Gene Editing for Neurodegenerative Diseases-A review

By

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A thesis submitted to the Department of Pharmacy in partial fulfillment of the requirements for the degree of
Bachelor of Pharmacy (Hons.)

The Department of Pharmacy
Brac University
October, 2021

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Declaration

It is hereby declared that

1. The thesis submitted is my own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
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Approval

The project titled “Gene editing for neurodegenerative disease– A Review” submitted by Anika Islam Sharna (17346055) of Summer, 2017 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy (Hons.) on October, 2021.

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Ethics Statement

This study does not involve any human or animal trial.

Abstract

Gene editing is quickly gaining traction as a promising treatment option for a variety of neurodegenerative diseases, including Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), Amyotrophic Lateral Sclerosis (ALS), and Frontotemporal Dementia (FD). Clinical investigations have so far failed to establish that there is any change in result other than the placebo effect. Efforts to discuss on some key areas: vectors and the identification of novel vector serotypes, the identification of new therapeutic targets, current techniques and some clinical uses of gene editing. To increase efficacy, these advancements are being evaluated separately and in combination. Certain advancements might pave the way for gene editing to be successful in treating these diseases.

Keywords: Neurodegenerative disease (ND), Alzheimer’s disease (AD), Parkinson’s disease (PD), Amyotrophic Lateral Sclerosis (ALS), Huntington’s disease (HD), Frontotemporal Dementia (FD).

Dedication

Dedicated to my parents

Acknowledgement

I would like to begin by thanking the Almighty Allah, our creator, the source of our life, strength, knowledge, wisdom, blessings and mercy. All praises to the Almighty Allah for blessing me with immense patience and strength to complete this project. This project would not have been completed without the support of the people who are recognized here.

After that I would like to express my deepest gratitude to my supervisor, Namara Mariam Chowdhury, Lecturer, Department of Pharmacy, Brac University for her expert guidance and suggestions and for giving me all kinds of support, encouragement and motivation at any moment.

Last but not least, I would like to thank my family for being with me and supporting me to work harder in every phase of life. Without their prayer and unconditional love, I would not have come this far.

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List of Abbreviation

ND	Neurodegenerative Disease
PD	Parkinson's Disease
HD	Huntington's Disease
AD	Alzheimer's Disease
ALS	Amyotrophic Lateral Sclerosis
FD	Frontotemporal Dementia
CNS	Central Nervous System
ApoE	Apoliprotein E
TALEN	Transcription-like effector nucleases
ZFN	Zinc finger nucleases
NHEJ	Non-homologous end joining
FDA	Food and Drug Administration
CRISPR	Clustered regularly interspaced short palindromic repeats
MoMLV	Moloney Murine Leukemia Virus
AAV	Adeno- associated virus
HDR	Homology directed repair
TFIIIA	Transcriptional factor III A
RVD	Repeat variable residue
UPR	Unfolded protein response
RGCs	Retinal ganglion cells
ER	Endoplasmic reticulum
TEEB	Transcription factor EB
DBDs	DNA binding domains
DSB	Double strand break

TMZ	Temozolomide
MGMT	Methyl guanine methyl transferase
iPSCs	Individualized pluripotent stem cells
LDL	Low circulating lipoprotein

Chapter 1 Introduction

1.1 Background of neurodegenerative disease

A disease in which the central nervous system's cells stop operating or die. Neurodegenerative diseases are frequently progressive and incurable. They could be caused by a tumor or a stroke, or they could be genetic. Alzheimer's disease is an example of a neurodegenerative disorder (Fan et al., 2018).Neurodegenerative diseases are a diverse collection of multi-system illnesses that affect the central nervous system and eventually result in neurodegeneration (Chen et al., 2020).The main pathological feature of acute and chronic neurodegenerative conditions, including Alzheimer Parkinson's disease, neuro tropical viral infections, strokes, etc., is neurodegeneration and the slow and progressive dysfunction and loss of neurons and axons within the center despite various triggering events chronic immune activation is a common feature, especially of microglia, the central nervous system's residential macrophages (Choi et al., 2015). In addition to the pathogenic role of immune responses, there is proof that immune responses are also critical to neuro regenerational nervous system (Costa et al., 2013).

The most feared diseases are neurodegenerative diseases (NDs), including, in any case, Alzheimer's, Huntington's, and Parkinson's, as there are no particular indicative tools or specific remedies for these weakening conditions. This review presents the clinical indexes of 3 different NDs and the use of genetic engineering in these weakening diseases (Mesuraca et al.,2018).

Neurodegeneration is the overall term for medical conditions involving gradual neuronal network failure and eventually neuronal death in the motor, sensory and cognitive functions. Neurodegenerative (ND) disorders, including at least Parkinson's (PD) disorder and Huntington's

(HD) disorder and so on, complex and multifactorial conditions are endangering Human health and no diagnostic tests or therapies are effective (Cappella et al., 2019). There are several mechanisms underlying NDs of pathophysiological pathophysiology, including an over excessive accumulation of structurally atypical aggregated proteins; and/or lysosomal pathways of

autophagy; apoptosis and Autophagy; transporters of glutamate; free radicals of calcium, Mitochondria and so on (Chen et al., 2016). These multi-faceted systems suggest that the NDs come from a complex interaction between many Genetic factors, each acting individually or symphonically leading to clinical characteristics. A quick, cheap and costly RNAi Method for knocking down a specific gene highly successful. Cell lines primary cultures, or animal models have been used for the same technique (Blanc et al., 2020). Gold standard RNAi was used for gene silence and gene function studies. But several inconveniences include the following in respect of this technique:

(1) The effects of the mutant selective RNAi targeting a single nucleotide may vary, be incomplete or temporary in different nucleus, in different ways

(2) This technique is difficult to transfer several genes in cells/animals in vitro or genes into an adult animal in vivo;

(3) The RNAi cannot produce stable gene knockouts or epigenetic modifications specific to the site; and

(4) The off-target effects of this technique may be unpredictable. These defects may restrict clinical practice use of RNAi. HR in embryo stem cells of the mouse is a common and popular genetic engineering method modeling of human diseases by modified animals (Dever et al., 2019).

However, include the following disadvantages of this technique:

(1) Time consuming HR and labor intensive;

(2) Low efficiency HR and

(3) potential mutagenic effects not unwanted HR (Fan et al.,2018).

1.2 Types of neurodegenerative disease

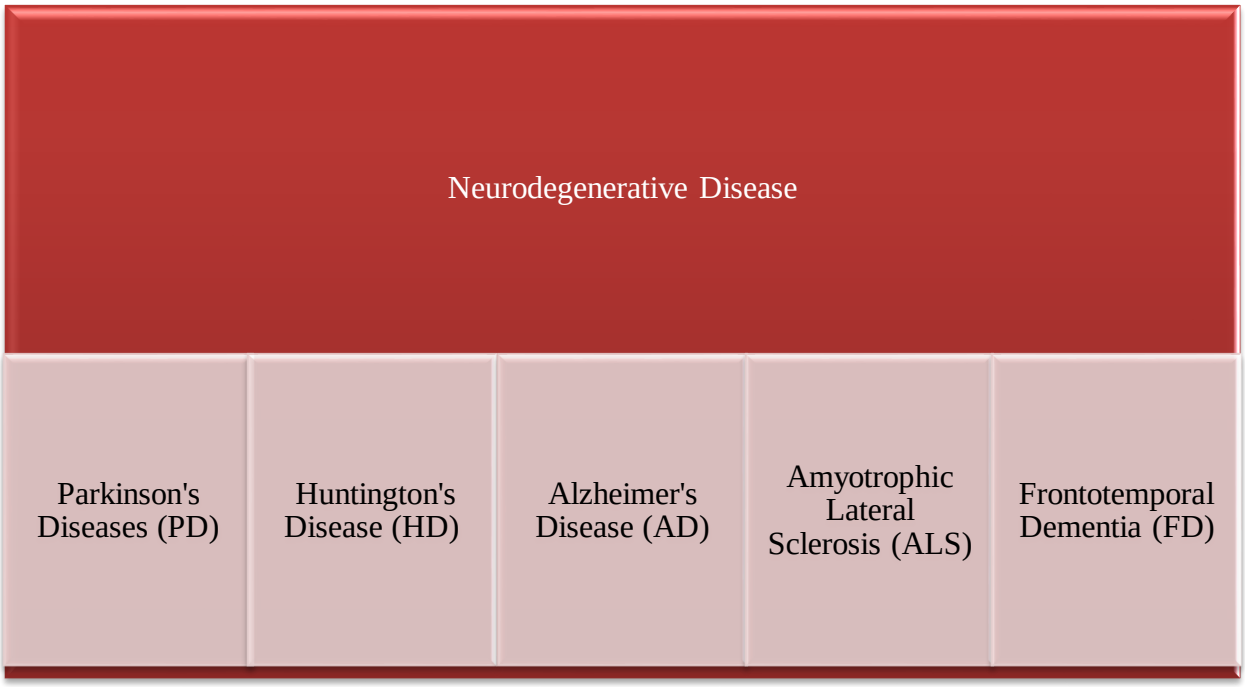


Table 01: Types of the neurodegenerative disease

There are no accurate diagnostic methods or clear treatments for these severe diseases, neurodegenerative disorders (NDs), including Alzheimer's, Huntington's, and Parkinson's diseases, have become the most feared ailments. The rising prevalence of NDs, as well as their significant impact on social–economic and medical care, has prompted the government to establish regulations to mitigate the burden (Barman et al., 2020).

Parkinson's diseases (PD) is a disorder of degenerative age, progressiveness, disability and motor neuron. Prevalence estimated Prevalence Over 1% of the 60-year-old PD persons 4% and 4% of Over 8018 those ages. PD prevalence is 100 to 250/100, 00019, and 118.7/Japan in North America and Europe. Taiwan 84, 8/100, 00021 and 100, 00020. A growing trend Asia reported annual PD prevalence rates and Europe (Artyukhova et al., 2019).

Pathophysiology: Nerve cell in the brain produces dopamine in substantia nigra decreases in a number. In progressive stage 80% of dopaminergic cells are damage.

MAO-B also continuously breaks down, the small amount of dopamine that left. Since the amount of acetylcholine remains normal in basal ganglia.

Imbalance in the number of Acetylcholine and dopamine results producing uncoordinated movement and tremor.

Huntington's disease (HD) is a infrequent and rapidly increasing neurodegenerative disease dominating automotive disease. HD prevails in Europe, the United States and Canada, at 0.1 to 0.8/10000025–27. 0.3 to 0.3 to the figures 0.69/100, 00028, 29 and Japan 0.42/100, 00031. 0.42/2000. The mean age when symptoms begin is 30 to Fifty-two (Taran et al., 2020). The development of HD is the main feature of Korea, dystonia, bradykinesia, incoordination of motors Comport ability or personality psychiatric elements Changes, poor attention, decreased cognition, annoyance and dementia. The development of the disease leads to total dependence in everyday life and requires full time care and death after the onset of the disease at around 15-20 yr (Ratican et al.,2018).

Pathophysiology: Degeneration of the inhibitory medium spiny neurons in the corpus striatum and atrophies of the caudate nucleus occurs in Huntington disease.

Mutation in the huntingtin (*HTT*) gene (on chromosome 4), causing abnormal repetition of the DNA sequence CAG, which codes for the amino acid glutamine is responsible for resulting this disease. The more CAG repeats, the earlier the onset of disease and the more severe its expression.

Alzheimer's disease (AD) is the most common source of dementia and is a long-lasting and rapidly increaing neurodegenerative disorder. The onset of AD mainly takes place in 60s28 elderly people. Researches that dependably examined age have publicized that AD prevalence and incidence increased with time of life and incidence (Ghosh et al.,2017). An estimated 5% of 65-year-olds are AD-borne and 30% of people are aged 85 or 85. Dementia may develop older 29. The process of degeneration 2 transplants of cells. I genes is related to the central nervous system (CNS); (ii)B amyloid (Ab) processed from the precursor amyloid Protein (APP) by b-secretase sequence cleavage (ii) neuronal enclosures which are microtubule-associated protein tau hyper

phosphorylated; (iii) (MAPT) and other changes in neurodegenerative diseases associated with this; (iv) swellings; (v) gliosis; (vi) oxidative stress; (vii) neuronal dystrophy; (viii) neuro deficiency; (ix) synapse loss; Cell cycle (xii), apoliprotein E (apoE) 32. (xii) cell cycle; These pathological changes are linked to the gradual memory deficiency, mainly short-term memory loss in Stadium Early (Fan et al., 2018).

Pathophysiology: Progressive cortical atrophy which leads to the neurofibrillary tangles in the neurons and senile plaques. In the affect’s parts of the brain both neurofibrillary tangles and senile

plaques are found in large numbers in which “the plaques disrupt neural conduction containing fragments from beta-amyloid precursor protein”. Amyloid plaques consist of abnormal proteins and fragments of nerve cells that are attached to other nerve cells. After the death of nerve cell the amyloid protein is embedded in the cell membrane and when it breaks off a fragment of the protein is still present, and it builds up in the brain. It’s the destruction and death of the nerve cells and the deposits of the protein on the membrane that causes memory failure, personality changes and carrying out activities of daily living.

