"ADVANCES AND RECENT TRENDS IN GENE EDITING TECHNOLOGIES FOR GENETIC DISORDERS"

M.Sivasankari[1*], Dr.D. Senthil Rajan[1], A. Nandhini[1], D.Rajeswari[2], M.Tamizharasu[2].

Professor[1*], Professor[1], Professor[1], Student[2], Student[2]

Department of Pharmaceutics and Research, Swamy Vivekanandha College of Pharmacy, Tiruchengode, Namakkal-637205, Tamil nadu, India. (Affiliated to The Tamilnadu Dr.M.G.R. Medical University), Chennai.

Contact no: 9585946159

ABSTRACT:

Recent advances in gene editing technologies have revolutionized the treatment landscape of genetic diseases by enabling precise, targeted, and efficient modification of disease-causing genes. Technologies such as CRISPR–Cas systems, base editing, and prime editing have demonstrated significant potential in correcting both monogenic and polygenic disorders. These tools offer improved specificity, reduced off-target effects, and expanded therapeutic applications compared to traditional gene therapy approaches. Recent trends focus on the clinical translation of gene editing for diseases such as sickle cell anemia, muscular dystrophy, hemophilia, cancer, and neurodegenerative and cardiovascular disorders. Furthermore, the integration of artificial intelligence, epigenome editing, and non-viral delivery systems has enhanced editing accuracy and safety. Despite these advancements, challenges related to ethical considerations, delivery efficiency, immune responses, and long-term safety remain critical. This review highlights recent trends in gene editing technologies, their therapeutic applications in genetic diseases, and future prospects for precision medicine.

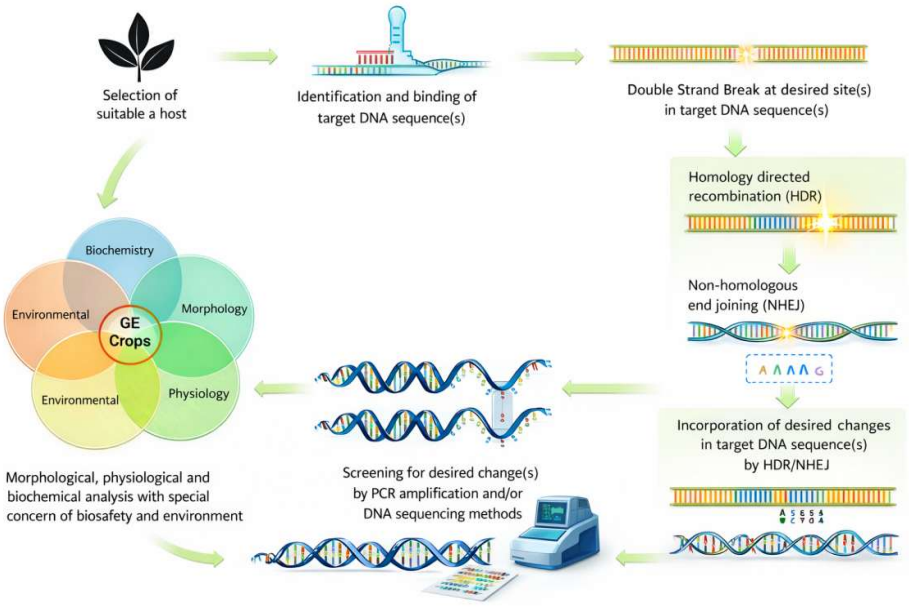
KEYWORDS:

CRISPR–Cas9, Monogenic disorders, Polygenic disorders, Precision medicine, Genome engineering, Gene therapy.

