Gene Therapy for Rare Genetic Disorders

By

Atia Tanjum
15346034

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

School of Pharmacy
BRAC University
October 2022

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.

4. I have acknowledged all main sources of help.

Student's Full Name & Signature:

Atia Tanjum
15346034

Approval

The thesis titled "Gene Therapy for Rare Genetic Disorders" submitted by Atia Tanjum (15346034) of Summer 2015 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy on October 2022.

Examining Committee:

Supervisor:

_____
Tanisha Tabassum Sayka Khan
Lecturer, School of Pharmacy
BRAC University

Program Coordinator:

_____
Namara Mariam Chowdhury
Lecturer, School of Pharmacy
BRAC University

Assistant Dean:

_____
Professor Dr. Hasina Yasmin
Assistant Dean, School of Pharmacy
BRAC University

Dean:

_____
Professor Dr. Eva Rahman Kabir
Dean, School of Pharmacy
BRAC University

Ethics Statement

The study does not involve any kind of animal or human trial.

Abstract

Genetic disorders are widespread. However, many of these genetic illnesses have minimal information, making therapy difficult. Gene therapy has emerged as a major breakthrough in the field of medical science to treat diseases resulting from defective genes. The FDA has received over 900 gene therapies for hereditary disorders. Rare genetic illnesses are life-threatening and impact 5 per 10000 people worldwide. Researchers are conducting clinical trials to investigate treatments for rare genetic disorders despite a paucity of knowledge. With sufficient clinical trial data showing signs of improvement among the patients and safety of gene delivery systems, gene therapy can be the most efficient treatment strategy for rare genetic disorders. In this review paper, a number of rare genetic disorders and the prospects of gene therapy in the treatment of those disorders were discussed.

Keywords: Gene therapy; vectors; rare genetic disorders; clinical trial

Dedication

Dedicated to myself

Acknowledgement

I am grateful to the Almighty Allah for all the blessings and allowing me to successfully complete my undergraduate thesis.

My sincere appreciation goes to my respected supervisor, Tanisha Tabassum Sayka Khan, Lecturer, School of Pharmacy, BRAC University for her guidance and support throughout my project work.

At last, I would like thank all my faculty members for their valuable guidance and support throughout my undergraduate study.

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List of Acronyms

RD   Rare Diseases

CAR-T   Chimeric Antigen Receptor T

FDA   Food and Drug Administration

GMP   Good Manufacturing Practice

AAV   Adeno-Associated Virus

HSV   Herpes Simplex Viruses

PEG   Polyethylene Glycol

UDP   Undiagnosed Disease Program

ICD   International Classification of Diseases

CF   Cystic Fibrosis

IRD   Inherited Retinal Dystrophy

ADA   Adenosine Deaminase

SCID   Severe Combined Immune Deficiency

SNV   Single Nucleotide Variations

LOF   Loss-of-function

GWAS   genome-wide association studies

UDP   Undiagnosed Disease Program

Chapter 1

Introduction

1.1 Background

In order to address genetic disorders, a treatment method called gene therapy involves transferring functional gene into the diseased cells. The goal is to change the genetic makeup of the patient's disease-causing cell, restore that cell to health, then repeat the process in other cells (Maldonado, Jalil, & Wartiovaara, 2021). The term rare diseases (RDs) refers to conditions that are either fatal or severely disabling and affect a limited number of people (less than 5 per 10,000). A sizeable fraction of the global population is collectively affected by rare genetic illnesses. Limited information is available for many disorders, making it challenging for clinicians to distinguish between diseases that share clinically similar symptoms. This study is crucial because genetic engineering has gained prominence as a possible treatment for uncommon genetic illnesses, especially those with a monogenic etiology. The purpose of this review paper is to provide an overview on gene therapy and discuss its potential for treating rare genetic disorders.

1.2 Objectives

The objectives of this study are:

- to provide an overview on gene therapy

- to provide a comprehensive insight on rare genetic disorders and the current development and prospects of gene therapy for the treatment of rare genetic disorders.

1.3 Rationale

There have been 3500 genes associated with various genetic diseases. One of the most extensively researched therapeutic approaches in the area of medicine, genetic engineering is a potent option for treating genetic disorders. Most medicines have been created for people who have long-term and recurrent illnesses as well as for diseases that impact a significant number of people. Patients who are detected with rare diseases thus frequently have little or no therapeutic choices available to them. For uncommon genetic illnesses, especially those with a monogenic origin, gene therapy has become a feasible treatment option. The purpose of this paper is to explore the potential of gene therapy for the treatment of rare genetic diseases.

