperphosphorylated Tau protein, a microtubule-

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associated protein. This interplay between Aβ plaques and Tau-related NFTs is widely regarded as the classical hallmark of Alzheimer's disease.(77,78).The traditional β-amyloid hypothesis has served as a foundation for exploring potential disease-modifying treatments aimed at inhibiting Aβ formation and facilitating the clearance of toxic proteins, including Aβ, from the brain.[79].Despite extensive efforts to develop disease-modifying therapies based on animal models of Alzheimer's disease, numerous setbacks and failures have been encountered in clinical trials.[77,81].As a result, in recent years, CRISPR/Cas9 technology has emerged as a popular tool in Alzheimer's disease research due to its efficiency, relatively short experimental duration, and low resource consumption. It is being widely employed for various purposes such as creating Alzheimer's disease models, screening for pathogenic genes, and developing targeted therapeutic approaches.[82]

While most Alzheimer's disease cases are sporadic, a small fraction, termed Familial Alzheimer's Disease (FAD), stem from dominant autosomal mutations in three genes: amyloid precursor protein (APP), presenilin-1 (PSEN1), and presenilin-2 (PSEN2).[83,84]Mutations in PSEN1 are the main cause of familial Alzheimer's disease and often lead to an earlier onset of symptoms compared to mutations in the other two genes. These mutations frequently result in increased production of Aβ42, which is more prone to aggregation than Aβ40.[85].The overproduction of Aβ42 is implicated in the formation of Aβ plaques, a characteristic feature of Alzheimer's disease. Recent research suggests that CRISPR/Cas9 technology holds promise in rectifying autosomal dominant mutations. Its success in correcting similar mutations underscores its potential for genetic modification in Alzheimer's disease and beyond.

2-Relevance OfCRISPR/Cas9 In Parkinson's Disease:

Utilizing the CRISPR-Cas9 system to delete the A53T-SNCA gene has shown substantial improvements in Parkinson's disease-related conditions. These include addressing the overproduction of α-synuclein, mitigating reactive microgliosis, reducing dopaminergic neurodegeneration, and ameliorating motor symptoms associated with Parkinson's.[86]

3-Relevance OfCRISPR/Cas9 In Huntington's Disease:

CRISPR/Cas9 represents a gene therapy approach utilized to suppress the expression of mutant HTT genes, offering potential in the treatment of Huntington's disease.[87]

4-Relevance OfTalens, Zinc Finger Nucleases:

ZFNs and TALENs rely on protein-DNA interactions for recognizing specific DNA sequences, whereas Cas proteins utilize RNA guidance for DNA specificity. To direct Cas proteins to particular genomic sites, researchers employ single-guide RNAs (sgRNAs) or dual-guide RNAs.(88,89,90,91,92).Cas proteins offer the advantage of rapid design and generation. Additionally, they enable the simultaneous use of multiple sgRNAs for editing multiple genes, facilitating the study of genetic interactions and the modeling of multigenic disorders. This capability eliminates the need for multiple cloning and complex protein engineering steps previously required with ZFNs and TALENs.

GENE EDITING IN NEURODEGENERATIVE DISEASE RESEARCH

Gene Editing In Neurodegenerative Disease

For the treatment and prevention of the various neurodegenerative disease gene therapy or gene editing technologies in drug development are in practice which is trending now.

Gene-editing tools or the processes are used such as zinc finger nucleases (ZFNs), mega-nucleases, and transcription activator-like effector nucleases (TALENs), and CRISPR (clustered regulatory interspaced short palindromic repeats)-Cas9 (CRISPR-associated), which can edit, exchange , transform, modify defective areas on the genome and obtain a great idea of new fascination as a means of treating certain neurodegenerative diseases.[100]

Applications of gene editing in elucidating the genetic basis of neurodegenerative diseases, including studies on disease modeling and functional genomics:

Most wide and interested application of gene editing is prevention and treatment of the prevalent neurodegenerative disorders include Alzheimer's disease(AD), Parkinson's disease (PD), Huntington's disease (HD), Frontotemporal dementia(FTD), and Amyotrophic lateral sclerosis (ALS). [101]