INTRODUCTION:

Gene therapy is a method of treating disease by introducing DNA and RNA into cells.The DNA/RNA transfer into the cells acts into three ways and first it enables the expression of transferred gene and second it inhibits the expression of target gene final stage it modifies the target gene.Cells and organism can manipulated with the help of genome editing to change specific gene sequence(1).Over the last few years, the genome editing has revolutionized research on the human genome and has contribution of a single-gene product to disease in an organism genome editing can be achieved in vitro or in vivo by delivered the editing machinery in situ, which powerfully and "correct" genes as well genomic modification(2).Genome editing technologies have the double strand DNA and at specific genomic loci it has recruit endogenous repair machinery for either non-homologous end-joining(NHEJ) it has four major classes of nucleases, meganucleases and their derivatives and zinc finger nucleases(ZFNs), transcription activator like effector nucleases(TALENs), and CRISPR-associated nuclease Cas9 have been developed to enable site-specific genome editing(3).Gene editing therapies, including CRISPR-dependent base editing can treat a broder range of genetic disease as they can directly correct pathogenic mutations in the genomic DNA(4).Viral vectors are the most commonly usec method of inserting the genetic information into the target cells.The aim of this systemic review was to

provide a broad understanding of the perceived acceptability of gene therapy and gene editing for human use and to highlight factors that influence acceptability[5].



DIGRAMMATIC REPRESENTATION OF GENE EDITING

TOOLS OF GENE EDITING :

Since the original study in 1971 to determine the "ω" self-splicing intervening sequence located within the mitochondrial gene-coding large ribosomal RNA of yeast, Saccharomyces cerevisiae.such nucleases which can recognize a unique DNA target site among the whole genome are now as homing endonucleases defined as microbial DNA- cleaving enzymes that mobilize their own reading frames by generating double-strand breaks at specific genomic invasion sites[6].Compared with their are individual in size as recognize the fully symmetric DNA target sites multiple engineered nucleases, including meganucleases,Zinc finger nucleases(ZNFs),transcription activator-like effector nucleases(TALENs) and the CRISPR-Cas9 have to modify the genomes of model organisms and humans[7].

1.CRISPR-Cas9:

The CRISPR -Cas9 system ,an RNA - guided, nuclease - mediated form of genome editing , represents a major breakthrough in genomic engineering and offers a revolutionary approach to alter the human genome [8]. (Figure no.1: CRISPR-Cas9**)** The CRISPR RNA and CRISPR -associated protein (Cas) systems are now confessed as key components governing bacterial adaptive immune response which consists of three main stages : adaptation. Expression,and interference[9].

Molecular Mechanism of CRISPR-Cas9:

CRISPR-Cas9 gene editing relies on three core components:

- **Cas9 endonuclease** – a programmable DNA-cutting enzyme
- **Single guide RNA (sgRNA)** – a chimeric RNA composed of crRNA and tracrRNA
- **Protospacer Adjacent Motif (PAM)** – a short DNA sequence (5′-NGG-3′ for SpCas9) required for target recognition

Upon sgRNA binding, Cas9 undergoes a conformational change that enables sequence scanning of the genome. Once a PAM is recognized, the sgRNA hybridizes with the complementary DNA strand, activating the HNH and RuvC nuclease domains, which introduce a site-specific double-strand break (DSB).

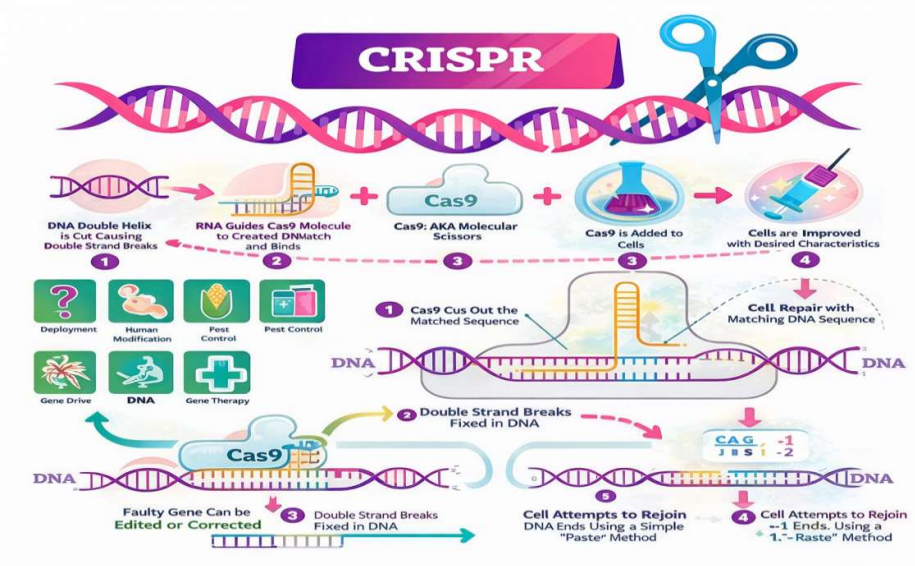


Figure no.1: CRISPR-Cas9

2.TALEN S and 2FNS:

Thus, generation of a unique, site-specific genomic DSB had remained the rate limiting step in using homology-directed repair (HDR) for robust and precise genome modifications of human cells, that is, until the creation of zinc finger nucleases (ZFNs) – the first of the programmable nucleases that could be designed to target and cleave custom sites[10].To cleave a specific site in the genome, ZFNs are designed as a pair that recognizes two sequences flanking the site, one on the forward strand and the other on the reverse strand. (Figure no.2: TALEN S and 2FNS) Upon binding of the ZFNs on either side of the site, the pair of FokI domains dimerize and cleave the DNA at the site, generating a double-strand break of zinc finger nucleases[11].

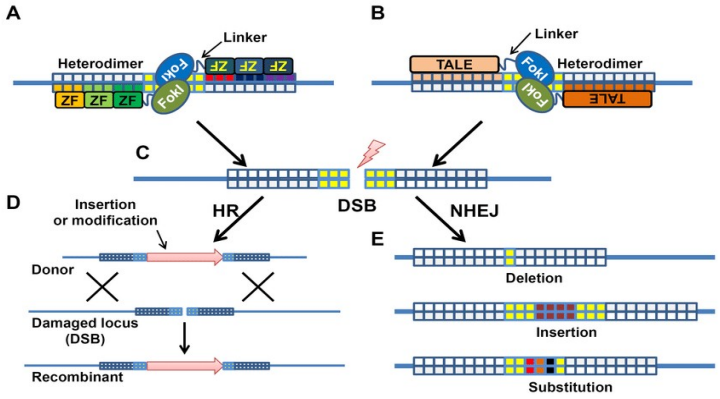


Figure no.2: TALEN S and 2FNS:

3.PRIME EDITING:

The prime editing retains CRISPR'S targeting specificity but carries with it, additional cargo in the form of an edit containing RNA template as a contiguous extension of the guide RNA(known as the pegRNA).It has the high efficiency of editing by single base substitution and provide (C>T; T>C; A>G; G>A) and recent expanded this to include two transversions(C>G and G>C)[12].It is first precise genome-editing technology that allows all the 12 possible base-to-base conversions as well as insertions and deletions that does not require DSBs or donor DNA.It is broad editing spectrum and

human genetic disease and it is conjugated and reverse transcriptase paired with a prime-editing guide RNA and has targeted site (Figure no.3: Prime Editing) [13].

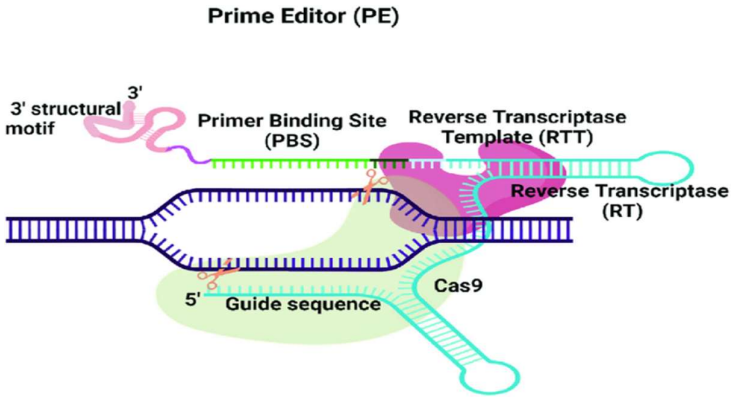


Figure no.3: Prime Editing

4.RNA EDITING:

RNA editing processes are confined to the world of eukaryotes and have been recognized in microrganism and identified in bacterial genes including some in Escherichia coli, post transcriptional changes in RNA editing may also occur in prokaryotes.These modification are most prevalent in tRNA and rRNAs and are often essential for biological function[14]. Inactivation of ADAR gene family members has significant physiological consequences as seen in phenotypic alterations of ADAR gene mutants created in various species one type of RNA editing converts adenosines to inosines(A→I) in double stranded RNA and has protein-coding sequences of a limited number of mammalian genes in recoding and subsequent alterations of their functions. (Figure no.4: RNA Editing)[15].