Amyotrophic lateral sclerosis (ALS): About 1 in 100,000 people suffer from Amyotrophic Lateral Sclerosis (ALS), an incurable neurological illness affecting motor neurons. Although 5-10% of cases are familial ALS, the majority of ALS cases are sporadic. The degradation of cortical and vertebrae motor neurons involves both sporadic and family ALS (FALS) degeneration. ALS is still unclear in its genesis. However, the most prevalent cause of FALS was called superoxide dismutase 1 mutations (Wang et al.,2018). ALS is divided into two different types. Sporadically (90–95%), the most frequent type does not have a clear genetically modified component. The remainder of the cases are ALS (FALS) because of its genetically dominant heritage component (Diaz et al.,2015). Symptoms often start between 50 and 65 years of age. Muscular weakness, twitching, and clamping are the most typical signs of both forms of ALS, which can eventually lead to muscle deterioration. ALS patients experience dyspnea and dysphagia symptoms in the most severe stages (Zarei et al.,2015).

Pathophysiology: The death of both upper motor neurons (located in the motor cortex of the brain) and lower motor neurons (located in the brainstem and spinal cord) causes ALS. In ALS with frontotemporal dementia, neurons throughout the frontal and temporal lobes of the brain die

as well. The pathological hallmark of ALS is the presence of inclusion bodies (abnormal aggregations of protein) known as Bunina *bodies* in the cytoplasm of motor neurons.The gross pathology of ALS, which are features of the disease that can be seen with the naked eye, include skeletal muscle atrophy, motor cortex atrophy, sclerosis of the corticospinal and corticobulbar tract, thinning of the hypoglossal nerves (which control the tongue), and thinning of the anterior roots of the spinal cord.

Frontotemporal dementia (FD) : Since Pick originally defined it in 1892, FTD has experienced several modifications in nomenclature and classification methods. FTD now includes clinical problems including behavioral abnormalities, language, executive control and engine symptoms (Hodges et al., 2014). In this section we utilize the word to define the fundamental FTD spectral disorders: comportabal FTD (bvFTD), primary progressive aphasia (nfvPPA) nonfluent/graphic variant, and semantinal PPA (svPPA). Related FTD illnesses include motor neuronal disease (TDN), progressive supranuclear paralysis (SSP) and corticobasal disease (CS) were examined. Related FTD diseases will be explored (CBS). For pathological reasons which induce degeneration of the frontal and temporal lobe, the term "Frontotemporal Lobar" (FTLD) is used. FTD is a diverse disease linked with several neuropathologic substrates with different clinical phennotypes (Chare at el., 2014).

Pathophysiology: Accumulation of amyloid and Taw within neurons that mainly affect on frontal and temporal anterior lobes of the brain. And that causes neuronal death. As death occurs reduction in the neurotransmitters also occurs. As a result symptoms of frontotemporal dementia are seen.

1.3 Current treatment

Gene editing, also known as genome editing or genome engineering, is a newer and more precise genetic engineering technology or process that allows an organism's genome to be edited to change particular target genes. Gene editing technology, in a nutshell, is a method of inserting, removing, and replacing genes (Afshar Saber et al.,2020). Nuclease, an enzyme that cuts a specific DNA sequence in cells, is used in gene editing technologies to delete, repair, or replace disease-related genes. These technologies include homing endonucleases (or mega nucleases), zinc finger nucleases (ZNF), transcription-like effector nucleases (TALEN). Repeats of palindrome (CRISPR). The principle lies in those artificial endonucleases are used to target DNA dual-beaches accurately and specifically cut them to induce site-specific DNA dual bearings (DSB). When DSB is produced, two main natural repair mechanisms are initiated by activated cells. The unregistered end Joining (NHEJ) and repairing homologous recombinants (HDR) (Bjorklund and T, 2018).

Cells are usually mainly repaired by NHEJ. NHEJ can generate random insertions and indexes of bases at the DSB site in the repair and reconnection of broken DNA, leading to frequent inactivation of genes in frame-shift mutations which lead to the knockout of the destination gene. Whether an exogenous, The NHE J mechanism will link the donor gene sequence to the double beach break site of the DSB (Ahfeldt et al., 2020). Targeted exogenous knock-in genes are realized thereby. If it exists, the cell will also use H DSB in remediation of a recombinant donor with a homologous arm. By means of the homologous recombination process the foreign target gene of the donor is fully integrated into the target location, Careful insertion, removal or substitution from a particular site, without random insertion or loss of base (Garcia-Leon et al., 2019).

1.4 Limitations and prospects

Since the first clinical gene-therapy experiment, the topic has gotten a lot of attention and shown a lot of promise. Significant governmental and corporate sector investment has been made, as well as increasing levels of scientific effort. Several preclinical animal model studies have given proofs of concept for a variety of possible clinical uses (Arias-Fuenzalida et al.,2017). Clinical progress, on the other hand, has been slow. In September 1999, the research suffered a major setback when a well-publicized death was announced as a result of a gene-therapy trial (Svetoni et al., 2016).

Jesse Gelsinger, an 18-year-old male, died in a clinical experiment at the University of Pennsylvania, in which the gene for ornithine decarboxylase, a defective hepatic enzyme, was delivered via a modified Ad5 vector. According to an investigation by the university, Gelsinger died from a massive immune reaction to the Ad5 vector. This widely publicized case led to congressional and Food and Drug Administration (FDA) hearings on the conduct of clinical gene-therapy trials as well as a transient hold, subsequently lifted, on all adenoviral-vector clinical trials. The FDA found multiple possible violations in the manner this clinical research was conducted and supervised during its examination. The matter was settled in February 2005, after a five-year inquiry. Gene therapy underwent a period of severe criticism and mistrust as a result of the Gelsinger case. (Calvo-Garrido et al., 2019)

Fortunately for the gene therapy field, the first report of a spectacularly successful gene-therapy trial was released less than a year after Gelsinger's death. Cavazzana-Calvo and her Paris colleagues published the findings of a study involving two infants with a severe combined immunodeficiency condition (SCID-XI), which forced them to live in an isolated environment. (Cavazzana-Calvo et al., 2000). These researchers employed a MoMLV vector to ex vivo transfer

a curative gene (c cytokine receptor subunit) into the patients' lymphocytes, then returned the cells to the patients after amplification. Both individuals were discharged from the hospital and returned to their regular lifestyles. Several other patients were treated and appeared to be cured as a result of these trials. There was, however, a drawback (Levy el at., 2020).

Three of the eleven early patients who received the MoMLV vector developed leukemia as a direct effect of the gene-transfer process (Dunber et al., 2017). The MoMLV vector has obviously integrated in a nonrandom manner around the LM02 (LIM domain only 2) gene in all of these individuals. The LM02 gene was activated as a result of this integration, resulting in leukemia. After the patients' leukemia was treated, a broad, joint scientific effort was launched to learn more about the mechanisms that drive MoMLV integration (Zarei et al., 2015).

1.5 Gene editing technology in neurodegenerative disease

The possibility of altering a gene in a live cell provides several potential advantages, including the treatment of hereditary illnesses, better knowledge of what certain genes do, more robust cultivation and even the identification of species. Gene therapy for neurodegenerative illnesses has progressed steadily over the last few decades (Luo et al., 2019). With a better understanding of the pathogenic mechanisms underlying these diseases, major improvements in critical technologies, such as the identification of novel treatment targets and vectors, have become possible. Viral and non-viral based gene therapy is used to treat this disease. AAV-based vectors, Adenovirus, retroviruses, lentiviruses are used as viral vectors. To avoid gene toxicity and insertional

mutagenesis, these insertions should be regulated under tight settings and more research should be done (Malankhanova et al., 2017). Non-viral vector-based gene therapies have the potential to

overcome several of these problems, particularly those related to safety. Non-viral delivery vectors can be classified as lipid-based vectors or polymeric vectors based on the material composition of the carriers. Lipid-based vectors are the most widely used non-viral gene carriers (Medvedev et al., 2017). Endoplasmic reticulum stress and unfolded protein response, mTOR signaling, epigenetic regulation, mitochondrial function, autophagy, stem cell therapy, neuronal progenitors, or microglial and astrocyte function are methods of target identification for neurodegeneration (Long et al., 2016).

1.6 Current gene editing technology

Specific DNA-binding domains (DBDs) and nonspecific DNA cleavage domains combine to form gene editing (DCDs). DBDs provide for well-organized and accurate sequence binding. DCDs cleave the targeted DNA which activates cellular DNA repair processes like NHEJ and HDR. The HDR looks for homologous donor to restore the original DNA sequence. NHEJ connects the broken strands immediately (Axelsen et al., 2018). ZFNs is one of the mutual conserved structures in humans is the ZF domain. ZFNs have been used in cell and animal biotechnology, as well as in the development of potential medicines. Hundreds of assembled ZFNs are used by the MA to target dozens of genomic locations. Although the OPEN program has a greater success rate than the MA, the sophisticated, time-consuming, and labor-intensive techniques and skills required to screen combinatorial libraries have limited its use (Barman et al., 2020). Finding of Xanthomonas DNA-binding element was a watershed moment in gene-editing techniques. Xanthomonas bacteria secrete virulence factors (TALEs) that bind to the plant's genomic DNA and cause catastrophic harm to plants including rice, pepper, and tomato. TALENs can be tuned to target specific DNA sequences. CRISPR-CAS is a groundbreaking gene-editing technique. CRISPR is a property of prokaryotes that consists of an array of short repetitive sequences divided by spacers containing

inimitable sequences (Batista et al., 2016). CRISPR/CAS is a type of innate immune system that can detect and destroy invading DNA or RNA. CRISPR/CAS relies on PAM to recognize and

delete foreign DNA, it is a unique and crucial component of the invading DNA (Chare et al., 2014).

1.7 How gene editing technology can help neurodegenerative disease

The technique for gene editing is employed to delete the gene encoding a protein called PD-1. This protein is the target of cancer medications such as control points inhibitors on the surface of immune cells, since certain tumor cells can bind the protein to prevent an immune reaction to malignancy (Calvo-Garrido et al., 2019). CRISPR/CAS functions may be separated into three stages, which include adjusting, expressing and interfering. Firstly, 'adaptation' means that foreign DNA is recognized and integrated as a new spacer inside the CRISPR locus. The protospacer comprises a brief (2–5 bp) length of preserved nucleotides, acting as a motive for identification. A single copy of spacer of about 30 bp is inserted on the front side of the CRISPR array and duplicated (Choi et al., 2015). CRISPR mediated immunity against pathogenic assaults may be interfered with by mutations in PAMs of the viral genome. In 'expression,' the CRISPR arrays with the aid of CaS Proteins (CAS1, CAS1, CAS9 and CAS4) and a transactivating CRNA molecule (TRRNA) are being translated to precursors of CRISPR RNAs (pre-crRNAs) under renewed maturation (Compagnucci et al., 2017). The crRNA consists of a repeat serving and a DNA (protospacer) invader serving. The tracrRNA is also involved in pre-crRNA processing. The tracrRNA will be joined to create a tracrRNA-crRNA complex with crRNA via basic complementarity. TracrRNA enables pre-crRNA to be processed into mature crRNA. The processed CRNAs are part of the CRISPR Antiviral Defense Complex (CASCADE) and assist in identifying a specific foreign DNA target area (Ekman et al., 2019). CrRNA guides the cassa-

caught foreign nucleic acid proteins at places complementing the crRNA spacer sequence into tiny pieces of DNA in the 'interference' process to treat external genes. The CRISPR repeat spacer arrays are closed for the placement of the tiny clusters of case genes (Costa et al., 2013). More than 45 families of genes exist. Nuclearase, RNase, and/or DNase activities, helicases, and RNA-binding proteins were included in CAS protein functions. CAS1 protein is a metal dependent, basic DNase that integrates the spacer DNA in the CRISPR locus. CAS3 is a complicated component of the cascade. Adaptation involves both CAS1 and CAS2 proteins. The spacer purchase involves Cas4, a RecB-like exonuclease. The mysterious repeatedly-assigned protein (RAMPs) involved in crRNA processing can be linked with CAS5, CAS6, and CAS7 (Cullis et al., 2017). CAS9 is used to digest crRNA and cleaves the destination DNA. CAS9 protein has helicase, includes 2 domains of endonublease: the endonuclease (HNH) and (RuvC). Each domain fastens a strand of double-stranded DNA and induces DSBs to start repairing cellular DNA. CrRNA processing and DNA splitting are associated with the CAS10 Protein (Hong et al., 2019). The system CRISPR/CAS is divided into 2 main classes with 5 systems types, each using distinct molecular mechanisms for recognizing and divorcing nucleic acids, and can link the diversity of CAS proteins. Class 1 is divided into Type I, Type III and Type IV and Type II and Type V. CRISPR, CAS1 and CAS2 proteins are commonly used in Type I, II, and II (Gaj et al., 2017).

1.8 Aims of the study

This review aims to find out what is the existing and new gene editing technology in neurodegenerative diseases. And also find out how the gene editing technology can curve the future of neurological disease treatment.