Chapter 2

Methodology

The keywords "gene therapy," "gene therapy for rare genetic condition," "treatments for rare genetic disorders," "gene therapy and genetic disorders," "rare genetic disorders," and "gene therapy and sophisticated treatments procedures" have been used for the search of scientific articles.The articles were collected from journals indexed in databases such as PubMed, Google Scholar and Scopus. Prior literature search, an outline was created for data collection in a systematic manner. Many articles were screened and only the ones relevant to the topic were used for this review paper.

Chapter 3

Gene Therapy

3.1 What is Gene Therapy?

By altering a person's DNA, gene therapy can be used to treat or perhaps eradicate some disorders. There are several ways by which gene therapies can work:

- injecting a patient with a novel or modified gene to treat a disease

- replacing a faulty gene with a functional one.

- knocking in or silencing a faulty gene.

- More than 1700 authorized clinical trials of gene therapy have been conducted worldwide and 20 years have passed since the first trials were conducted. Despite the challenges gene therapy has experienced, more success stories are emerging (Wirth et al., 2013).

Currently, gene therapy is used to treat cancer more frequently than any other disease. Following single gene and systemic diseases, it makes up more than 60% of all clinical studies of gene therapy that are now being conducted worldwide. There have been or are presently being done more than 1800 authorized clinical gene therapy research trials worldwide. Adenoviral vectors, retroviral vectors, and bare plasmids have been the most frequently used gene transfer vehicles in clinical trials (Wirth et al., 2013).

3.2 Types of Gene Therapy

Gene therapy can of two types: somatic cell gene therapy and germ line gene therapy, and this classification is based on the type of cell into which the gene is delivered. A gene inserted into the somatic cells is known as somatic cell gene therapy. The goal of somatic cell gene therapy is to treat the patient's illness, with little or no chance of gene transfer to the offspring. In germ line gene therapy, DNA is inserted into the reproductive cells-egg or sperm. This method of

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treatment makes it possible to fix disease-causing gene mutations, which are passed down from parents to offspring in a manner that cannot be avoided. This kind of gene editing has safety concerns as well as moral dilemmas related to any unintended consequences that could result in undesirable modifications that could be passed onto future generations.

Genetic material can be introduced into the diseases cells via two approaches.

1. *In vivo* approach, where the functional copy of the gene is injected directly into the diseased cells using a vector.

2. *Ex vivo* approach, where the diseased cells are isolated from the patient, genetically modified in the laboratory and reintroduced into the patient's body.

3.3 Applications of Gene Therapy

Over 30 million people in the US are affected by much more than 7,000 rare conditions, which are hereditary disorders brought about by gene abnormalities. Numerous scientists have argued for more than 30 years that genetic changes might give efficient treatments for a wide range of hereditary human disorders, giving long-lasting and potentially curative clinical effects. The chimeric antigen receptor T (CAR-T) cell treatments (Yescarta and Kymriah) and Strimvelis (the gammaretrovirus licensed for ADA-SCID) are some of the gene therapies that have been approved for commercialization and are currently accessible. Numerous more therapies are undergoing clinical testing (Mendell et al., 2021). The first adeno-associated virus gene therapy to accomplish this feat was Glybera, which received Food and Drug Administration (FDA) approval in October 2012. Glybera treated genetic Lipoprotein Lipase Deficiency, which causes eruptive fat-filled patches, pancreatitis, and recurrent stomach pain as a result of extremely high triglyceride levels. However, it was extremely challenging to continue delivery of the therapeutic commercially due to the disease's rarity, its cost to the patient, and the