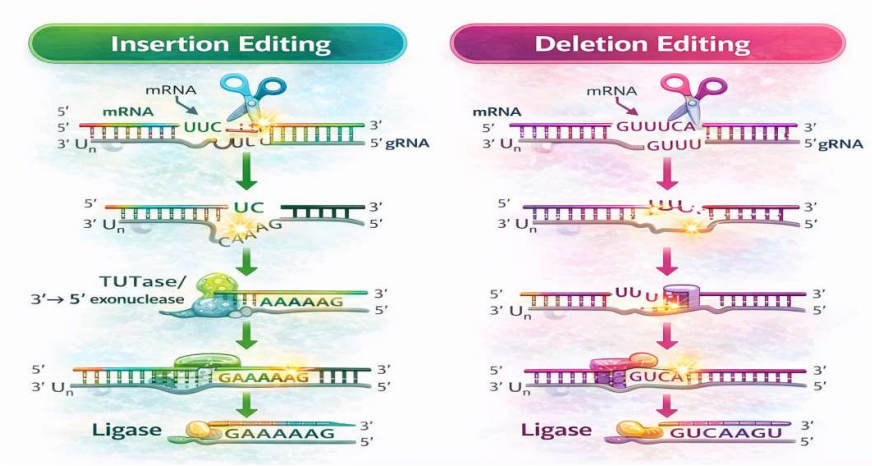


Figure no.4: RNA Editing

5.BASE EDITING:

Base editing holds great potential for the treatment of numerous genetic diseases, through either temporary RNA or permanent DNA base alterations. Recent advances in the specificity, efficiency, precision and delivery of DNA and RNA base editors are revealing exciting therapeutic opportunities for these technologies. We expect the correction of single point mutations will be a major focus of future precision medicine[16]. Base editing directly makes targeted and irreversible base conversion without creating double-strand breaks (DSBs). (Figure no.4: Disease treating of gene Editing) This technology has recently gained quick acceptance and adaptation because of its precision, simplicity, and multiple capabilities[17].

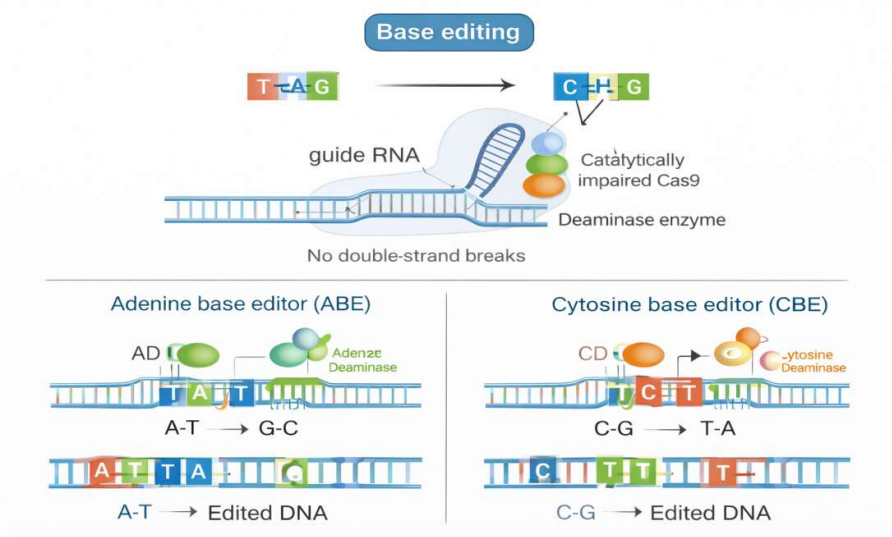


Figure no.5: Base Editing

DISEASE TREATING OF GENE EDITING:

- Monogenic gene editing
- Polygenic gene editing

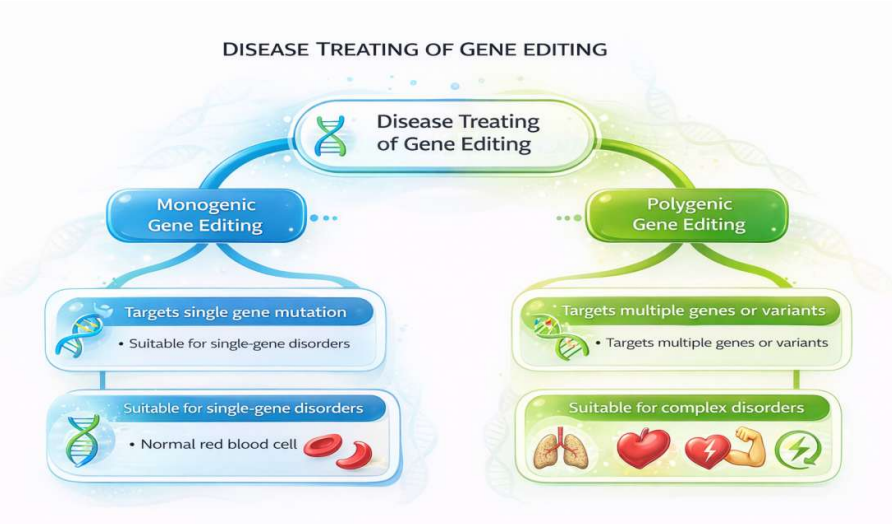


Figure no.6: Disease treating of gene Editing

1.SICKLE CELL DISEASE:

United states and several million around the world are affected by both acute and chronic manifestations of SCD such as frequent pain crises,silent cerebral infarct,stroke, and end organ damage and early death.interferes with red blood cell biconcave architecture and flexibility, resulting in crescent shaped cells with enhanced adherence to the vascular endothelium, and hemolysis that obstructs the blood flow[18].The technology offers the potential to permanently repair disease - causing mutations through correction,deletion, addition, and disruption of the specific sequence mediated targeted DSB generation followed by non- homologus end joining (NHEJ) or homology - directed repair (HDR)[19].

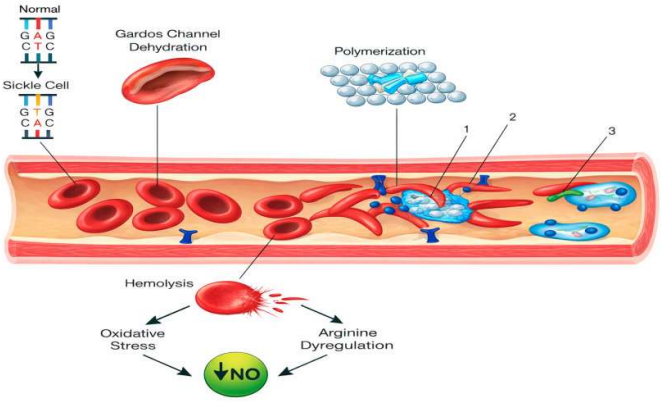


Figure no.7: Sickle cell disease

2.MUSCLE REGENERATION:

Recent advances in gene editing technologies are enabling the potential correction of devastating monogenic disorders through elimination of underlying genetic mutations. CRISPR gene editing has provided new opportunities to ameliorate the disease by eliminating DMD mutations and thereby restore dystrophin expression throughout skeletal and cardiac muscle. Proof-of-concept studies in rodents, large mammals, and human cells have validated the potential of this approach, but numerous challenges remain to be addressed, including optimization of gene editing, delivery of gene editing components throughout the musculature, and mitigation of possible immune responses[20]. (Figure no.8: Muscle Regeneration) This additional strategies are needed to reduce the dystrophic pathology and restore muscle mass and func tion.These include, but are not limited to, inflammation prevention, muscle growth and regeneration, fibrosis, and improving mitochondrial function[21].