1.9 Objectives of the study

The objective of the review is to create a concise an informative review that will address the topic of interest to summarize existing cloning study the topic of interest.

Chapter 2 Research Methodology

To collect all of the information included in this review study, a thorough literature review was conducted. The data came from a variety of trustworthy sources, including peer-reviewed journals, online scholarly databases, and newspapers. The following is a summary of some of the several databases that were thoroughly searched for the current study.

- Journal database
- Library catalogue
- Subject specific professional websites
- Newspaper database

Chapter 3 Delivery of gene editing technology

Gene therapy for neurodegenerative illnesses has progressed steadily over the last few decades. With a better understanding of the pathogenic mechanisms underlying these diseases, major improvements in critical technologies, such as the identification of novel treatment targets and vectors, have become possible (Garcia-Leon et al., 2019)

3.1 Vectors: viral and non-viral based gene therapy

3.1.1 Viral vectors

Almost entirely, AAV-based vectors have been used in clinical studies of gene therapy for neurodegenerative disorders. AAV serotypes are a primary factor of bio distribution, tissue tropism, and sensitivity to neutralizing antibodies developed in vivo, all of which are important properties of successful AAV-based gene therapy (Cross et al., 2014). To build a dependable and predictable gene therapy technique, researchers must first figure out how distinct serotypes distribute gene cargos to their intended locations for vector delivery. In humans and nonhuman primates, more than a hundred AAV variants with 13 serotypes (AAV1e13) have been found. AAV2 has been used in multiple clinical trials and is currently considered a suitable vector for gene treatment of neurodegenerative illnesses due to its relative safety profile and persistent expression in neurons (Biella et al., 2020).

Adenovirus (Adv) is an icosahedral capsid virus that is 70 to 100 nanometers in diameter. Adv is unable to implant its gene into the host genome, resulting in transgenic expression that is only temporary but has a superb safety profile (Herzog et al., 2020). Adv's therapeutic potential efficacy for CNS gene therapy is limited due to innate immune reactions to it. Although few studies have used Adv as a gene therapy vector to treat neurodegenerative illnesses, it should be noted that in

these trials, Adv has been well tolerated with few adverse effects (Jarosz et al, 2017). Unlike AAV and Adv capsids, retroviruses and lentiviruses (LVs) were able to fully integrate DNA into the host genome via reverse transcription, resulting in more stable and longer transgene expression in vivo (Jin et al., 2018).

To avoid gene toxicity and insertional mutagenesis, these insertions should be regulated under tight settings. The use of a lentivirus vector, which can carry bigger DNA cargos for PD, is the only important clinical trial to date (Mishima et al., 2018). ProSavin, a lentiviral vector-based gene therapy aiming at restoring dopamine production, improved motor function and was found to be safe in all patients with advanced PD, according to their findings. Given the lack of clinical data, more research on Adv and LVs-mediated gene therapy for neurodegenerative illnesses is urgently needed (Morelli et al., 2020).

3.1.2 Non-viral vectors

Non-viral vector-based gene therapies have the potential to overcome several of these problems, particularly those related to safety. Furthermore, despite the fact that only a few of these techniques Non-viral delivery vectors can be classified as lipid-based vectors or polymeric vectors based on the material composition of the carriers (Luo at el., 2019).

Lipid-based vectors are the most widely used non-viral gene carriers. Neutral lipids such as cholesterol, DOPE, and DSPE have been used as a "helper lipid" among liposomal components in order to improve liposome stability and transfection capacity have been tested in humans, it is critical to take advantage of innovative vectors, particularly nanoparticles and liposomes (Pahan & K, 2019). Cationic lipids, such as DOTAP, DODAP, DOTMA, and DC-cholesterol, have three

major domains: hydrophobic tails, connecting groups, and cationic cap groups, which have been employed for gene therapy. The primary drawbacks of cationic lipids are their cytotoxicity and suboptimal pharmacokinetic bio distribution due to nonspecific binding and fast elimination (Zarei et al., 2015). Optimized cationic lipids with acceptable pKa values have been created to alleviate these disadvantages. Exosomes, magnetic nanoparticles, and lipidoids (lipid-like compounds) have also showed promise as gene delivery vehicles for neurodegenerative illnesses (Wykes et al., 2018).

3.2 Target selection for neurodegenerative disorders

Neurodegenerative disorders are characterized by gradual neuronal malfunction in specific areas of the central nervous system (CNS), eventually leading to disability and death. The number of newly discovered targets is rising, which broadens the range of potential clinical applications (Vanderwall et al., 2020).

3.2.1 Endoplasmic reticulum stress and unfolded protein response

Almost all neurodegenerative illnesses share the same pathophysiological feature: aberrant protein mis-folding. Endoplasmic reticulum (ER) stress and ER-associated degradation are two unfavorable effects of aggregating misfolded proteins. Misfolded proteins that collect in the ER lumen, such as amyloid b oligomers and a-synuclein, disrupt ER calcium homeostasis and distort unfolded protein response (UPR) signaling, which is supposed to restore cellular proteostasis but instead causes proapoptotic responses and neuron death (Fan et al., 2018). Importantly, researchers, including ourselves, have shown that gene treatments to lower ER stress by targeting UPR signaling to improve protein folding are more likely than antibodies and small chemicals to deliver long-term, local therapeutic effects. Glaucoma, optic neuritis, and traumatic optic nerve injury cause optic nerve degeneration and apoptotic death of retinal ganglion cells (RGCs). AAV given to the mouse retina to down regulate CCAAT/enhancer binding homologous protein (CHOP) or activate XBP-1 inhibits this. However, long-term safety studies will be required for the clinic before this method can be considered (Wang et al., 2018).

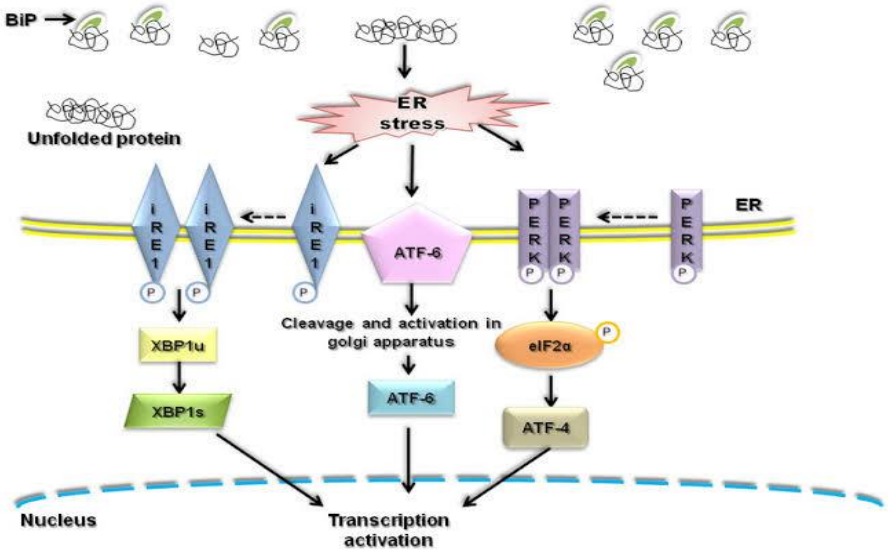


Figure 1: Endoplasmic reticulum stress and unfolded protein response pathway (Fan et al.,2018).

3.2.2 mTOR signaling

Mammalian target of rapamycin (mTOR) signaling has been linked to a variety of neurodegenerative illnesses, including Alzheimer's disease, Parkinson's disease, Huntington's disease, and traumatic brain and optic nerve injury (Costa et al., 2013). Although abnormal mTOR signaling may have diverse impacts in different brain cells, such as those in the significant nigra, caudate nucleus, retina, and entorhinal cortex, all of these cells will eventually degenerate if they are unable to eliminate harmful protein accumulation (Levy et al., 2020). Our previous research has shown that AAV-mediated delivery of positive regulators or effectors of mTOR signaling (such as AAV2-AKT and AAV2-S6K1) to the retina, as well as AAV-mediated deletion of negative regulators of mTOR signaling (such as PTEN) from the retina, can prevent RGC death and promote CNS axon regeneration following traumatic optic nerve injury. Several studies have demonstrated that mTOR signaling is hyperactive in HD and AD, and that restoring abnormal mTORC1 activity can help to reverse neurodegeneration (Kolli et al., 2017). As a result, future research should concentrate on the cellular and molecular pathways that link mTOR signaling to neurodegenerative diseases (Kang et al., 2019).

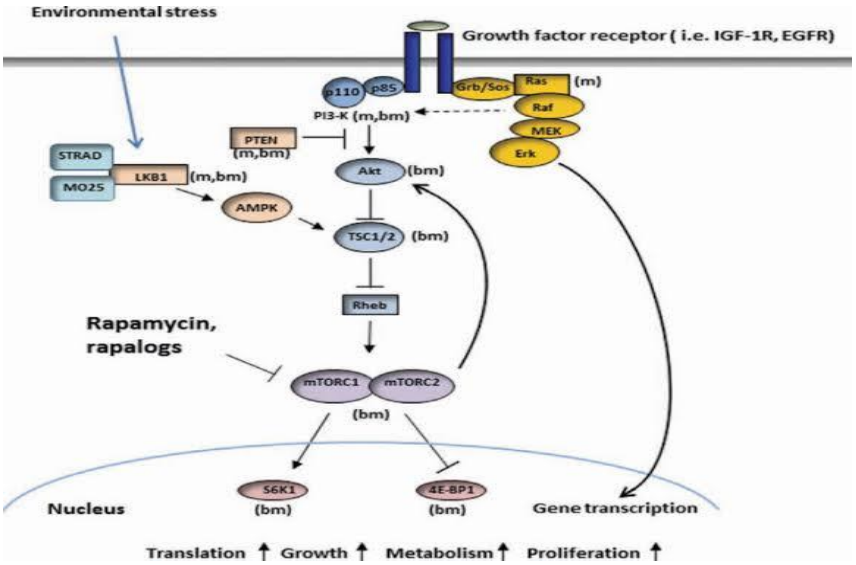


Figure 2: mTOR signaling pathway (Levy et al., 2020).

3.2.3 Mitochondrial function

Mitochondrial respiratory failure has been linked to a variety of neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease, etc. Limited regulation of mitochondrial quality, oxidative damage, NAD depletion, disturbed ATP generation, and protein aggregation are all symptoms of mitochondrial respiratory dysfunction in these illnesses and imbalanced of mitochondrial calcium homeostasis (Mohamed et al., 2019). In animal models, therapeutics that block mitochondrial damage or stimulate mitochondrial biogenesis, such as CoQ10, Bendavia, MitoQ, and NAM, reduce neurodegeneration. Furthermore, gene therapy that overexpresses regulators of mitochondrial oxidative stress and dynamics, such as PGC-1a, HSP70, and TFEB, can lessen neurotoxicity in PD and HD models, implying that similar treatments could be useful for additional neurodegenerative diseases (Taran et al., 2020).

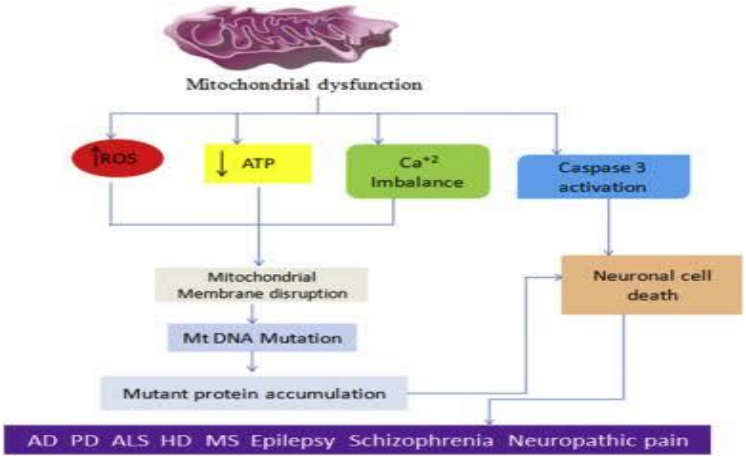


Figure 3: Mitochondrial function (Taran et al., 2020).

3.2.4 Epigenetic regulation

Plentiful features of axonal development and neuronal survival have been linked to epigenetic regulatory mechanisms such as chromatin transformation, DNA methylation, histone variants, and histone post-translational change (Chung et al., 2019). Changes in H3K27ac or H3K4me3 were linked to genetic variations in AD in one study, suggesting that immune-associated enhancers and promoter proteins play an essential role in defining AD susceptibility (Sun et al., 2019). Another study found that H4K16ac, a histone linked to DNA repair and neurodegenerative diseases, is dramatically lower in the cortex of Alzheimer's patients, implying that the elderly brain is unable to upregulate H4K16ac. H4K16ac. It's also worth noting that loss of H3K4me3, a protein linked to gene activity, has been linked to the deterioration seen in PD and HD, but that overexpression of H3K4me3 can speed up AeT mutation, which mitigates behavioral problems and neurodegeneration (Ross et al.,2014).