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1- **In Alzheimer's Disease**

Production of the amyloid beta protein and tau protein are enhanced that causes the Alzheimer. CRISPR technique used to construct the RNAs (gRNAs) that targeted to destroy to the specific genomes .CRISPR related protein cas9 specially enters and breaks the exact location of the DNA. The DNA pairs break through two ways either by the non homologous end joining (NHEJ) or by the homology directed repair(HDR).[112]

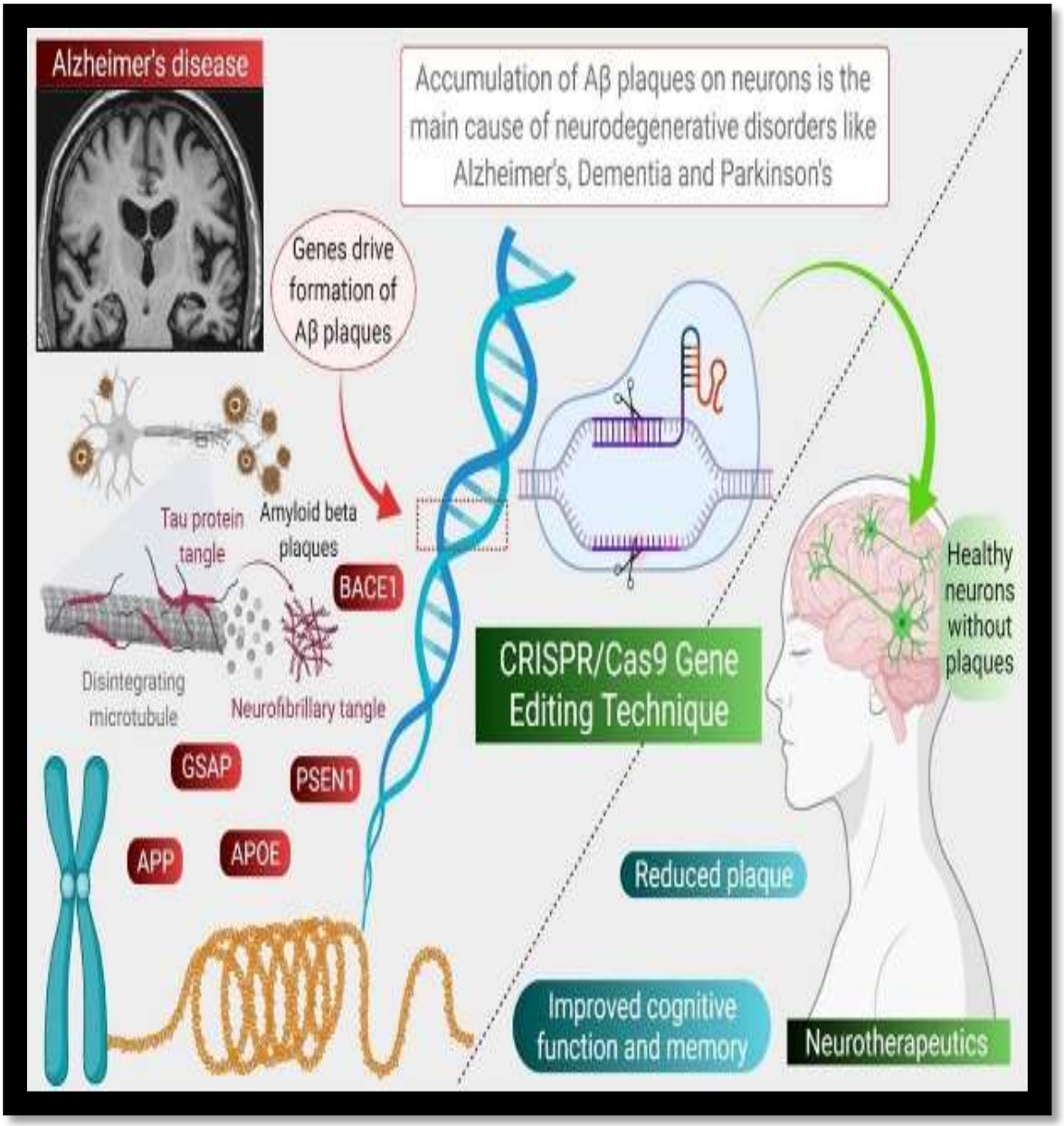

Fig10.Alzheimer disease

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Organism	Cell type	In vivo/in vitro	Application	Inference and conclusion	Delivery methods	Strategy Used	Source
Human	iPSC cells	In vitro	2A-GFP reporter was inserted before the stop codon of the MYF5 gene	Achieved 55% efficiency in the insertion of the GFP gene. Used Cas9-nickase, and suggested less off-target effect	Electroporation	HDR	Wu et al., 2016[102]
Human	HEK293	In vitro	Tested Knock-in rates between 3% and 8%	Cas9D10A mutant, functions same as a nickase in vitro, and yielded similar HR but lower nonhomologous end joining (NHEJ) rates. Deactivating one of the Cas9 nuclease domains increased the ratio of HR to NHEJ and reduced toxicity.	Lentivirus	HDR	Mali et al., 2013[103]
Mouse	Bone marrow stem cells	In vitro	Edited human Huntingtin transgene using 2 different gRNA, separately in the cells.	Every single cell expressed CRISPR-Cas9. gRNA1 and gRNA2 reduced mutant huntingtin protein up to 79% and 57%, respectively. gRNA location (Kozak sequence) play an important role in efficient gene-silencing. NHEJ resulted in large number of nucleotide	Lentivirus	NHE	Kolli et al., 2017[105]

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				deletions ranging from 72 to 1, in randomly sequenced cells			
rat	hippocampal slice cultures	In vitro	Edited GluN1 subunit of NMDA receptor and GluA2 subunit of the AMPA recepto	More than 90% of the mutations led to out-of-frame insertions or deletions resulting in elimination of NMDA receptor and GluA2 function.	Lentivirus	NHE	Incontro et al., 2014[105]