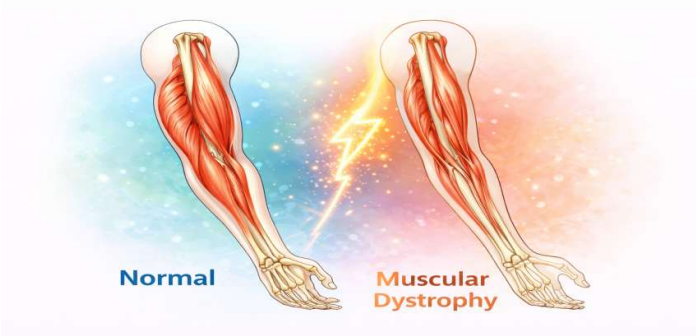


Figure no.8: Muscle Regeneration

3.CYSTIC FIBROSIS:

New genetic technologies and advances in molecular medicine allow huge amounts of information to be generated from individual sample in the form of genomic, transcriptomic, and proteomic data.However analysis and interpretation of this flood of findings, most notably putting them into the right biological context, represent one of the main challenges for research and medicine today this review is to provide insight into role of modifier genes as an important source of phenotypic variability in so-called monogenic disorder example of cystic fibrosis[22] .The pancreas is one of the earliest-and most commonly -affected organs in people with cystic fibrosis was named after the fibrocystic lesions observed in the pancreas from pediatric autopsy cases cystic fibrosis the reproductive system with a majority of adult male patients showing congenital bilateral absence of vas deferens[23].Cystic fibrosis is a rare genetic disease affecting the people the gene encodes a chloride/bicarbonate channel thet plays

an essential role in the fluid and electrolyte balance across secretory epithelia (Figure no.9: Cystic Fibrosis)[24].

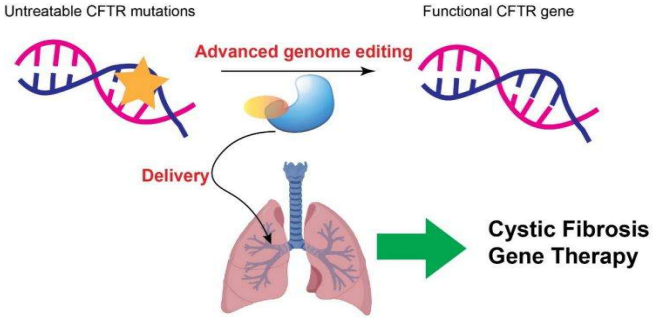


Figure no.9: Cystic Fibrosis

4.HEMOPHILIA A and B:

Hemophilia is caused by various mutations in blood coagulation factor genes, including factor (FVIII) and factor IX(FIX), that encode key proteins in blood clotting pathaway hemophilia, an inherited bleeding disorder, can be caused by deficiency in various blood coagulation factor proteins[25]. Deficiency of the factor leads to prolonged bleeding that occurs spontaneously or after injury the incidence of hemophilia B in the world is traditionally estimated at 1 in 30,000 male births worldwide, as males are the most affected, and females serve as carriers[26]. (Figure no.10: Hemophilia A and B) General treatment for hemophilia involves the use of recombinant proteins, but repeated administration is required and recombinant cogulation factors with extended half-lives have been developed to overcome the short-term therapeutic effect of recombinant clotting factor adeno-associated virus(AAV)-mediated gene therapy has reduced bleeding events and prolonged bood coagulation factor synthesis and gene therapies of high tissue-specific tropism and efficient transduction potential[27].

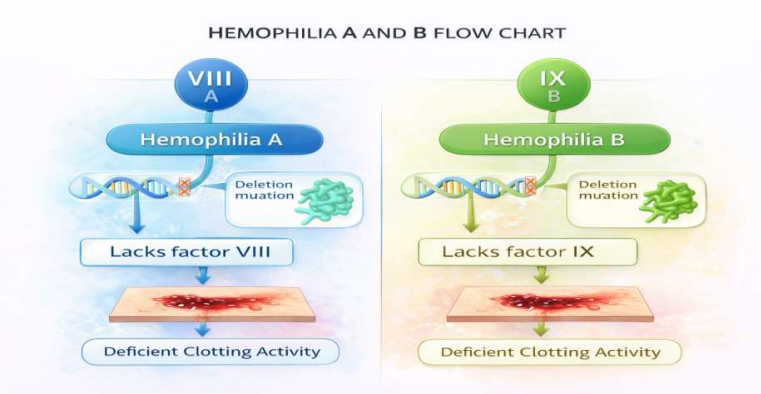
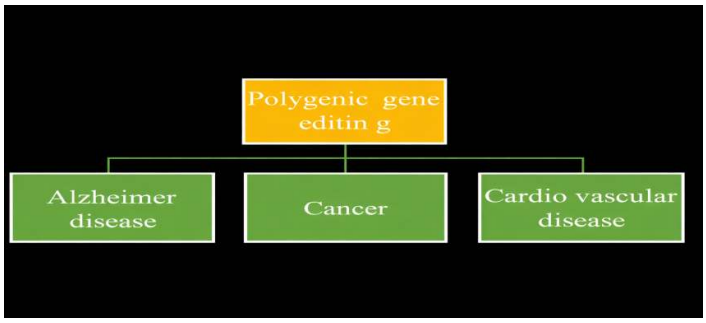