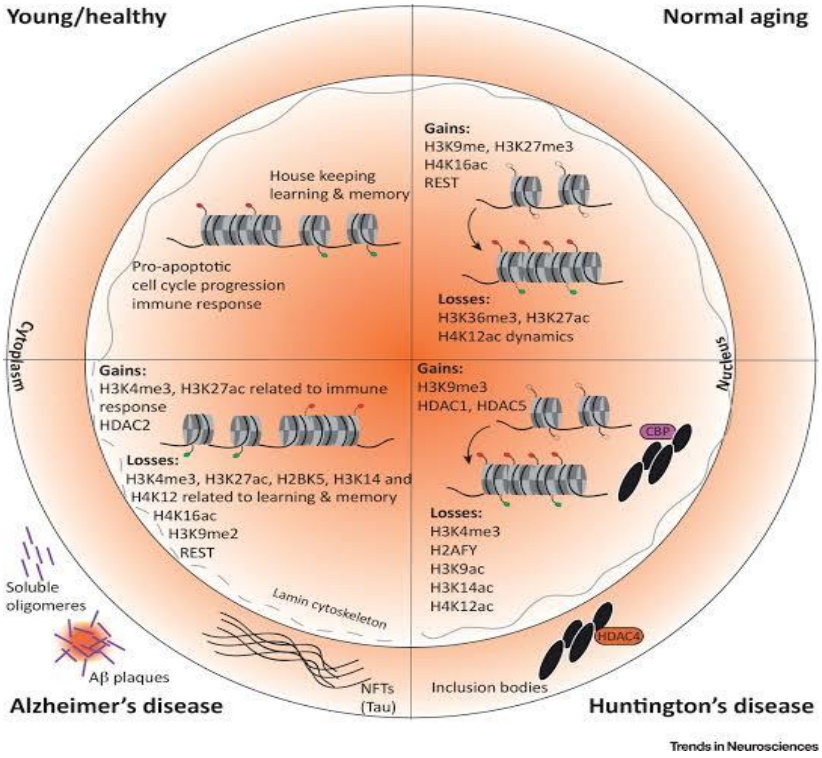


Figure 4: Epigenetic regulation pathway (Chung et al.,2019).

3.2.5 Autophagy

Autophagy is the process by which evolutionarily conserved intracellular machinery degrades dysfunctional organelles and denatured proteins in lysosomes and has been linked to the intensity of neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, high blood pressure, glaucoma, and ALS (Schmid et al.,2019). Autophagy protects the brain primarily by removing misfolded proteins such as tau, HTT, and a-synuclein. Previous research has shown that AAV2-mediated PTEN-induced overexpression of putative kinase 1 (PINK1) stimulates autophagy, which aids in the clearance of defective mitochondria, hence alleviating mitochondrial function loss and cognitive decline and induced synapses in experimental Alzheimer's disease by amyloid b oligomers (Price et al.,2016). Similarly, inducing lysosome biogenesis and chaperone-mediated autophagy by overexpressing the transcription factor EB (TFEB) or lysosome associated membrane protein 2a via intracerebral injection of AAV vectors can effectively alleviate a-synuclein-induced neurodegeneration in PD by enhancing axon regeneration and neuron survival.

Despite the fact that several lines of evidence indicate gene therapies' ability to cure neurodegenerative illnesses by controlling autophagy, the strategy is hampered by challenges in target selection and a lack of knowledge of the underlying mechanisms (Ratican et al.,2018).

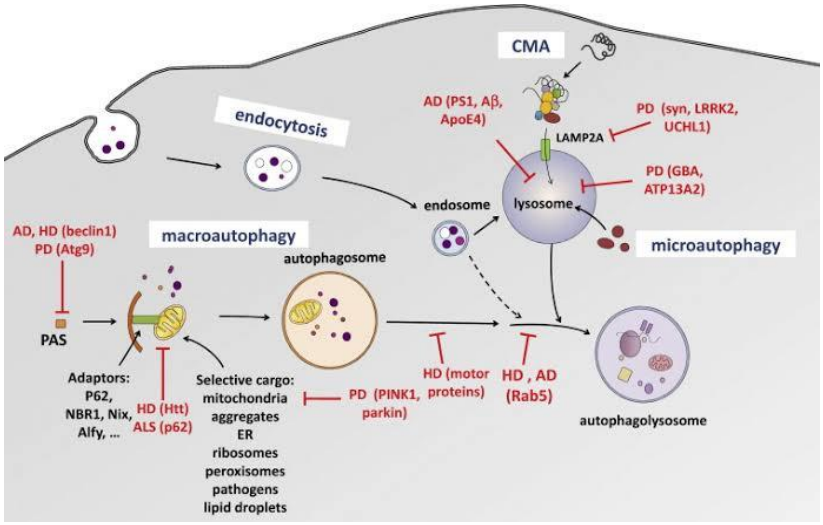


Figure 5: Autophagy pathway (Schmid et al.,2019).

3.2.6 Microglial and astrocyte function

As the key neuro-immune cells, microglia execute a variety of important functions, including housekeeping functions that maintain neuronal health and neuronal networks, sentinel functions that involve constant perception of environmental changes, and defensive functions that are critical for neuroprotection (Mustafa et al., 2020). Disruption of these microglial processes and neuro inflammation causes neuronal damage in Alzheimer's disease, Parkinson's disease, Huntington's disease, etc (Mesuraca et al.,2018) As a result, avoiding dysregulation of these functions could be a therapy option. Variants of microglial surface innate immune receptors such complement

receptor 1 (CR1), CD33, and triggering receptor expressed on myeloid cells 2 (TREM2) have been linked to an increased risk of AD (Poon et al., 2016).

Furthermore, AAV-mediated overexpression of soluble TREM2 increases microglial migration, proliferation, and amyloid b protein degradation, reducing amyloid plaque deposition and rescuing impaired spatial memory in an AD model. Furthermore, lentivirus-mediated haplo insufficiency of progranulin over expression prevents neuronal loss and spatial memory deficits in AD mice through altering microglial activity(Nityanandam et al., 2015)

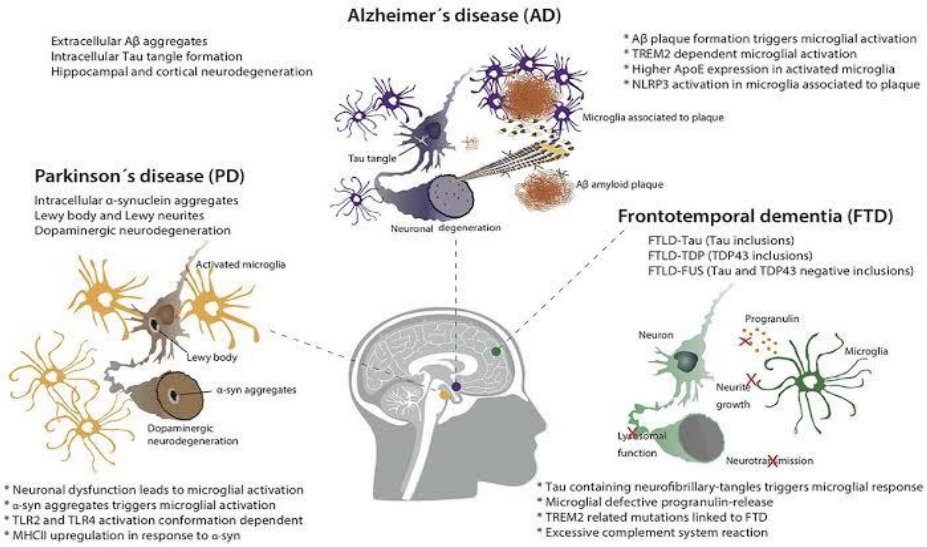


Figure 6: Microglial and astrocyte functional pathway (Mesuraca et al.,2018).

3.2.7 Neuronal progenitors or stem cell therapy

Transplanting neuronal progenitors or stem cells into patients with neurodegenerative disorders could transform treatment options. However, greater regulation of their proliferation and amelioration of their engraftment, as well as better differentiation and survival, are critical(Wu et al., 2020). Delicate gene regulation by gene therapy procedures is developing as a safe method for

modulating stem cell function., for example, used a CRISPR-Cas9 tool based on LVs to delete DNMT1 in neural progenitor cells, resulting in viable, proliferating cells and implying a novel gene therapy in human brain disease and development. For example, used LVs to insert functional genes into hematopoietic stem cells ex vivo and found that transplantation of these modified HSCs suppressed and relieved metachromatic leukodystrophy symptoms (Svetoni et al., 2016). Despite the fact that neural progenitors and stem cell therapy have previously showed clinical effect, these treatments are frequently limited, especially in the case of disorders caused by cell autonomous abnormalities (Chen et al., 2020).

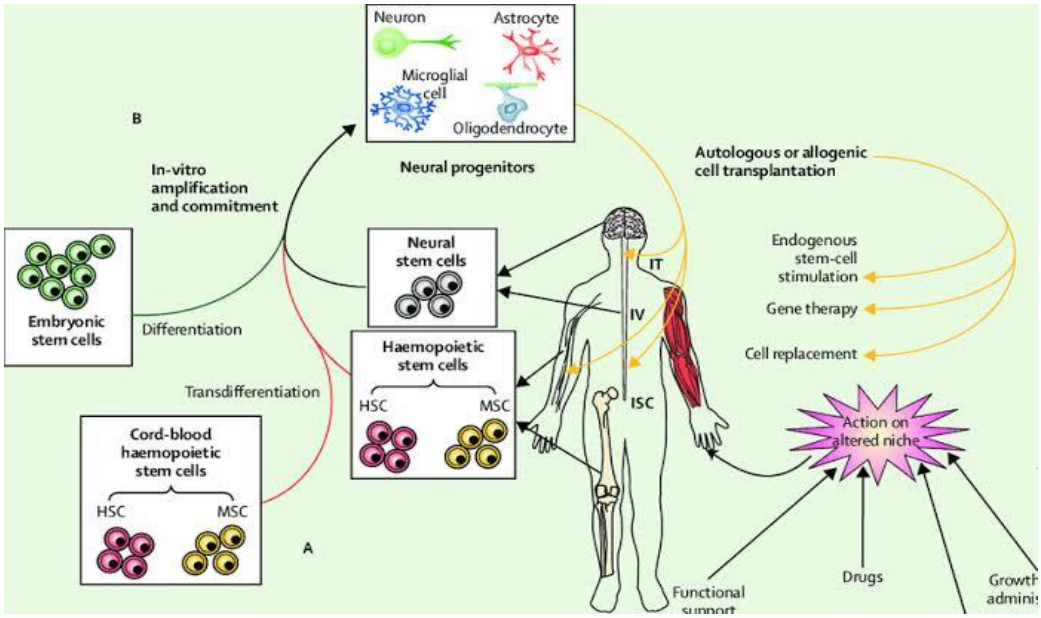


Figure 7: Neuronal progenitors or stem cell therapy pathway (Chen et al.,2020).

3.3 Current technique for gene editing

Precise DNA-binding domains (DBDs) and nonspecific DNA cleavage domains combine to form gene editing (DCDs). DBDs provide for efficient and precise sequence binding. DCDs, like genomic scissors, cleave the targeted DNA spot, causing a double-strand break (DSB), which activates cellular DNA repair processes such as error-prone no homologous end joining (NHEJ)

and homology-directed repair (HDR) (Rittiner et al., 2020). The HDR looks for homology between the scratched DNA sequence and sister chromatids, homologous DNA strands, or other related DNA as templates and copies the fragment's sequence between the two broken ends of the damaged sequence fragments to restore the original DNA sequence at DSB sites, whether or not the fragment contains the original sequence (Raikwar et al., 2019). The designed DNA can then be introduced into the targeted cleavage site using the machinery, and NHEJ connects the broken strands immediately. Errors in the repair process can result in minor insertions, deletions, and/or rearrangements. However, repair processes are essential for maintaining genomic integrity and generating genetic variety (Mesuraca et al., 2018).

ZFNs is one of the mutual conserved structures in humans is the ZF domain. ZF was first recognized as a DNA-binding motif in Xenopus laevis transcription factor IIIA (TFIIIA). DNA recognition, lipid binding, mRNA trafficking, transcriptional activation, chromatin remodeling, protein folding and assembly, apoptosis regulation, zinc sensing, cytoskeleton organization, epithelial development, and cell adhesion are just a few of the functions of ZF (Pantera et al., 2015).