Mouse and rat	pyramidal neurons (mitotic precursor cells)	Mouse = in vivo Rat= In vitro	knock-out Grin1, the gene encoding NMDA receptor subunit protein GluN1	Two different gRNAs, one located on the plus strand and the other gRNA located on the minus strand were tested, separately. gRNA on the plus strand have shown greater efficiency than the gRNA located on the minus strand	Mouse: in-utero electroporation of the plasmid construction on Rat: Biolistic transfection	NHE	Straub et al., 2014[106]
Mouse	Neurons	In vitro	In vitro and in vivo comparison of Mecp2 editing Tested multiplexing strategy in vivo for editing Dnmt1, Dnmt3b, Dnmt3a	In in vitro, Cas9 and CRISPRMecp2 vectors in primary mouse cortical neurons colocalized ine75% cells. In vivo delivery of the same vectors resulted in indel mutations ine 68% after two weeks of viral delivery. In vivo multiplied-CRISPR and Cas9 vector resulted in indel mutations for	AAV 1 & 2	NHE	Swiech et al., 2015[107]

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				two genes ine62% of the cells, whereas for all the three genes, indel mutation was found in onlye35% cells.			
Mouse	Neural progenitor cell, Neurons	In vitro	Explored the efficiency of short-lived Cas9 ribonucleoprotein (RNP) complex	RNP complex can overcome the problem with the cas9 permanent integration in the target cells. Variants of Cas9 with multiple SV40 nuclear localization sequences has shown a tenfold increase in the efficiency of editing in vivo	In vitro e nucleofection In vivo e injection of RNP into the brain	NHE	Staahl et al., 2017[108]
Human cells & transplanted into mouse	Human myoblasts	In vitro	Multiplexing strategy to perform exonskipping. Restoration in the expression of dystrophin gene in cells (DMD	Tagging T2A skipping peptide to the Cas9 vector resulted in generation of a single large deletion (exon 45e55) that can correct up to 62% of DMD mutations	Plasmid electroporation N	NHE	Ousterout et al., 2015[109]
Human	iPSCs	In vitro	Generated two isogenic disease-free iPSCs cell lines by knocking-out FUSþ/G1566A, and SOD1þ/A272Cgenes that cause ALS	The gene targeting efficiency at FUS gene and SOD1 gene was about 1% and 20%, respectively. However, the reason for differential efficiency in HDR with these two gees was not explained	Electroporation	HDR	Wang et al., 2017[111]