company's cost to maintain therapeutic readiness. After 2018, when only 31 patients had received treatment worldwide, this type of gene therapy was no longer offered. In order to treat individuals with biallelic RPE65 mutation-associated ocular dystrophy, a rare form of inherited blindness, Luxturna was granted FDA approval in 2017 and the approval in 2018. About 1,000 in 2000 people in the U.S. who carry a variation in both versions of the gene RPE65 are affected by the illness. Luxturna gives patients' retinal cells a healthy copy of RPE65, enabling them to produce a protein required for translating light into electrical signals and regaining their vision. FDA authorized Zolgensma in May 2019 for children under the age of two who have spinal muscle atrophy (affecting around one in 10,000 people globally). In just one therapy, Zolgensma gives a patient's motor neurons a healthy version of the normal SMN gene. For the treatment of refractory or relapsing large B cell lymphoma, Yescart received approval by the FDA in 2017 and EC in 2018. It was created to treat a malignancy called big B cell lymphoma. The treatment, which is a component of a technique that is called CAR-T cell treatment, entails making use of a virus in order to insert a gene into a patient's T cells that creates CARs. This gene is responsible for producing CARs. When these cells are given to the patient's body, they are equipped with CARs, which give them the ability to connect to cancer cells in circulation and destroy them. Kymriah was created for those suffering with B cell lymphoblastic leukemia, a disease of the white blood cells that primarily affects children and young adults. FDA and EC approval for Kymriah came in 2017 and 2018, respectively. It functions by giving a patient's own T cells a particular gene that gives them the ability to locate and eradicate cancer cells.

3.4 Strategies for Delivery of Gene Therapy

3.4.1 Viral Vectors

The target place where a scientist will wish to deliver gene therapy to cure a genetic abnormality is a host cell, and viruses serves an appropriate tool for doing this. The inherent

structure of a virus makes it particularly efficient at invading a cell. Many viruses have had their whole genomes—the entire collection of genes that make up an organism—mapped or generated by scientists. They are able to separate the virus genome's elements that are efficient at entering cells from the virus genome's potential pathogenic components. Only a few, harmless components of the initial virus blueprint are utilized, and these components by themselves are insufficient to spread a virus. Although there are numerous viral blueprints, the selection of a vector depends on factors including the length of expression of genes, the amount of genetic material it can accommodate, and immunogenicity. The vector enters the target cell, crosses the cell membrane, moves inside the cell, and eventually reaches the nucleus where it disassembles. The genetic material guides the cell to produce the desired therapeutic protein. The vector is then naturally broken down by the cell and eliminated by the body.

There is a very wide range of viral vectors, facilitating both short-term and long-term gene expression. In addition, there is a wide variety of vectors, including RNA viruses and DNA viruses, each having either a single- or double-stranded genome.

3.4.1.1 Types of Viral Vectors

3.4.1.1.1 Adenovirus: These are undoubtedly the foundation of the most widely utilized viral vectors. Naked dsDNA adenoviruses are capable of packaging 7.5 kb of foreign DNA, which is sufficient for rapid episomal transcription of the target gene in many cell types. In contrast to the robust immune responses elicited by the original viral vectors, it has been found that the gutted second and third generation vectors having deletions are far less immunogenic. Developing packing cell lines for mass synthesis of Good Manufacturing Practice (GMP)-grade recombinant particles to assist clinical trials has received a lot of attention (Slade, 2018).

3.4.1.1.2 Adeno-associated virus (AAV): Only 4 kb inserts can be packaged since AAV vectors have a short ssRNA genome. AAV is typically thought to provide minimal levels of

pathogenicity and toxicity and, through chromosomal integration, to offer long-term transgene expression. The immunological response brought on by repeated delivery is one of the uses of AAV that is restricted. Introducing a different AAV serotype for every re-administration has solved this issue. The restricted ability of foreign DNA to be packaged into hybrid AAV particles is a different problem. Dual AAV vectors have been developed to solve this weakness (Slade, 2018).

3.4.1.1.3 Herpes simplex viruses (HSV): HSVs are huge enveloped dsDNA viruses that cause life-long latent neuroinfection and long-term transgene expression due to their catalytic and delayed nature of infection. Expression vectors with nontoxicity and great packaging capacity for >30 kb foreign DNA were created by deleting the HSV genes (Slade, 2018).

3.4.1.1.4 Retrovirus: In contrast to HSV, retroviruses have an envelope-enclosed ssRNA genome. As previously mentioned, the therapy for SCID patients has been difficult since retroviruses typically randomly integrate into the host DNA. This flaw, however, has spurred the creation of safer vectors with focused integration and better helper cell lines. These have served as the industry standard carriers for long-term applications of gene therapy because they can hold up to 8 kb of viral inserts. The inability of retroviruses to infect non-dividing cells is one of their drawbacks, which has increased interest in using lentivirus carriers for gene therapy. Despite being members of the retrovirus family, lentiviruses can infect both proliferating and non-dividing cells with little damage. Lentiviruses have grown in popularity for therapeutic applications which require long-term expression because they have the same packaging ability and chromosome integration as conventional retroviruses (Slade, 2018).