In a complex genome, ZFNs, the first genome-editing tool utilized in zebra fish, may create gene point mutations, deletions, insertions, inversions, duplications, and translocations. ZFNs have been used in cell and animal biotechnology, as well as in the development of potential medicines. ZFNs are a chimera fusion of a customer-designed ZFDBD and a nonspecific DCD in terms of structure (Soldner et al., 2016)

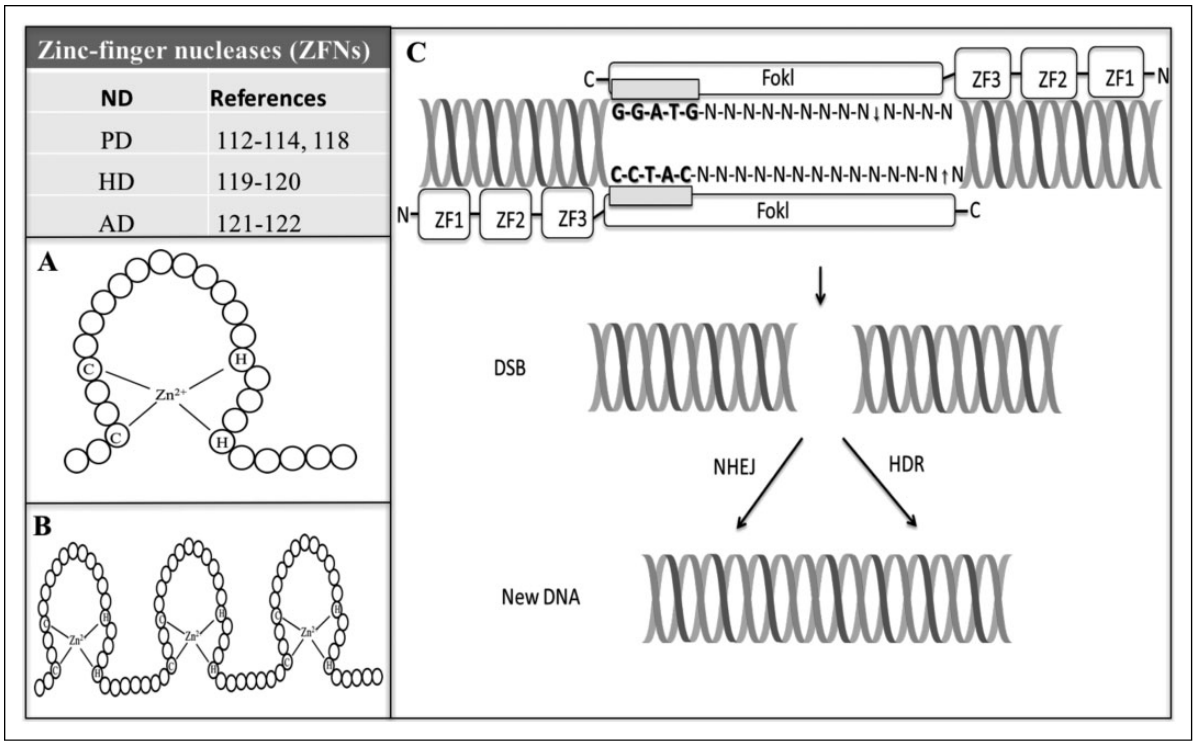


Figure 8: Schematic diagram of zinc-finger nucleases (ZFNs) (Soldner et al.,2016).

The references in the table pertain to the use of ZFNs in neurodegenerative diseases (NDs) such as Parkinson's disease (PD), Huntington's disease (HD), and Alzheimer's disease (AD). (A) Zinc finger structure (ZF). The paired cysteines (Cys) and histidines (His), (Cys2His2), bind a zinc ion tetrahedrally to form a compact structure. (B) An example of a C2H2 ZF motif arrangement. The ZFs' modular design allows them to be assembled into a linear array to target DNA. (C) ZFN structure. ZFNs are made up of two functional domains, one of which is a ZF DNA-binding domain with a chain of three finger modules (ZF1 to ZF3). Each ZF can recognize 3 bp of DNA; a DNA cleavage domain is made up of the FokI nuclease domain. However, because the FokI needs to form a dimer before it can function properly, this technique requires two ZFNs to bind at or near the cleavage site. In addition, the target sequences must have been separated by 5 - 7 base pairs to permit formation of the catalytically active FokI dimer, resulting in a double-strand break at a specific sequence that activates the cell DNA repair machinery, including homology-directed repair and nonhomologous end joining, to repair the abnormalities that

result in targeted gene disruptions or gene integration.

Selection-based procedures, on the other hand, are labor-intensive and time-consuming. Hundreds of assembled ZFNs are used by the MA to target dozens of genomic locations. The MA is simple and quick in theory, but it is said to have a very low chance of effectively generating active ZFNs (Schmidt et al., 2015). For binding of randomized ZFs to each triplet in the targeted sequence, the OPEN program uses several and parallel low stringency selections, and ZFs from these pools are linked, and the products are selected at high stringency for binding to the final target. Although the OPEN has a greater success rate than the MA, the sophisticated, time-consuming, and labor-intensive techniques and skills required to screen combinatorial libraries have limited its use (Axelsen et al., 2018).

DCD is found in Flavobacterium okeanokoites and is made up of the type II restriction enzyme FokI, which has a molecular weight of 65.4 kDa. FokI starts out as an inactive monomer, but when it becomes an active dimer, it transforms into genomic scissors, cleaving the targeted DNA spot and causing a DSB. As a result, two ZFNs were required to bind opposite strands of DNA with their C-termini separated by a specific distance, and the cleavage domain required the 50 edge of each binding site to be separated by 5 to 7 bp. When ZFNs and donor DNA are ready, they are delivered to target cells using viral vectors, liposome transfection, or electroporation. When ZFNs bind to duplex DNA at the 50-GGATG-30 recognition site, the FokI endonuclease, a nonspecific DCD, cleaves at the first strand 9 nucleotides downstream and the second strand 13 nucleotides upstream of the recognition site to induce DSBs at particular loci . To digest the ends of the strands formed at DSBs, exonuclease might be used. To produce new DNA mutations, the created DNA

defects require DSB repair systems. FokI variants that require heterodimerization to increase ZFNs and target DNA sequences have also been created (Fan et al., 2018).

The discovery of the Xanthomonas DNA-binding element was a watershed moment in gene-editing techniques. Xanthomonas bacteria secrete virulence factors (TALEs) that bind to the plant's genomic DNA and cause catastrophic harm to plants including rice, pepper, and tomato. (Chen et al., 2016). TALEs act as transcriptional activators, allowing pathogenic bacteria to colonize by upregulate or downregulate the expression of target genes. TALEs have a DBD that is made up of 33 to 35 amino acid tandem repeats that are nearly similar. The repeat variable diresidue (RVD) is made up of the 12th and 13th amino acid positions, which have substantial variabilities and a specific DNA recognition. By developing appropriate RVDs, TALENs can be tuned to target specific DNA sequences.

Transcription activator like effector nucleases (TALENs)	
ND	**References**
PD	137
HD	138
AD	139-141

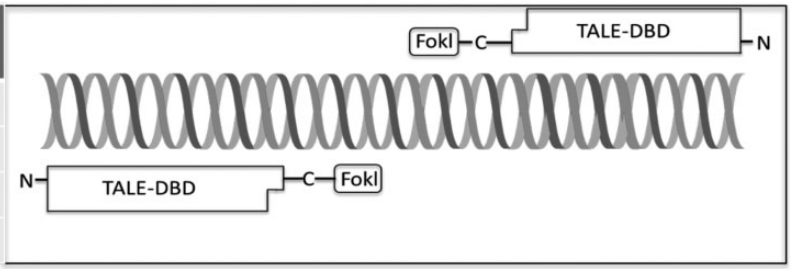


Figure 9: Schematic diagram of transcription activator-like effector nucleases (TALENs) (Chen et al.,2016).

Table indicates references regarding applications of TALENs in neurodegenerative diseases (NDs) including PD, HD, and AD. Structure of TALENs consists of 2 functional domains including transcription activator-like effector (TALE) and DNA cleavage domain (DCD). TALE is shown as long squares with a final carboxy-terminal truncated “half” repeat. TALE amino- and carboxy-terminal domains required for DNA-binding activity are shown as “N” and “C,” respectively. The DCD, including the FokI endonuclease, is shown as a small square. (Fan et al., 2018).

CRISPR-CAS is a groundbreaking gene-editing technique. CRISPR is a property of prokaryotes that consists of an array of short repetitive sequences separated by spacers containing unique sequences (Rehbach et al., 2020). Ishino and his colleagues initially noticed these patterns in Escherichia coli. A proto spacer is a sequence in an external nucleic acid element that corresponds to a CRISPR spacer. A highly conserved any base-Guanosine-Guanosine (NGG) motif-proto spacer neighboring motif frequently flanks the proto spacer. The majority of PAMs have two to five highly conserved nucleotides (Barman et al., 2020).

Because CRISPR/CAS relies on PAM to recognize and delete foreign DNA, it is a unique and crucial component of the invading DNA. The dairy industry explained the functions of the CRISPR/CAS system. Bacteriophage contamination has been a major issue in the dairy industry, as lactic acid bacteria like Streptococcus Thermophiles are utilized to ferment milk into a variety of products. The dairy processes are severely hampered after the bacterio phages infect the S. thermophile. To combat the infection, the dairy sector sequenced bacteriophage-resistant strains and discovered several short, partially palindromic DNA repeats (CRISPR repeats; Fig.9, black rectangle) in the bacteria by mistake. CRISPR/CAS is a type of innate immune system that can detect and destroy invading DNA or RNA.

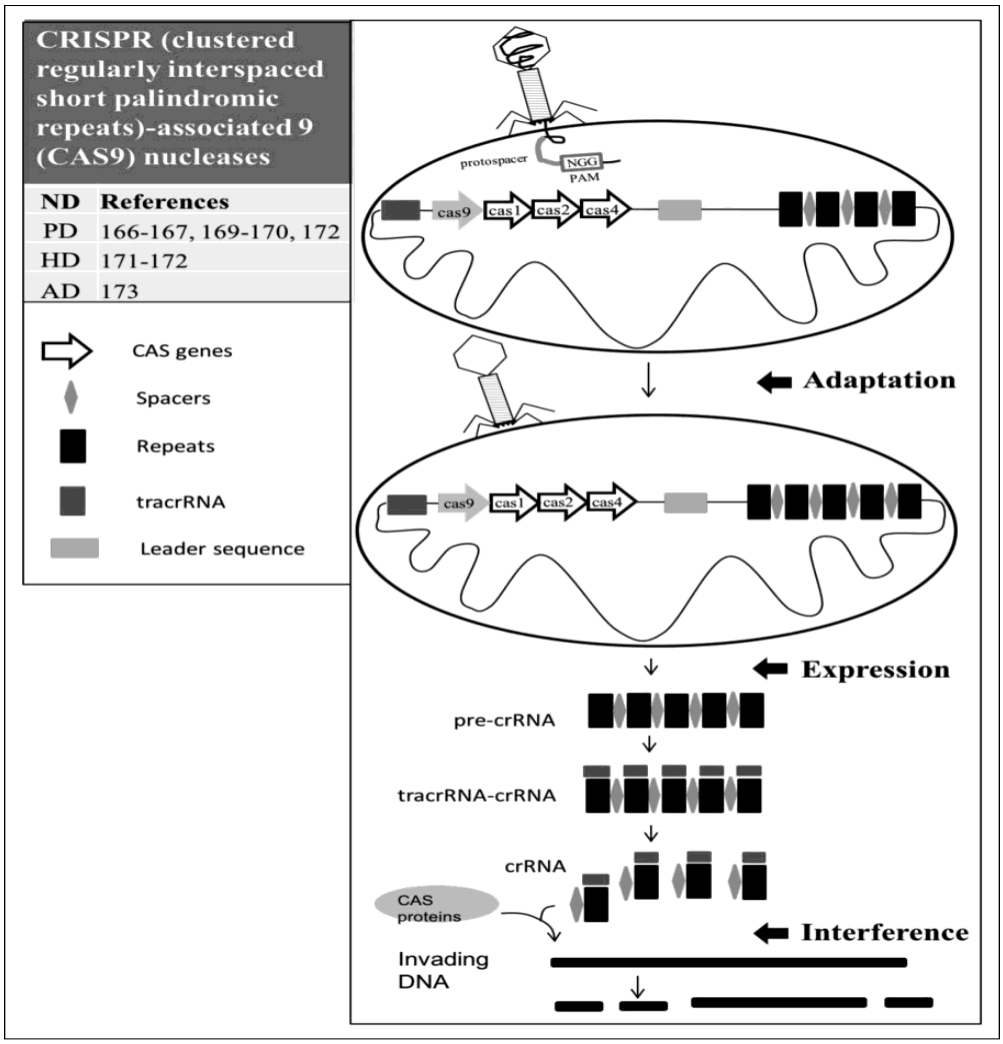


Figure 10: Illustration of clustered regularly interspaced short palindromic repeats–associated nucleases (CRISPR/CAS) (Barman et al.,2020).

Table indicates references regarding applications of CRISPR/CAS in neurodegenerative diseases including PD, HD, and AD. CRISPR is an array of short repeated sequences (black rectangles) separated by spacers (gray diamonds) with unique sequences. When a segment of a bacteriophage's genome invades and integrates into the cellular DNA, the processes of the CRISPR/CAS mediated immunity against the integration is initiated, including adaptation, expression, and interference. "Adaptation"—the invading bacteriophage's DNA contains 2 to 5 bp protospacer adjacent motif (PAMs) acting as a recognition motif. The new single copy

of spacer (green diamond) occurs at the leader side of the CRISPR array and is followed by its duplication. Any mutations in the protospacers or PAMs of the bacteriophage will interfere with the CRISPR/ CAS-mediated reactions. “Expression”—the repeats, the invader DNA (green diamond), and spacer sequences are transcribed to the precursor of CRISPR RNAs (pre-crRNAs), which turn into the crRNA through the help of CAS proteins (CAS1, CAS2, CAS9, and CAS4) and the trans-activating crRNA (tracrRNA) molecule. The tracrRNA and crRNA form a tracrRNA-crRNA complex. “Interference”— crRNA guided CAS proteins to cleave the invader DNA into small DNA fragments (Fan et al., 2018).