Figure no.10: Hemophilia A and B



5.ALZHEIMER DISEASE:

Alzheimers disease(AD) is the most common and age-related form of dementia it has most possible therapeutic target is mostly unsuccessful and the products of several pharmaceutical giants development for alzheimers treatment it has mainly inhibitors of acetylcholine esterase(AChE) directed to boosting cholinergic transmission in the brain of patients[28]. Alzheimer's disease (AD) is a progressive neurodegenerative disorder and the most common cause of dementia, characterized by memory loss, cognitive decline, and behavioral impairment. The disease results from complex interactions among amyloid pathology, tau dysfunction, neuroinflammation, synaptic failure, and neuronal death. (Figure no.11: Alzheimer disease**)** Alzheimers disease is generally categorized into two groups: early-onset alzheimer disease(EOAD) that usually starts in one's life and late-onset(LOAD) or sporadic alzheimer disease it is different non-genetic factors including oxidative stress, inflammation, lipid metabolism, and gene-environment interaction are responsible for inducing the disease[29].The basics of CRISPR/Cas9 neurodegenerative disease have the RNA-guided clustered regularly short palindromic repeats associated nuclease 9 system revolutionary genome editing tool derived from the bacterial type 2 adaptive immune system and it has the guided spacer sequence known as "Protospacer adjacent matif" it is viral gene and share all the common data in one end the ultimate goal of the system to treat genetic disease including alzheimer disease[30].

- **Amyloid-β Accumulation**- Abnormal APP processing leads to toxic Aβ oligomers and plaque formation, causing synaptic damage.
- **Tau Hyperphosphorylation**- Tau detaches from microtubules and forms neurofibrillary tangles, disrupting neuronal transport.
- **Synaptic Dysfunction**- Aβ oligomers impair synaptic plasticity and neurotransmission, leading to memory loss.

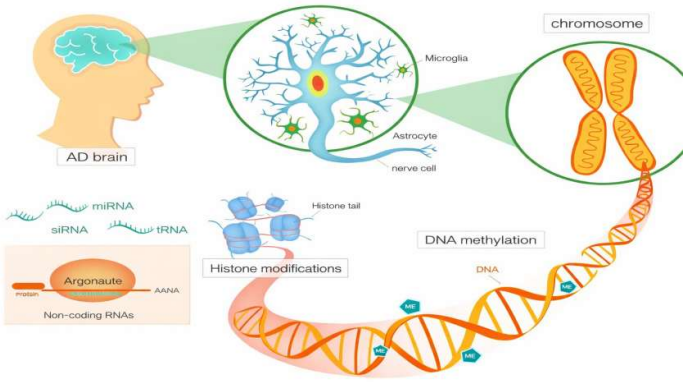


Figure no.11: Alzheimer disease

6.CANCER:

CRISPR-based gene editing technology has the potential to revolutionize the way for treating cancer by allowing for precise and efficient manipulation of the genome totarget specific genetic mutations that drive the growth and spread of tumors the identification of specific gene target and illustrates the design and construction of CRISPR guide RNA libraries, essential for precise genomic targeting[31]. Cancer is a refractory disease with high mortality and global attention the malignant tumor causes 1 out of 6 deaths globally thus threatening lives of thousands of human beings in the field of cancer therapy, including surgery, radiotherapy, chemotherapy, targeted biotherapy and new combination therapies, high post-operative recurrence and radiation chemotheraphy resistance and harmful toxic side effects continue to be barriers to survival time and quality of life[32].Genome editing holds graet potential for cancer treatment due to the ability to precisely inactivate or repair cancer-related genes the first cancer limitless relicative potential a cancer hallmark that have expansive tumor burden whereby editing a small number of cells would not be able to reverse disease symptoms (Figure no.12: Cancer disease)[33].