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Table no 1.Examples of recent applications of CRISPR-Cas9 for gene correction and gene editing

2- In Parkinson's Disease :

Gene which involve in the development and occurring of the Parkinson's disease are SNCA (encoding alpha synuclein), and LRRK2,PARKIN.

In a sagittal slice of the human brain in substantianigra (SN), adeno-associated virus (AAV) carrying genes encoding by the induction of the AAV for glutamic acid decarboxylase (GAD), a gamma-aminobutyric acid (GABA)-producing enzyme, were delivered to the subthalamic nucleus (STN), to neuronal cells and that ostensibly inhibiting hyperactive putaminal (PUT) neurons of the striatum Nigra.[112]

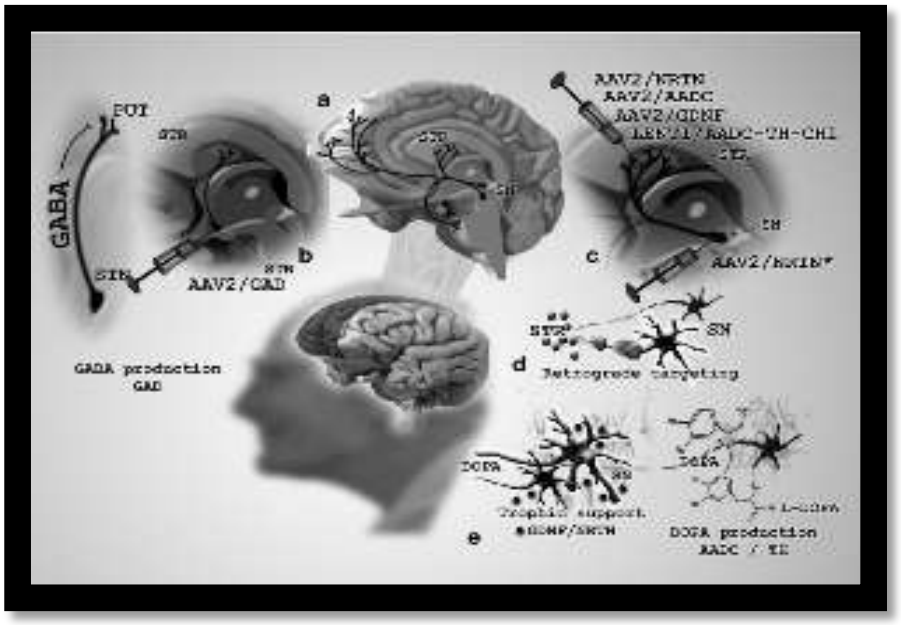

Fig 11.Parkinson's disease gene therapy

GENE THERAPY APPROACHES FOR NEURODEGENERATIVE DISEASES

Overview Of Gene Therapy Strategies For Neurodegenerative Disease Including Gene Editing Based Technologies :

Due to technical limitations, our initial understanding of neurodegenerative disease (NDD) was initially restricted to the pathological manifestations of abnormal protein aggregation, such as Aβ protein in Alzheimer's disease (AD), huntingtin (HTT) protein in Huntington's disease, α-synuclein in Parkinson's disease, and neurofilament in amyotrophic lateral sclerosis. Over the last few decades, gene therapy for neurodegenerative disease has made straight forward progress . However, treatment for this needs tageting abnormal protien level which on set back in clinical trials. At the end of 20th century the increase in knowledge has led down the targeting multiple genetics intravention root cause of neurodegenerative disease[113]

Gene silencing to handle gain of function mutations and Gene overexpression to handle loss of function mutation the advent of gene editing technique has facilitated ability of researcher to specify and target selected genome and alter the eukaryotic genome It involves breakdown of Double stranded DNA in specific genome[114] .Gene therapy holds promise for treating neurodegenerative diseases, which are characterized by the progressive degeneration of neurons in the brain or spinal cord. Several gene therapy strategies, including gene editing-based approaches, have been explored for these conditions. Theses are discussed below [115,116,117].