The CRISPR/CAS system is separated into two primary groups, each with five different types of systems. Each system uses different molecular methods to recognize and cleave nucleic acids, and the diversity of CAS proteins may be connected. Types I, III, and IV make up class 1, whereas types II and V make up class 2. Only three of the five categories had been thoroughly investigated (Fan et al., 2018).

Table 1. Different Classes of the CRISPR/CAS System and Cas Proteins.

Class	Type	CAS Proteins	Target
1	Type I	CAS1, CAS2, CAS3, CAS5, CAS6, and CAS7	DNA
2	Type II	CAS1, CAS2, CAS3, and CAS9	DNA
1	Type III	CAS1, CAS2, CAS6, CAS10, and RAMPs	DNA/RNA
1	Type IV	CAS1, CAS2	?
2	Type V	?	?

Table 02: Different classes of the CRISPR/CAS System and Cas Proteins

Chapter 4 Current clinical uses of gene editing

The first clinical gene delivery was published 25 years ago, involving insertion of a marker gene into lymphocytes in cancer patients. We describe progress in gene therapy since then in this review. Now, patients with a few legacy single-gene defects can be treated with a viral vector that carries the missing gene with their own bone marrow stem cells(Younesi et al., 2015). Local or systemic viral vector injection also may be used for patients with inherited retinopathy and hemophilia B. There are also a number of promising cancer and infectious disease approaches to gene therapy. We forecast improvements in gene supply vectors and in gene-editing technologies for the next 25 years in the safety, effectiveness and manufacture of the clinical system(Vermilyea et al.,2020).

4.1 Bone marrow transplantation replacement gene therapy

Toxic purine metabolites accumulate in patients with adenosine deaminase (ADA) deficiency. Their most immediate problem is a severe combined immune deficiency (SCID), leading to several life-threatening early childhood infections. Effective treatment involves either transplantation of bone marrow if a suitable donor is available or regular injection of recombinant enzymes before the advent of gene treatment. Two children without adequate bone marrow donors who had no funding for recombinant enzyme therapy were treated for their first successful gene therapy (Tagliafierro et al., 2016).

In the past, however, patients also received low-intensity myelosuppression, due to insufficient numbers of cells being grafted. Magnetic beads with antibody to the surface marker CD34 are used to isolate the bone marrow stem cells from the patients. The cytokines and an MLV vector were grown for 4 days and transmitted with ADA cDNA (Schmidt et al., 2016).

Bone marrow can also be used to treat neuro metabolic disorders, since the blood-brain barrier is crossed by microglial cells or their precursors. The lack of a carrier involved in peroxisomal degradation of high-chain fatty acids through oligodendrocytes and microglia, for example, causes x-linked adrenoleucodystrophy (ALD) (Retican et al., 2018). This disrupts the cells' preservation of myelin, leading to severe neurological consequences and childhood death. Metachromatic leukodystrophy is an arylsulfatase a deficiency that results in sulfatide build-up, leading to oligodendrocytes and microglia cytotoxicity. Bone marrow transplantation may treat ALD, although the most serious MLD cannot (Hatton et al.,2020). Gene therapy with lentiviral vectors for translating bone marrow stem cells in either condition is effective; in fact, gene therapy with early-stage MLD gene therapy is the unique efficient therapy (Morelli et al., 2020).

The enhanced safety of lentiviral vectors is because lentiviral vectors are engineered to remove any enhancing activity from LTR, thereby reducing the risk of expression activation of adjacent genes compared to LTR-containing MLV vectors. However, a different mechanism for cellular gene upregulation, involving the cellular cut-off from mNRA by supplying a lentiviral splice acceptor in the lentiviral vector, was seen when the lentiviral vector has been used to treat a painting with β- thalassemia. Lentiviral vector design work is underway to remove splice donor and splice acceptor (Malankhanova et al., 2017).

Gene therapy (GT) with the presence of nonmyeloablative conditioning and ADA Enzyme Replacing Treatment (ERT) before autologous transplantation of the α-retrovirus-patient BM CD34+ cells can provide significant long-term advantage to the adenosine deficiency–deficient combined immune deficiency (ADA-SCID). These variables were studied in Ada gene knockout mice (Ada−/-) in order to assess the relevance of conditioning and ERT cessation for therapeutic

outcomes (Luo et al., 2019). With the transplantation of an ADA-deficient marrow, mice were transplanted without preconditioning or with a complete ADA-expressed retroviral vector, or with a total body radiation of 200 cGy or 900 cGy and assessed 4 months later. In the tissues tested in all animals receiving 900 cGy, the number of vector copies (VCNs) was 100 to 1000 times higher compared to 200 cGy (P <-05) (Mishima et al., 2018). VCN was comparable in mouse receiving 200 cGy, whether ERT was discontinued or delivered 1 to 4 months after GT. With and without ERT, survival was reduced in unconditioned mouse, and VCN was extremely little to undetectable. Only patients receiving ERTs (1 or 4 months) have identifiable vector sequences in thymocytes when they were conditioned with 200 cGy and got a transducted linear depreciation marrow (Li et al., 2020).

In conclusion, cytoreduction is crucial in the grafting process of genetically modified HSC, and short-term ERTs do not decrease the ability for engrafting and persistence of geneally corrected cells (Long et al., 2016).

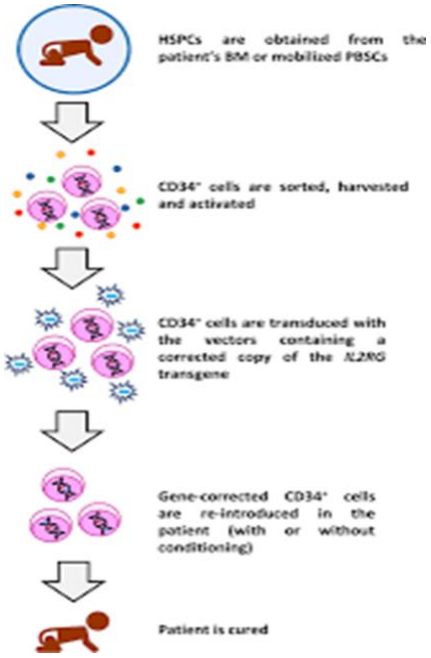


Figure 11: Bone marrow transplantation (Long et al., 2016).

4.2 Direct injection of gene replacement therapy adeno-associated virus vectors

The eye is an attractive target for direct gene delivery. It can be accessed by an eye only if it is toxic, a small number of vectors are needed and the eye is an immune-privileged place for the suppression of inflammation and immunity (Pahan & K, 2019). The retina is, in addition, an inherited defect in one of more than 60 genes that leads to retinal degeneration or blindness in about 1 in 3000 of the population. In addition, it is complex tissue. A RPE65 gene defect was the first disease to be treated with gene therapy. The protein RPE65 is expressed in the retinal pigment epithel and needs to be converted into 11-cis retinal for all-trans retinal generated during light-emitting photoreceptor responses (Afshar et al., 2020). Three groups have injected vectors sub-retinally very similar to AAV serotype 2 vectors, with a total of 21 patients reporting results. The treatment was safe and retinal function and visual performance improvements were identified. In patients treated for deficiency in the Rab escort protein REP1 improved visual acuity was also safety of these trials will encourage the treatment and ongoing pre-clinical work is developing

treatment for the younger patients, where further improvements are expected. AAV vectors were also used for the treatment of Factor IX (F.IX) deficient blood-clotting cascade patients with hemophilia B. These patients rely on the prevention of spontaneous bleeding using prophylactic or on-demand plasma or recombinant F.IX injection and continue to experience progressive damages to the joints and life threats like intracranial hemorrhage. Due to the cost, this therapy in less-developed countries is not available. The first clinical trial used AAV2 encoding of F.IX injected into muscle, as many cells can secrete F.IX when transduced through AAV. Around 1% of the normal F.IX levels could be detected, but there was limited clinical benefit(Cappella et al., 2019).

In order to transmit hepatocytes into the cells that normally generate F.I X, the same investigators then added a higher dose of the même vector to the hepatic artery. In this case, the highest vector dose of F.IX therapy was reached, but it declined over the next two months as a result of immune responses to the AAV2 capsid, eliminating hepatocytes that were transduced. More recently, the use of an intravenously injected AAV8 complementary vector has shown considerable promise, with a number of patients with prophylaxis discontinuation for up to three years (Choi et al., 2015).

The better results with this vector are due to the efficient self-complementary vector translation and the use of the AAV8 serotype which makes gene transmission to hepatocytes more efficient. This allowed a relatively moderate vector dose intravenous administration. In the population, exposure to AAV8 is also considerably lower than the AAV2 level, and in the patients in the recent clinical trial there was no evidence of prior immunity to AAV8 (Dever et al.,2019).

The glycosylated cell surface recipients of the host are identified by Adeno-associated virus (AAV). This causes clathrin-mediated endocytosis of the virus. AAV transfers the cytosol across

the cytoskeleton network. The area VP1/VP2 experiences a conformational change due to the somewhat low pH environment of the endosome. AAV is transported into the nucleus and uncoated following an endosomal escape (Gaj et al., 2017). Proteolysis of proteasome may also occur to AAV. Two kinds of recombinant AAVs (rAAVs) are in use: one-stranded AAVs (ssAAV) and one-stranded AAVs (scAAV). SsAAV's are either packaged as sensory (plus-stranded) or anti-sense genomes (Joshi et al., 2017). These unilaterally shaped shapes are still transcriptional

inactive once they reach the nucleus. As a condition for transcription, they must be transformed into double-stranded DNA. The second strand synthesis can be carried out by host DNA polymerases or by the strand annealing of plus and minus strands co-existing in the nucleus. Since scAAVs are already designed to be double stranded, they may be transcribed instantly. Viral inverted terminal repeats (ITRs) in RAAV can lead to a re-combination of inter-molecular or intra-molecular genomes that can survive in the nucleus, which are circularized. Also, vehicle genomes with very low frequencies indicated with the dotted line can integrate into the host genome (Garcia-Leon et al., 2019)

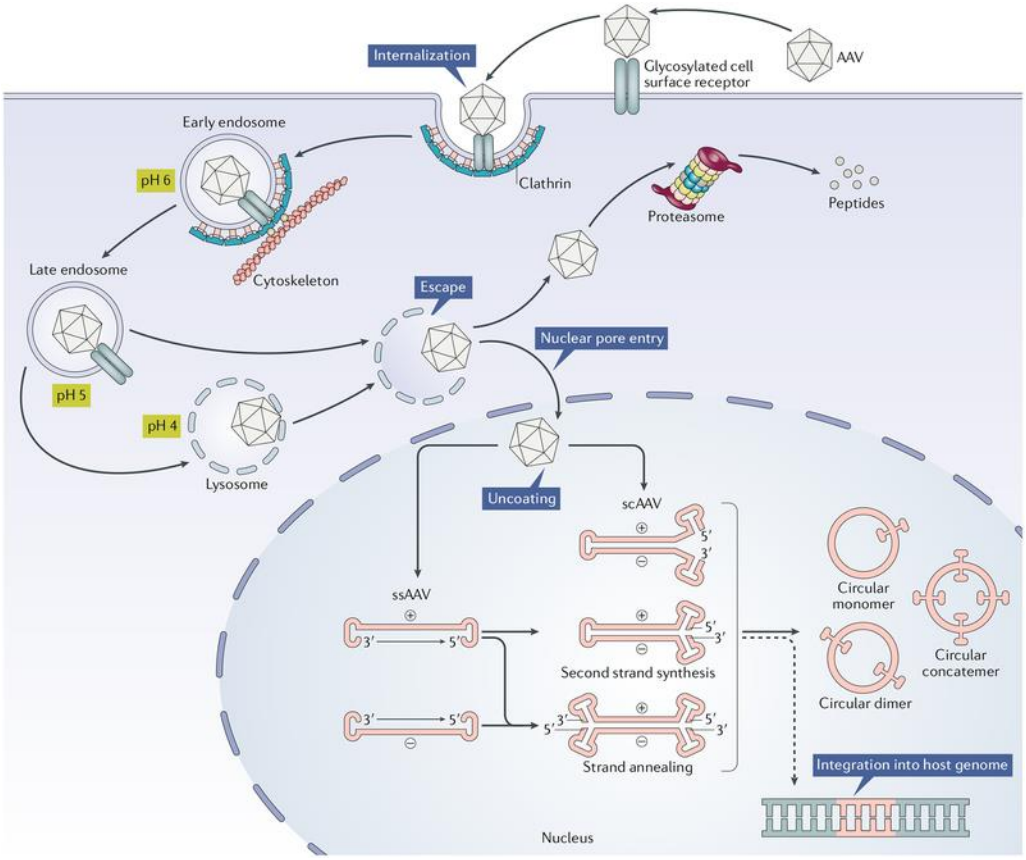


Figure 12: Diagram of rAAv transduction pathway (Afshar et al., 2020).