3.4.2 Non-viral Vectors

A delivery method without the aid of a virus serves as a non-viral vector. Non-viral vector and their capacity to transfer genetic information to the cell are mostly being researched chemically

and physically. Researchers can directly deliver genetic information to specific cells using physical methods (for example, using an injection needle). Chemical techniques are less likely to cause immunological reactions since they employ organic or artificial materials that are friendly to the human body. Nanoparticles, polymers, and fat molecules are all in this category.

Non-viral vectors might make it easier for therapeutic genetic material to be expressed over an extended period of time, albeit this hasn't been demonstrated by researchers. Non-viral vectors are now being examined for use in clinical trials for gene therapy.

3.4.2.1 Physical methods

Due of their simplicity, physical methods of delivering gene material are more appealing to researchers studying gene therapy. These techniques use physical force to overcome the cell membrane barrier and enable the transfer of genetic material inside cells.

3.4.2.1.1 Needle: A syringe with a needle is used to inject the genetic information of interest into the target tissue or into the body as a whole. There is no need for a carrier, and the process is quick and simple. Solid tumors, liver, muscle, skin, heart muscle all provide good candidate tissues. However, the effectiveness is poor because mononuclear phagocytes quickly degrade the substance via nucleases in serum and remove it (Ramamoorth & Narvekar, 2015).

3.4.2.1.2 Ballistic DNA: Other names for ballistic DNA include micro projectile gene transfer, particle bombarding, and gene gun. Initially, this method was used to introduce new genes into plant species. The concept behind the method is to rapidly transport large amounts of metal coated in DNA across the tissue of interest. The required velocity can be achieved using high-voltage spark discharge, electronic discharge, spark discharge, or helium force discharge. The crucial factors in influencing the effectiveness of gene transfer are particle size, gas stress, and dose frequency. Metal particles with an average diameter of 1 m include gold, tungsten, and silver. The accurate distribution of DNA dosages is the gene gun's main benefit. It is most

frequently employed in studies on ovarian cancer gene therapy (Ramamoorth & Narvekar, 2015).

3.4.2.1.3 Electroporation: It is also known as electro gene transfer, electronically mediated gene therapy, gene electro injection, and electrode position. When an electric field exceeds the capacitance of the cell wall, charges of opposite polarity align along the two sides of the membrane, generating a potential imbalance. As a result, the membrane tears, creating a pore that lets the molecule pass. Pore creation takes place in about 10 nanoseconds. Depending on the field intensity and pulse duration, the membrane's porosity may be reversible. Cells survive if the condition is reversible; otherwise, cell death occurs. In cancer treatment, irreversible electroporation is utilized to kill cancer cells. The intensity and duration of the pulse regulate how permeable the membrane is to gene transfer. Currently, there are two types of field strengths being used: high field strengths and low field strengths, with either long or short pulses (milliseconds). This set of parameters is determined by the target tissue. Cancer cells often need a long pulse and a low field strength, whereas muscle fibers need a short pulse and a high field strength. One trustworthy physical technique for transferring plasmid DNA is electroporation. The treatment can be administered intratumorally, intramuscularly, or both (Ramamoorth & Narvekar, 2015).

3.4.2.1.4 Sonoporation: It is a non-invasive site-specific method that momentarily opens up the cell barrier to allow DNA to enter the cell. Interest-related genetic material is integrated into a microbubble and given to the body's circulatory system. Ultrasound is then applied externally after that. The cavitation of the microbubble by the ultrasonic wave within the target tissue's microcirculation causes biological events that lead to the precise transfection of the therapeutic gene. Small bubbles are made out of a high molecular weight gas filled core (air, inert gas, nitrogen), such as perfluorocarbon or sulfur hexafluoride. Lipids, or synthetic biopolymers, proteins make up the outer layer, which is composed of biocompatible

substances. Red blood cells in motion are similar to microbubbles (mean diameter: 2-4 m). The sonoporation technique is typically applied to the tissues of the brain, cornea, kidney, peritoneum, and muscles and heart (Ramamoorth & Narvekar, 2015).