1-**GeneReplacementTherapy**: in this therapy replacement by functional or required gene for defective or missing gene which cause neurodegenerative disease . For e.g : in spinal mascular atropy (SMA) gene repalcement therapy has done by develping survival motor neuron which is deficient in SMA patient

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2- **GeneSilencingTherapy**.gene silencing technique involve reducing or suppressing mutant disease which cause neurodegenerative disease like huntington's disease etc suppresion of mutant gene done by technique like RNA interference(RNAi) & antisense oligo nucleotides' etc

3-**GeneEditing-basedApproaches**: the another very effective approach for treating neurodegenerative disease is to edit mutant gene of disease by using various techniques like CRISPR-Cas9

4-**NeuroprotectiveGeneTherapy**: Some gene therapy approaches focus on enhancing neuronal survival and function through the delivery of neuroprotective genes. For instance, growth factors like brain-derived neurotrophic factor (BDNF) or glial cell line-derived neurotrophic factor (GDNF) have been investigated for their potential to promote neuronal survival and regeneration in neurodegenerative diseases like Parkinson's disease and ALS.

Clinical application of gene editing in treating neurode generative disease:

CRISPR application in Alzheimer's disease: Neurodegenerative disease mainly Alzheimer's disease mainly caused by accumulation of extracellular amyloid plaques and neurofibrillatoryprotiens (hyperphoaphorylated Tau protien)[118]. so the classical β-amyloid hypothesis provided a framework for the development of potential disease-modifying therapies that would prevent Aβ formation and promote the elimination of toxic proteins (such as Aβ) from the brain [119]·although due to its disease modifying defective gene its is gain more popularity due to short experimental duration and low consumption [120]

CRISPR application in Huntington's diease:

It is the progressive neurodegenerative disease caused by mutation in single CAG(cytosine- adenine- guanine)in which CAG sequence repeated due to which formation of an elongated polyglutamine strand in the N-terminal region of the huntingtin protein due to which aggregation of protien in brain leads to loss of various cellular funtions causes chorea,dystonia and incoordination etc[121,122].further studies are conducted showing that CRISPR-Cas9 to disrupt the mutant HTT gene in mice , that carry exon 1 of the human HTT gene with around 115-150 CAG repeats – resulted in a 2-fold reduction in the formation of neurotoxic inclusions[123]

CRISPR Application In Parkinson's Disease:

Parkinson's disease (PD) is the second most prevalent neurological disorder in humans, following Alzheimer's disease.PD is characterised by progressive loss of dopaminergic neuron in substantia nigra cause tremor,rigidity& bradykinesia [124].missense mutation called Ala53Thr (A53T) in SNCA is recognized as one of the most prominent risk factors for early-onset PD[125].In 2022 studied conducted which shows that CRISPR-Cas 9 delete the A53 and SNCA gene related to parkinson's disease and promote production of dopamine to recover symptoms of it[124]

CRISPR Application In Amyotrophic Lateral Sclerosis(ALS):

Amyotrophic lateral sclerosis (ALS), which is also referred to as "Lou Gehrig disease," is a rapidly progressive neurodegenerative condition that impacts the human motor system. This condition is caused by the degeneration of motor neurons in the central nervous system .The most frequent genes associated with it C9orf72, SOD1, TARDBP, and FUS[121].More ever hex nucleotide repeat expansion(HRE) is noncoding of C9orf72 cause both sporadic (5-6%) and inherited (40%)ALS.In a study adenovirus vector used to deliver CRISPR/cas 9 which remove C9orf72 from located genomes[128].

Another study by usiing CRISPR/Cas 9 conducted to correct SODI E100G mutation[129] .