4.3 Gene therapy for cancer and infectious conditions

Visitors to cancer perhaps the most numerous clinical gene therapies to date have been instigated by the most seriousness of the condition. Many of these studies use ingenious viral vectors to test for cancer vaccines (Kang et al., 2019). These are often considered as gene therapies, as opposed to the use of similar vectors for infectious diseases, such as prophylactic or therapeutic vaccines. A remedial approach to cancer-gene therapy has been proposed to correct the genotype tumor cell by, for example, restore the function of the tumor suppressor gene (Latour et al., 2019).

However, this autonomous approach to tumor cells will be very challenging as it will be very difficult for gene delivery to each tumor cell. In addition, at the time of diagnosis many tumors are widely diffused, so that they are systemic. Gene therapy therefore makes it more logical to approaches which create a tumor-hostile systemic environment (e.g. by increasing immunity from tuberculosis) (Mazzara et al., 2020). Infusion of monoclonal antibodies can stimulate some forms of tumor immunity; in particular, targeting tumor cell receptors like her or inhibiting immune system retroactive mechanisms such as engagement with PD1/PD-L1 (Monteys et al., 2017). There are however clear evidence that the number of T-cells in the effector infiltrate tumors is linked to the clinical result. This means that a large amount of T cells can be generated by engineering the T cells themselves to identify the tumor, so that the gene modification can be used (T Rohn et al., 2018).

The initial AIDS gene therapy proposals included policies to inhibit the viral replication, for example by providing HIV-infected cells with dominant negative viral proteins. Another idea was that an HIV controlled promoter should also be linked with a cytotoxic gene to kill virally infected cells (Shuvalova et al., 2020). However, following an understanding of AIDS pathogenesis, the better objective would be to supply HIV-resistant cells. The transplantation of an HIV infected person with marrow from an individual homozygote in the bone marrow was an elegant demonstration, leading to a relatively common deletion of the CCR5 HIV receptor, leading to the apparent HIV cure. T-cells of people infected by HIV were taken in the first gene therapy clinical trial to use genome editing and ZFN was used to target the CCR5 HIV binding site (Wang et al., 2018).

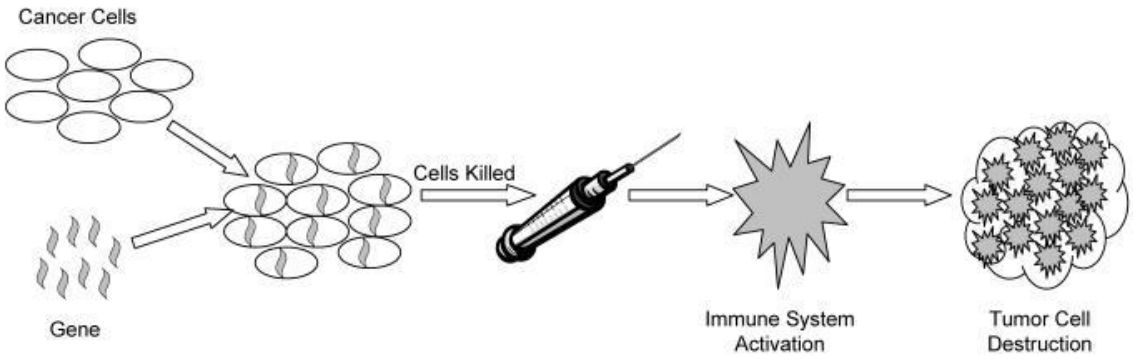
Furthermore, if a gene editing protocol was to be implemented on bone marrow stem cells, it would

then be necessary to minimize off-target effects that might be oncogenic (Chen et al., 2016).

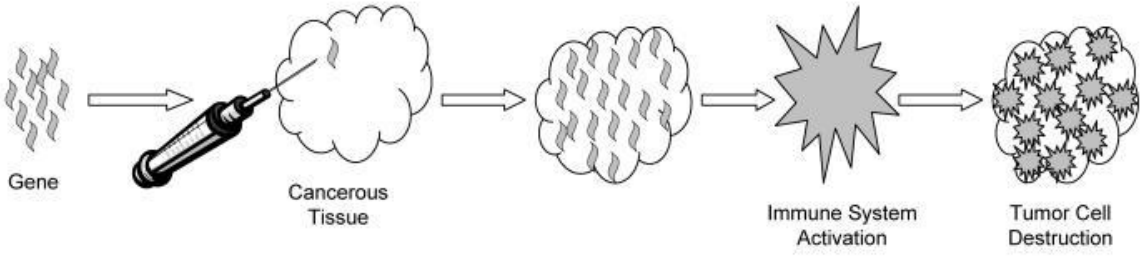
Gene therapy for recombinant cancer vaccines is now being employed. These vaccinations are not designed to prevent or curb disease, as opposed to infectious agent vaccines, but by exercising the immune system, the patient's immune system is intended to identify cells by showing high-antigénic and immunostimulatory cell debris (Chare et al.,2014). The patient (autologous cells) or the cancer cell (allogeneic) are first collected from cancer cells and cultivated on in vitro.The cells have been designed to be more recognized in the immune system by adding one or more genes, which frequently are cytokine genes which generate chemicals that stimulate pro-inflammatory immunes or highly antigenic proteins. The modified cells are cultivated in vitro, destroyed and integrated into a vaccine by the cellular content (Figure 13A). Involvement of immuno-stimulatory gene (especially cytokines) is also being attempted in vivo. The technique of delivering a gene into the tumor varies, and the gene transfer part of this examination is described in greater depth. Once these genes have been involved in cancerous cells, they will generate proteins that will expose immune escape cells and stimulate anti-tumor growth (figure 13B). Another unique gene therapy immunotherapy method is to directly modify the immune system of the patient in order to raise his or her awareness of cells of the malignancy. One technique utilizes the patient to collect mononuclear blood cells or bone marrow. The specified cell type will be then added to a tumor antigen or other stimulating gene. These modified cells are now ready to induce an immune response to cancer cells to eradicate cancer (figure 13C). Otherwise, a targeted delivery mechanism, such as the altered viral component, can be used to introduce the gene in vivo.(Arias-Fuenzalida et al., 2017)

Initial studies utilizing first-generation vaccinations have yielded mixed results that indicate both the promise for the treatment and the areas to be further refined prior to the development of these ingenued cancer vaccines.

A



B



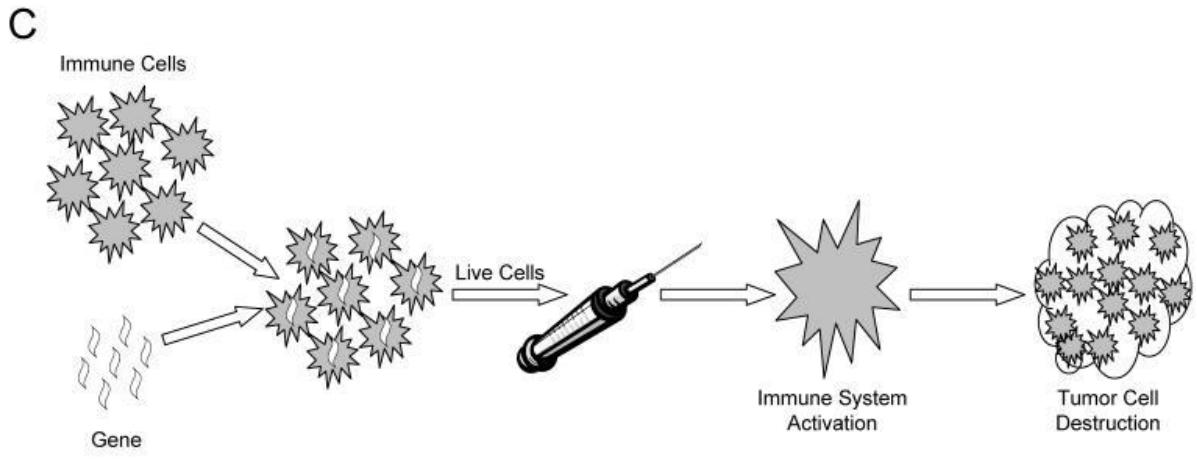


Figure 13: Immunotherapy schematic diagram. Pathway A is immunotherapy of cancer cells that have been modified. Pathway B stands for in vivo gene-immunotherapy. Pathway C means the use of altered immune cells in immunotherapy (Chen et al., 2016).

4 .4 Gene therapy as another conventional formulation

AAV1 vector containing human lipoprotein lipase (LPL), to be injected intramuscularly in treatment for patients with LPL deficiency is in fact the first licensed gene treatment drug, alipogene tiparvovec (Glybera). There has been much interest in the treatment of infectious diseases with muscle antibody gene delivery (Bjorklund &T, 2018)

For a persistent virus such as HIV, permanent expression of a widely-neutralizing antibody may be more effective than the so far failed vaccination. The effectiveness of this was shown in a humanized mouse model. Influenza prophylaxis was tested in mice, both in the muscle and intranasal (Sakuru et al., 2019). These technologies were also applied. There may also be an advantage compared to vaccination, especially in elderly people who do not respond effectively to flu vaccination. Prophylaxis-type gene therapy for antibodies may also be very effective in a

rapidly expanding pandemic where vaccination could be too slow to protect key health workers. For example, A small molecule that can regulate in vivo the gene expression would be very useful for this type of application. The in-vitro antibiotic system (such as the system 'Tet-on') are in vivo unsuitable as the bacterial Trans activator controlled by tetracycline is immunogenic and leads to the removal of transduced cells. A modified human protein responding to a small molecule that is clinically suitable is a major step forward in this field (Raikwar et al., 2019).

Gene delivery also enables active medicines to be generated where necessary. Parkinson's disease, for example, is caused by dopamine deficiencies in the brain, so that dopaminergic neurons are lost and tremors are weakened. A tablet that provides a dopamine precursor with drug in conjunction to enhance blood–brain permeability is a common treatment for Parkinson's disease (Ratical et al., 2018). In early stage conditions, this works well, but decreases in efficacy. The enzymes can be given a more stable local dopamine concentration if they are used to synthesize dopamine directly in the brain. The first lentiviral study and the safety and effectiveness reported are used, but a more efficient delivery or increased gene expression is required in order for full assessment to be carried out. Several efforts have been made to enhance tissue vascularization after cardiac ischemia through gene provision, which encodes vascular growth factors (Svetoni et al., 2016).

Recently, in patients with heart failure, promising results have been reported in local gene delivery using AAV1 encoding the sarcoplasmic recital calcium ATPase (SERCA2A) gene. Improved contractile function and unexpected decrease of arrhythmia have been reported. Additional testing of both AAV1 and SERCA2A is in progress. Gene therapy concentrated in the case of lung disease on the replacement of the gene of the CFTR in patients with cystic fibrosis. Several small studies

have shown CFTR expression and local chloride conductance rehabilitation. In the only clinical effect trial using AAV2 aerosol, however, lung function was not improved. In cystic fibrosis patients due to mucus deposits, the gene delivery to the lung epithelia is particularly difficult (T Rohn et al., 2018). The efficacy of existing drugs can also be enhanced by gene therapy. In combination with O6 Benzyl guanine (O6BG) for example, Temozolomide (TMZ) is used in treating glioblastoma to inhibit methyl guanine methyl transferase (MGMT). A lot of glioblastomas are excessively expressed and TMZ inactivates (De Gioia et al., 2020). The amount of O6BG which could be used is unfortunately limited, since it is very toxic to hematopoietic cells. In hematopoietic cells of the patients with glioblastoma, gene therapy was used to express MGMT mutant P140 K resistant to O6Bg, enabling them to be given more intensive chemotherapy (Cullis et al., 2017).

4.5 Applications of customizing gene

The rapid growth of genome-editing has over the last several years transformed human genome research and enabled researchers to better comprehend the role of a single-gene product to an organism illness. In the seventies, genetic engineering (DNA or RNA modification) created a new boundary in genome editing. Genome editing methods have been rapidly developed in the last 10 years based on manmade or bacterial nucleases and have begun to demonstrate

exceptional usefulness in several domains from basic Research to applied BI Research(Wykes et al., 2018).

4.5.1 Modeling Genetic Diseases in Cells

The conventional technique of genetic alteration in cells is least effective, in particular when the fluorescent reporters conduct targeted gene knockouts or knocks. Customisable nucleases enabled a range of genetic changes to mimic mammalian cells, including cancer, metabolic and neurological disorders (Pahan & K, 2019). Stem cells are commonly employed in labs since they can be differentiated by a certain media to any kind of cell but genome editing is less efficient by homologous recombination and is restricted for the modeling of illnesses.The production of pluripotent stem cells from patient somatic cells by reprogramming provided opportunities for the production of individualized pluripotent stem cells (iPSCs). iPSCs are commonly used to produce diseases that cause mutations in cell culture to imitate disease phenotypes that help to understand molecular and cellular alterations in disease progression. The iPSCs produced for large scale testing and CRISPR knockdown screening can also be distinguished from other cell types to detect novel gene networks that play a part in the progression or preventation of diseases (Schmid et al., 2019). In addition, pluripotent stem cells are frequently employed for the production of diverse organ types. In the in-vitro organoid system, gene-editing instruments are also utilized to simulate human diseases in order to understand both the genesis of the disease and the pharmacological tests. The cells utilized for genetically modified production include the brain, gut, liver, kidney and lung, in many different organ types (Ghosh et al., 2017).