3.4.2.1.5 Photoporation: This mechanical technique creates brief pores on the cell membrane using a single laser system to let DNA enter the cell. The laser's efficiency is controlled by the central focus and pulse frequency. According to this claim, electroporation and transgenic expression are both at the same level. There is no concrete proof for this procedure (Ramamoorth & Narvekar, 2015).

3.4.2.1.6 Magnetofection: Magnetofection is based on the idea that drugs could be delivered to specific magnetic regions. The process is based on joining healing genes with magnetic nanoparticles. The cell culture receives this complex. The field gradient that is produced by the rare earth electromagnets that are positioned underneath the cell culture expedites the process of transfection while also hastening the sedimentation of the complex. The medical gene-magnetic particles complex is given intravenously in the case of in vivo. The complex is drawn into and maintained at the target by powerful external magnets with high gradients. Enzymatic cleavage of the cross-linking molecule, charge interaction, or matrix degradation all result in the liberation of the genetic material. For disrupting the cell primary cells and cells that are challenging to transfect using other methods, this technique is mostly employed in in vitro research (Ramamoorth & Narvekar, 2015).

3.4.2.1.7 Hydroporation: It is a process for transferring genes hydrodynamically. The cell membrane is penetrated using hydrodynamic pressure in this method. By quickly infusing a high volume of DNA solution, hydrodynamic pressure is produced. As a result, the capillary endothelium becomes more permeable and pores are formed in the plasma membrane that surrounds the parenchymal cells. Through these membrane pores, the therapeutic desired gene

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can enter the cell. Later, these membrane pores seal, retaining the genetic information inside the cell. The majority of the time, this method is applied to studies on gene therapy in hepatic cells.

It is based on the concept that physical stimulation of the liver causes temporary membrane abnormalities for a few moments, which makes it easier for plasmid DNA to diffuse into hepatic cells. However, there is few research on physical massage as a mechanism of gene transfer, and the topic is hotly contested (Ramamoorth & Narvekar, 2015).

3.4.2.2 Chemical Methods

Inorganic particles, polymer-based, and peptide-based chemical vectors are the four basic categories into which they can be divided.

3.4.2.2.1 Inorganic particles: These are often nanoparticles that can be designed by changing their shape, and porosity in order to get through the reticuloendothelial system or to prevent the deterioration of a molecule that has been trapped inside. The most researched materials in this category are calcium sulfate, silica, magnetic compounds, gold, quantum dots, carbon nanotubes, fullerenes, and supramolecular systems (Ramamoorth & Narvekar, 2015).

3.4.2.2.2 Calcium phosphate: The initial particles utilized in this technique were calcium phosphate ones, both biodegradable and biocompatible. Because it is easily absorbed and has a high binding affinity, calcium plays a crucial function in endocytosis. However, as calcium phosphate nanocrystals develop over time, their storage capacity is reduced. Magnesium is eventually added to the mix to resolve this (Ramamoorth & Narvekar, 2015).

3.4.2.2.3 Silica: It is a key ingredient in many materials that people frequently use, including sand glass and sand. It is appealing to utilize as a genetic delivery device due to its very simple functionalization. Because of their low toxicity, amino silicones are utilized to functionalize nanoparticles to produce the silica that is most frequently used as a gene delivery agent. A

significant limiting issue is the media's reduced ability to transport it when serum is present because of the interactions between serum proteins (Ramamoorth & Narvekar, 2015).

3.4.2.2.4 Gold: Researchers were drawn to gold nanoparticles because of their easy synthesis, limitless surface characterization, and inert nature. Near-infrared light is strongly absorbed by gold nanoparticles. Tissues can be deeply penetrated by near infrared light. Using the photothermal effect, it is possible to transfect cells by changing the surface of gold with DNA. The light thermal effect-induced thermal denaturation aids in regulating gene release. Studies had demonstrated that the efficiency of transfection using gold is comparable to that of lipoplexes, despite having a lower level of toxicity in vitro. The main issue is that because of its great chemical stability, it is difficult to dissolve in cells, which causes buildup and could impair cell growth (Ramamoorth & Narvekar, 2015).