While CRISPR-Cas9 has gained significant attention in the field of gene editing, other technologies such as TALENs (Transcription Activator-Like Effector Nucleases) and ZFNs (Zinc Finger Nucleases) have also been explored for their potential applications in treating neurodegenerative diseases

TALENs (Transcription Activator-Like Effector Nucleases):TALENs are catalytic domain derived from bacterial proteins termed transcription activator-like effectors (TALEs) has new shed in genome editing. This correct the mutant gene .example(molther) TALENs were used to correct SOD1 gene associated with ALS [130]

ZFNs (Zinc Finger Nucleases):are DNA binding proteins that induces breaking of double strands. It genetically repair parkinson's associated with SNCA gene in patient derived ips cells[131]

A study (natbiotechnology) published shows that ZFN's edit mutant HTTgene in huntington's disease in patient derived fibrinoblast. In PD the

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missense mutation of SNCA and LRRK2 genes can be corrected by ZFNs in vitro[131]
ZFNs are engineered DNA-binding proteins that can induce double-strand breaks at specific genomic loci. They have been investigated for their therapeutic potential in correcting disease-causing mutations in neurodegenerative disorders.

CLINICALTRIALS:

Ongoing clinical trials utilizing CRISPR-Cas9 for treating diseases like sickle cell anemia and beta-thalassemia. These trials primarily focus on ex vivo editing of patient cells to correct genetic mutations before reinfusion. Additionally, there are trials exploring CRISPR-Cas9 for cancer immunotherapy and HIV/AIDS treatment.
However there is no ongoing clinical trials for ZFN and TALEN.
Most clinical trials involving gene editing technologies have focused on CRISPR-Cas9 due to its versatility and ease of use[132]

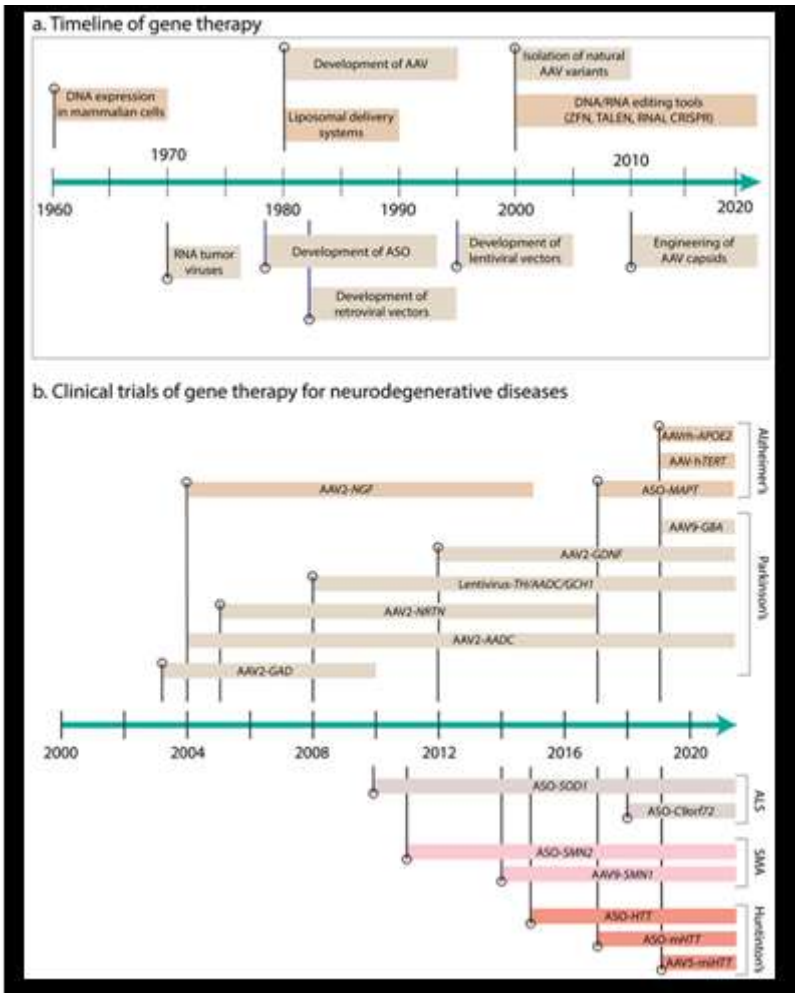

Fig 12.Gene therapy

Potential Benefits And Limitations Of Gene Editing Techniques Including Safety And Ethical Consideration:

Gene editing techniques hold significant potential for treating neurodegenerative diseases, but they also come with certain benefits, limitations, and ethical considerations

PotentialBenefits:

1-**Precision and effectivity** : Gene editing techniques offers precision for tageting specific gene in neurodegenerative disease and are more effective [133]

2-**DiseaseModification** : It potentially it target defective and mutant gene and and modify it to

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produce correct gene they modify gene so that it can well manage [134].

3-**PersonalizedMedicine and versatility** : Gene editing technique can be acts as a personalized medicine and specially CRISPR -cas 9 show remarkable versatility[135]

Limitations:

1-**DeliveryChallenges**:As gene editing techniques has delivery changes as it limits BBB due to which limited entry in therapeutic agent [136]

2-**Off-TargetEffects**: owing to targeting a mutant gene unfortunately it modify unintended genomic sequnces leading to adverse effect[136]

4-**ImmuneResponse**: It may influence immune response which produces inflammation etc[137]

Ethicalconsideration:editing gene raises untoward consequences for future generation so for proper use of it must understand that:

1- Whole knowledge like health benefits , treatment duration health consequence's risk, and these must be be taken on written consent from patient.
2- As gene editing is expensive technique one must understand to patient its issue of affordability, availability etc

SafetyConcerns: as it trigger the immune response of patient it may produce inflammation or therapy rejection .So to avoid this and ensuring safety and efficacy for the intended use GUIDE-seq and Digenome-seq has been developed [137]

Future Perspective:

Emerging trends and advancements in gene editing technologies for neurodegenerative diseases, including novel therapeutic approaches and technological innovations:

As of now there is no effective cure for this disease.Adeno associated virus extensively used for delivering gene therapies [141].there is intense focus on technologies that can reduce expression of spcific gene or alter a specific gene

The most challenging intravention is CRISPR-Cas 9 .but due to its short coming and additional limitations such as risk of off target there are some currently advances AAVs undergoing clinical trials for neurodegerative disease rAAVs -GDNE in single dose AVV vector based treatment by delivering GDNE which alleviate symptoms of PD and protect dopamine producing neurons in putamen and surrounding brain [142].

AMt130 is a single dose rAAV 5 vector produced by uniQue biopharma to modify disease couse of hutington'sdiseaseas trials shows that AMT130 reduces HTT protein and show improvement of symptoms of Huntington's disease [143] the selection of appropriate route if administration is imp factor to determine efficacy and safety of gene therapies the two main route of delivery to reach brain is intranasally or and intravenously or surgical intravention . Among this routes intracisternally, intracereboroventriculary , intrathecally has different level of efficacy and adverse effect [144,145].non invasive techniques are succesfully treat neurodegenerative disease whereas on surgical intervention's clinical trial's are going on because of higher risk and deliver vAAVs to direct to brain without other organ exposure [146]. recent clinical study demonstrated that HD patients showed dose-dependent reductions in concentrations of mutant huntingtin (HTT) after intrathecal injection of an antisense oligonucleotide (IONIS-HTTRx), suggesting this agent maybe a promising therapeutic[147]. Notably, syntheticshRNAs or microRNA produced from a single injection of AAV can produce a more lasting gene silencing than artificial siRNAs, these provided superior gene therapy approaches for neurodegenerative disorders. But owing to recent advancement one can make consent from pateint regarding: Risk,benefits ,ucercertainty regarding therapy etc

-Challenges and opportunities in developing effective gene editing therapies for neurodegenerative disorders, including interdisciplinary collaborations and funding priorities. Developing gene editing technique's for neurodegenrative disease produce a complexities in challenges and opportunities

Challenges:

As there is a no of genes in genome but it is become a challenge to target a specify gene owing to this if we are administering drug there is a challenge for crossing blood brain barreier for effective managemnt and by tageting specific gene of ftargettingeffecf ethical consideration immune rrsponse and host compatibility are taken into consideration. [147]

1.Delivery Methods:

One of the most basic primary challenges is delivering gene editing tools efficiently to the targeted cells within the central nervous system (CNS). The blood-brain barrier found as frightening and powerfull obstacle that limiting the delivery of therapeutic agents to the brain.[135]

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2. Off-Target Effects and potential effects:

Assurance of precision in gene editing is crucial to avoid undesirable mutations or off-target effects, which can expectedly and potentially worsen neurodegenerative conditions or lead new complications.[136]

3. Immune Response:

The immune response to gene editing therapies, specially in the CNS or neurodegenerative disease, is fully not understandable. Immune reactions can limit the efficacy of the treatment or cause adverse effects which can be worsen condition. Example: Editing of gene such as Cas proteins or viral vectors is crucial for long therapeutic efficacy.[137]

4. Ethical Considerations:

Negotiating the ethical considerations surrounding germline editing and potential undesirable consequences is necesasary for responsible translation to clinical applications.[138]

5.Long-term Effects and Durability:

Neurodegenerative diseases are chronic disease condition that has long duration for therapeutic interventions. To ensure the durability and stability of gene editing therapies in neurodegenerative disease over time is difficult challenge.[149]

Opportunities:

Gene editing technologies offer the potential for precision medicine approaches tailored to individual patients' genetic profiles and disease characteristics, paving the way for personalized therapeutic interventions. Ongoing advancements in gene editing tools, delivery systems, and genome editing techniques, coupled with innovations in CRISPR-Cas9 specificity and efficiency, offer new avenues for overcoming technical hurdles and enhancing the safety and efficacy of gene editing therapies.Involving patient advocacy groups and engaging with affected communities can provide valuable insights into patients' perspectives, needs, and priorities, ensuring that gene editing therapies are developed with patient-centric approaches and address unmet medical needs [148].

Intradisciplinary Collaboration: Intradisciplinary Collaboration Play A Crucial Role In Neurodegerative Disease

1-In Genetics and Genomics: Genetic research plays a crucial role in identifying disease-causing mutations and potential therapeutic targets for neurodegenerative disorders. So disciplinary Collaborations between geneticists and neuroscientists help to point specific genes in these diseases and guide the development of gene editing strategies.in genomincs studies have identified genetic mutations in Huntington's disease (HD and amyotrophic lateral sclerosis (ALS) laying the groundwork for targeted gene editing approaches [149,150]

2-neuroscientist and molecular biologist: as neuroscientist work on anatomy,physionlogy,and pathology of progression of disease by collaboration with molecular biologist helps in genetic discoveries and gene editing interventions (151)

Potential Impact Of Gene Editing Technologies On The Future Of Neurology And Personalized Medicine:

Gene editing technologies, particularly CRISPR-Cas9,(Versatile editing tech.) hold significant promise the field of neurology and personalized medicine. These advancements have the potential to address the underlying genetic causes of neurodegenerative diseases and pave the way for personalized therapeutic interventions tailored to individual patients.

•Potential impact on target gene mutation:as above discussed that the techniques allow Researchers to find out the disease causing mutation and correction gene in potentially gives impact on neurology such as defective gene in A53 and SNCA gene deletion of It increase production of dopamine in brain in case of parkinson's disease

•Potential impact on personalized medicine: As gene editing techniques are potential for personalized medicine as it target disease causing mutation ,modification of disease pathway and development of disease specific target therapies [149,150]

III. CONCLUSION

In conclusion , gene editing technologies, such as CRISPR/Cas9, Zinc Finger Nucleases (ZFNs), and Transcription Activator-Like Effector Nucleases (TALENs), hold promise for developing therapies for neurodegenerative diseases by facilitating precise modifications of genes. These technologies offer potential from precision medicine to genetic cures for conditions like Alzheimer's, Parkinson's, and Huntington's diseases, address the underlying genetic defects,

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and advance treatments for these complex conditions.

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