4.5.2 . Epigenetic Modifications

While the early applications of nucleases focused mostly on gene editing, they were rapidly diverted to other uses, including gene regulation. Zinc fingers and TALE have been fused into transcription activators, VP64 and p65 have demonstrated the most powerful in the targeted upstream and in promoter regions in the transcription startup site (Batista et al., 2016). In addition, catalytic dCas9 has been coupled with various transcription activators, repressors, modificers or fluorophores, in order to control the target gene expression, changing its epigenetic condition in the promoter regions**.**To suppress the expression of this gene, dCas9 was fused into the Krüppel-associated box (KRAB) transcription repressor domain, inducing the development of heterochromatin and chromatin modifications (De Leeuw et al., 2019). Silence genes, non-coding RNA and proximal and distal enhancing elements have been demonstrated to dCas9-KRAB (Jin et al., 2018). To activate the genes, numerous fields of activation of a transcription have been coupled to dCas9 or recruited utilizing a dimerized MS2 bacteriophage coat protein binding to gRNA tetraloop and stem-loop 2 to the minimum pin aptamer (Li et al., 2020). Under order to reprogram somatic cells in pluripotent conditions cellularly or for the direct development of fibroblasts into neurons, several genes were at the same time activated using this technique. In addition, the fusion of histone acetiltransferase p300 or the catalyst domain of TET1 to dCas9 methylcytosinseed has been carried out by other epigenetic changes, such as acetyllation and methyllation of the DNA. A recent work was performed with CRISPR and chemical epigenetic modificators to engage endogenous machinery for the activation of genes. A dosage dependent investigation was carried out (Mazzara et al., 2020).

For the first time in vivo, we have shown that Cas9 and transcription activation complexes may be

recruited into target loci by modified RNAs guidance to enable endogenous gene expression. In mice models, we cured acute renal illness, diabetes and dystrophy. She then activated the transgenic mouse model expressing a modified dCas9 system in vivo in order to control the transformation of astrocytes into functioning neurons of various neurogenic endogenous genes. Recently dCas9-VP64 using AAVs in a haploinsufficient mouse model to restore obesity traits.(Mishima et al., 2018). In addition, the transcriptional activation domain CRISPR-Cas12a

has been coupled to allow multiplexing knockout and transcriptive activation in vivo. The CRISPR technology also served to suppress in vivo gene expression. In recent years, several studies have succeeded in producing dCas9, fused to various transcriptional repressors, to reduce the expression of genes that play an important role in reducing the function of the brain, reduce the concentration of low circulating lipoprotein (LDL) and to rectify retinitis pigmentosa. The therapeutic potential for CRISPR-based epigenetic changes in vitro in improving the disease phenotypes is shown in these research (Ross et al., 2014).

4.5.3 Gene Editing in Human Embryos

Genetic mutations are transmitted to the following generation in the parents' germline. Some of these mutations can be deadly and can lead to early completion of embryo development. The mutation may lead to illness later throughout life, in some less severe situations. With advances in diagnosis and the availability of state-of-the-art medical procedures, many illnesses may be treated or prevented(Taran et al., 2020). Nevertheless, the majority of illnesses from parent-inherited genetic mutations still lack efficient techniques for the treatment, and correction of these mutations has been taken into account in the early stage of embryo development. However, in terms not just of repair mechanism of DNA, but also of long-term effects, the genome editing of somatic cells

and germlin differs considerably. Genome editing in somatic cells requires patient cells to be modified in order to treat the condition that can be accomplished by isolation and transplantation once mutation has been corrected (Chare et al., 2014). However, in the early phases of embryo development, a correction should be carried out during the germline editing and the changes can take place in all embryo cells, including germ cells, that will also influence future generations. Alternatively, just some cells in the embryo can be rectified, resulting in a mosaic embryo being generated (Barman et al., 2020).

Several research organizations have been engaged in gene editing in human embryos in recent years. Using human embryo to evaluate the efficacy and off-targets with a system of CRISPR-Cas, more than seven distinct research have been carried out until now (Latour et al., 2019). It is interesting that DSB's were repaired by the endogenous HDR and wild-type alleles as a template during pathogenic mutation correction in human embryos, which differ from the effectiveness of the HDR in pluripotent stem cells reported.In addition to harmful mutation repair, genes were also utilized to determine the role of pluripotent transcription factor OCT4 during early development in human embryos. In addition, basic editing technique has also been utilized to rectify harmful mutations in human embryos (Li et al., 2020). It is worth noting that greater correction effectiveness and increased conversion of homozygotic nucleotides with no overlapping mutations in human embryos of two and four cells compared to zygote have been reported (Kang et al., 2019).

The advent of long-term in vitro embryo culture systems (until 14 days) provided opportunities for a better understanding of early developmental issues for the cultivation of gene-edited embryos. In addition, pluripotent stem cells are currently grown in vitro on various cellular matrices such

that they are self-organized and structures termed synthetic embryos comparable to normal embryos and imitate natural embryo's early developmental programme. Recently, a single stem cell type has been employed to create blastocyst-like structures through the use of extended pluripotent mice and a 3D differentiation system (Dever et al., 2019). There is now a comparable method to the development of synthetic embryos utilizing human pluripotent stem cells. The use of synthetic embryos might be expected in the near future to replace the use of human natural embryos, particularly for gene editing, in fundamental research, to produce different disease models. In particular, the successful development of a synthetic human embryo can somehow circumvent ethical problems relating to the use of human embryos for fundamental scientific objectives (Levy et al., 2020).

Until recently, the goal of genome editing in human embryos was intended to better understand the efficacy of gene correction and early developmental problems without implanting the edited embryos. However, to prevent HIV infection, one researcher in China to prevent HIV infection attempted to modify the *CCR5* gene in the human embryos that were later transferred to a human resulting in the birth of twin babies. This controversial experiment reignited an international debate on the necessity and ethical issues on genome editing in human embryos(Ross et al., 2014).A ban on genome editing in human embryos for therapeutic purposes is presently available in various nations. In accordance with the guidance established by the National Academy, when performed within the regulatory framework and meeting a list of criteria that includes not enough alternatives, preventing the transmission of serious diseases, restricting genes conversion into t, clinical trials may be permitted for heritable genome publishing.Reports on the clinical use of the human germline genome editing coordinated by the American National Academy by the World Health Organization and the International Commission will take place later in this year.(Ratican

et al., 2018). In general, genetic diagnostic (PGD) before implantation may be used to identify mutation-free unmudated embryos for implantation and avoid the development of genome. The selection technique may nonetheless provide a difficulty for families that generate few embryos or when a homozygous autosomal dominant mutation occurs in one of the spouses (Wykes et al., 2018).

Chapter 5

Future studies

The delivery of therapeutic genes to treat neurological diseases has considerable potential in genes therapy. Currently one of the safest methods to treat CNS diseases is considering AAV vectors. Various serotypes are available and new AAV serotypes are rapidly needed, as the promise of gene therapy is becoming more and more acknowledged (Hong et al., 2019). New AAV vectors with enhanced transduction profile, better delivery, and greater transduction are well appreciated in target organs using less invasive methods. This obstacle might be addressed by genetically modifying AAV vectors and designing AAV vectors. Also crucial to the effectiveness of gene therapy is the choice of optimal delivery channels. The effective transfer of CNS is now favoured by direct injection of AAV vectors into the parenchyma(Luo et al., 2019). Although this is an intrusive method, the benefits from injections in the venous system or other fluid-filled compartments are obvious. Intraparenchymal administration gives high transgenic concentration, high local translation, reduced dispersion of transgenes to other organs and less danger of AAV or ectopic expressions of the transgenes for immune responses or toxicities (Ghosh et al., 2017). Management of AAVs via systemic or intrathecal methods requires greater dosages, thereby increasing the risk of toxicity. At the transgenic level likewise, there is much potential for improvement. For instance, new promoters and cis elements might be generated, the codons optimized, and transgenic design more efficiently may increase efficacy and limit expression in certain cell types of CNS (De Gioia et al., 2 020). .

Several AAV-based gene therapy studies have been covered throughout this review for CNS diseases. While all the research have shown that AAV is safe and well tolerated in human CNS, few of these studies have demonstrated effective therapeutic advantage. Too little transduction of

the target organs could have been crucial, but one key issue is the absence of strong predictive preclinical models to translate favorable preclinical results more precisely into the clinic (Fan et al., 2018).

After delivery of in vivo viral vectors, many areas of the CNS are hard to reach and result in the reduction of therapeutic transgenes that may not be adequate to achieve the necessary therapeutic levels overall (Joshi et al., 2017). In addition, the identification of biomarkers to identify new patients and better forecast illnesses would be crucial for early detection. In particular, the provision of previous treatments Neurodegenerative illnesses are crucial to the beginning of neurodegenerative diseases in order to improve the effectiveness of gene therapy. A greater knowledge of the variables that cause these disorders will also aid in the creation of more accurate pre-clinical models for predicting clinical success. Clinical trial results are improving, and the achievements and lessons learnt from previous and ongoing AAV gene therapy clinical studies will be extremely useful for applying to a broader spectrum of neurodegenerative and neuromuscular diseases (Latour et al., 2019).

In addition, its usage in regulating gene expression by altering the epigenetic marks on the promoters provides significant promise in addition to the application of individual nucleases for Gene Edits. Curiously, genetic disorders are not caused just by gene mutations, but also owing to

the decreased expression of these genes, which can impact the tissue and organ function (Mustafa et al., 2020). In particular, with ageing, epigenetic mark dysregulation leads to a reduction in expression of many genes that are necessary for normal functioning of cells and tissues, leading to disease phenotypes finally emerge. As the expression of a target gene without changing the genome sequences has been modulated by customized nucleases, they can be a very appealing way

to treat different illnesses in clinical settings (Chen et al., 2020). We have shown, in particular, that using the CRISPR-Cas system can allow in vivo and reverse disease gene activation. A CRISPR-Cas system for in vivo gene activation can also overcome numerous constraints of conventional gene therapy, including the size and quantity of transgenes that can be delivered (Axelsen et al., 2018). Dysregulation by one single gene or pathway does not cause age-associated disease and may require many genes to be active simultaneously. A multiplex method has to thus be created in which many gRNAs may be supplied to each cell and several genes activated concurrently, which assist to improve the cellular characteristics of aging and the functioning of the tissues. In addition, tissue or cell-specific promoters are used to avoid unwanted gene activations in other tissues and control Cas enzymes or gRNA expressions (Cappella et al., 2019). In conclusion, we have seen in the last several years not only the discovery and development of novel techniques to genome editing, but also their use in the treatment of different diseases. There are now a number of clinical trials using newly created gene editing tools, some of which will ultimately be utilized in clinics, not only to cure genetic disorders, but also to prevent or treat viral diseases like SARS-CoV-2 (Blanc et al., 2020).

Chapter 6

Conclusion

NDs, including Parkinson's disease, Huntington's disease, and Alzheimer's disease, are frequent in age-related, progressive, debilitating, and neuron degenerative illnesses. Advances in medical research and technology have increased the prevalence of NDs by extending human life expectancy (Choi et al., 2015). The absence of precise diagnostic techniques and viable therapies has hampered our knowledge of the pathophysiology of NDs. A growing body of data shows that a common pathogenic characteristic of NDs is the accumulation of aberrant misfolded protein complexes (Fan et al., 2018). The disrupted networks and dysfunctions of neurons may be repaired if the misfolded protein complex–associated genes can be corrected.In a variety of species, gene-editing techniques such as ZFNs, TALENs, and CRISPR/CAS may effectively snip away or add particular regions of DNA to accurately modify the sequence-specific gene (Barman et al., 2020). These approaches can be used to create a specialized ND animal model for studying human illnesses and evaluating prospective medicines on a wide scale. Furthermore, these approaches have the potential to deliver effective therapies for a variety of hereditary human illnesses previously considered to be incurable. ZFNs and TALENs, for example, are based on protein-guided DNA cleavage, which necessitates knowledge as well as time-consuming protein design, assembly, and selection, as well as validation (Ekman et al., 2019).

CRISPR/CAS, a low-cost technique that may concurrently alter genes at numerous locations, is a straightforward and easy procedure. These benefits have led to it being used more frequently than ZENs and TALENs in gene function research, transgenic animal generation with numerous gene mutations, and disease gene correction (Hong et al., 2019). Despite its many benefits, CRISPR/CAS was shown to have a reduced knock-in rate and a significant risk of off-target

alterations in human cellsIn the end, it is believed that by adopting a better gene editing approach, it is not only possible to break down ND riddles but also to fight cellular abnormalities which have accumulated and cause neurodegeneration by maximizing advantages and minimizing downsides (Chen et al., 2016).

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