3.4.2.2.5 Quantum dots: A semiconducting nanomaterial and supramolecular systems, as well as magnetic nanoparticles (supermagnetic iron oxide, primarily magnetite), carbon nanotubes (cylindrical fullerenes), fullerenes (soluble carbon molecules) and magnetic nanoparticles all reported some encouraging results in in vitro and animal models. These inorganic nanoparticles' surfaces can be coated to promote DNA binding. The idea is that tiny particles can effectively cross most cellular and physiological barriers, leading to increased transfection efficiency. To hasten its clinical implementation, more research is still needed on the long-term safety and surface functionalization effects of size, type, and form on transfection efficiency (Ramamoorth & Narvekar, 2015).

3.4.2.3 Synthetic or Natural Biodegradable

3.4.2.3.1 Cationic lipids: For the purpose of cationic gene transfer, hundreds of different lipids have been developed. Each one of them has a linker structure that joins a positive charge hydrophilic head and a hydrophobic tail. In nucleic acids, the positive charge head group

interacts with the negative phosphate group to form the distinctively compacted structure known as a lipoplex. The overall geometric form, the quantity of charged groups per molecule, the type of lipid anchor, and linker bondage all affect the efficacy of transfection. Lipoplexes, which have a positive charge, interact electrostatically with glycoproteins and proteoglycans found in cell membranes that have a negative charge, which may help facilitate the uptake of nucleic acids by cells. The genetic material is protected from extracellular and intracellular nucleases by positively charged lipids around it. Surface charge, on the other hand, is an issue since it shortens the ½ of lipoplexes in blood circulation, restricting their usefulness to vascular endothelial cells alone. For surface shielding, a neutral polymers like polyethylene glycol (PEG) is employed to reduce the excessive charge and lengthen the half-life. Even though they are thought to have little toxicity, lipoplexes turn harmful when the ratio of lipid to DNA exceeds 3:1 (Ramamoorth & Narvekar, 2015).

3.4.2.3.2 Lipid nano emulsions: These are examples of an emulsion, which is a dispersion of one liquid into another liquid of a different consistency that has been stabilized. They are 200 nm-sized particles made up of water, surfactant, and oil. Lipid nano emulsion is thought to have better scaling up and stability than liposomes, allowing it much more serum resistant. They are less hazardous than liposomes, according to the study (Ramamoorth & Narvekar, 2015).

3.4.2.3.3 Solid lipid nanoparticles: Lipid is the main component of solid lipid particles, which are solid at both temperatures. It benefits from cationic lipid and lipid nano emulsion in equal measure. It has been demonstrated that cationic solid lipid nanoparticles can successfully shield nucleic acids from being degraded by nucleases. It is now the preferred siRNA delivery method (Ramamoorth& Narvekar, 2015).

3.4.2.3.4 Peptide-based: This type of vectors are preferred over all other non-viral vectors because they are well packed, protect DNA, target certain cell receptors, rupture the endosomal membrane, and deliver genetic cargo to the nucleus. Basic residues such lysine and/or arginine are abundant in cationic peptides. Using polyplexes or lipoplexes with peptide ligands allows a vector to hit a specific target. The viral protein's short peptide sequence allows the vector to produce nuclear localization signals that facilitate the delivery of genetic material into the nucleus. Peptides are widely utilized to functionalize cationic polyplexes or lipoplexes because of these benefits (Ramamoorth & Narvekar, 2015).

3.4.2.4 Polymer-based Vectors

DNA and cationic polymers combine to produce nanoscale structures known as polyplexes. Lipoplexes are not as stable as polyplexes. Natural and artificial polymers are the two types of polymers.

Proteins, peptides, and polysaccharides are all natural.

Dendrimers, polyphosphoesters, and polyethyleneimine (PEI) are synthetic compounds (Ramamoorth & Narvekar, 2015).

3.4.2.4.1 Polyethylenimine (PEI): In both in vivo and in vitro gene transfer, polyethylenimine (PEI) is regarded as the gold standard. High-density density amine groups found in cationic polymers produce a protein sponge's effect that ultimately prevents the endosomal pH from becoming more acidic. As a result, there is an inflow of chloride into the compartment, which raises the osmotic pressure and causes the endosomal membrane to inflate and tear.

3.4.2.4.2 Chitosan: It is a cationic polysaccharide-based natural polymer, one of the non-viral vectors with the most research. Even at large quantities, it is harmless. It is a glucosamine-based linear cationic polysaccharide. Chitosan's positive charge interacts electrostatically with DNA's negative charge. Chitosan/DNA polyplexes are frequently employed in nasal and oral

gene therapy due to their mucoadhesive characteristics. Chitosan is linked to folic acid in order to successfully navigate intracellular obstacles.