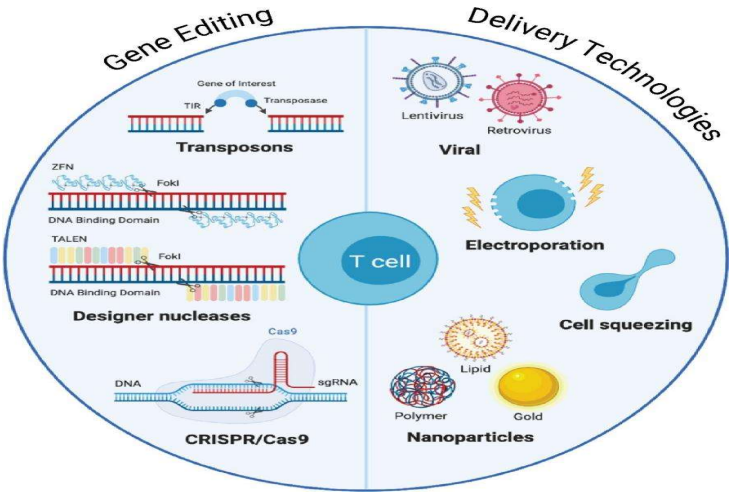


Figure no.12: Cancer Disease

7.CARDIOVASCULAR DISEASE:

Genome editing holds the potential to revolutionize the treatment of chronic cardiovascular disease such as coronary heart disease and existing pharmacological treatment for coronary heart disease require taking the pills daily or receiving injections every few weeks to months-for the rest of the lifetime in order to full therapeutic benefit genome editing results in permanent changes at the DNA level and offers the possibility of "one-and done"therapies that would confer long-lasting protection against disease[34].Cardiovascular disease are the most common causes of morbidity and mortality and frequency increase in upcoming years the major genome editing tools are nucleases, base editiors, and prime editors ana some gene editing strategies are CRISPR-Cas9 designed for pathogenic mutations like small insertions and nucleotide varients these mutations typically leads to a dysfunctional and non-functional expression of protein[35].Cardiovascular disease advances are still needed as to encompasses both hereditary and acquired diseases genome editing have causal editing of cardiovascukar disease with a single application it can permanently change the expression of a target protein that could result in life-long therapeutic benefit (Figure no.13: Cardiovascular disease) [36].

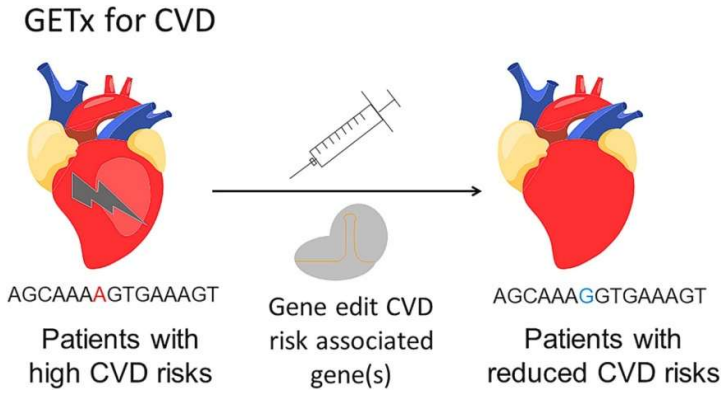


Figure no.13: Cardiovascular disease

CONCLUSION:

Recent trends in gene editing technologies have transformed the therapeutic approach to genetic diseases by enabling precise and efficient genome modification. Innovations such as CRISPR–Cas systems, base editing, and prime editing have expanded treatment possibilities for both monogenic and polygenic disorders. These advances have shown promising results in diseases including sickle cell

anemia, muscular dystrophy, hemophilia, cancer, and cardiovascular disorders. The integration of artificial intelligence and improved delivery systems has further enhanced editing accuracy and safety. Despite these developments, challenges related to ethical concerns, off-target effects, and long-term clinical safety persist. Continued research and regulatory refinement are essential for successful clinical translation. Overall, gene editing technologies hold significant potential for advancing precision medicine and personalized genetic therapies.

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