3.4.2.4.3 Poly (DL-Lactide) (PLA) and Poly (DL-Lactide-co-glycoside) (PLGA): These biodegradable polyesters are broken down in large amounts by water, which gives them a long-lasting effect. Citric acid cycle removes the breakdown products. The FDA has approved PLGA as a protein delivery vehicle. They are less than 10 m in size and easily phagocytosed by antigen-presenting cells, which triggers an immunological response.

3.4.2.4.4 Dendrimers: Dendrimer molecules have functional terminal groups and are symmetrical in size and shape. When polarized peripheral groups in physiological pH come into contact with nucleic acids, it bonds to genetic material. Because of its nanometric size, it may effectively interact with proteins, organelles, and cell membranes. The toxicity profile is determined by the positive charge density and terminal amino group.

3.4.2.4.5 Polymethacrylate: This type of polymers that can condense polynucleotides into nanometer-sized particles. But because of their weak capacity to interact with membranes, transfection is constrained.

Chapter 4

Rare Genetic Disorders

4.1 What are Rare Genetic Disorders?

Rare genetic disorders (RDs) are generally hereditary abnormalities that are chronically life-threatening or disabling and affect a small number of individuals (less than 5 per 10,000). Some have been reported cases of over 7000 unusual diseases thus far. In contrast to other common diseases, RDs have varied clinical outcomes that affect a small percentage of people in the general population yet are progressive, chronically disabling, and/or life-threatening. But recently, it has come to light that these are among the most significant global public health issues. RDs collectively affect a sizably higher percentage of the world's population. There are a number of difficulties that make RDs management and diagnosis difficult and obstruct RDs-related research. Despite various global efforts to address the RDs-related difficulties, much work remains to be done to solve this underutilized health sector (Angural et al., 2020).

Mutation that result in genetic diseases with strong penetrance often leads to-

i) Loss-of-function (LOF), which refers to a decrease in the amount and/or activity of a specific protein, is typically observed in illnesses that are recessively inherited.

ii) Gain-of-function (GOF), which is an elevation in expression levels and/or activity accompanied by the entrance of a novel pathogenic function frequently linked to the activation of a pathway, is typically observed in illnesses with dominant hereditary traits.

Once upon a time, rare diseases were viewed as medical curiosity with little relevance to public health. Although these illnesses are uncommon on their own, 25 to 30 million Americans have been believed to be impacted. The most perplexing medical cases sent to the NIH Clinical Center in Bethesda, Maryland, are the subject of the organization's Undiagnosed Disease

Program (UDP). Over 3,000 applications have been evaluated by UDP, who has also received close to 10,000 queries. Roughly 900 patients have been admitted to the NIH Clinical Center for in-depth, one-week examinations. We have learned more about common ailments like kidney stones, osteoporosis, and viral infections thanks to some of these people with unusual diseases. The NIH UDP has been expanded to include a range of sites around the nation, building on its early results. It is encouraging to see progress in the detection of rare diseases, yet it is not enough: Only around 500 of the 7,000 rare but also overlooked diseases have been identified for which we are aware of the molecular cause. The Treatments for Rare and Neglected Diseases program of the NCATS and other research projects are being carried out by the NIH in collaboration with a wide range of partners to hasten the development of efficient treatments.

Clinical Challenges, Lack of Reliable Patient-Registries and Inaccurate Epidemiological Data:

The diagnosis and therapy of RDs have become challenging due to clinical issues such as the lack of particular literature and evidence-based information or the difficulty evaluating knowledge sources (Schieppati et al., 2008). Lacking of standardized treatment guidelines, specific clinical infrastructure, and clinicians with sound knowledge and experience in clinical genetics are examples of inadequate clinical resources. As a result, needy patients are frequently forced to undergo unavoidable clinical investigations that lead to multiple, uncertain treatments (Knight and Senior, 2006; Zurynski et al., 2008). It goes without saying that a clinician's skill in illness management relates to how frequently they interact with patients. Due to the relative rarity of RDs, such clinical interactions are thought to be insignificant, which causes a significant clinical information gap. The lack of clinical information sources on the underlying cause, pathophysiology, and natural history of the majority of RDs, as well as the restricted availability and support of pertinent guidelines from affiliated clinical societies, all contribute to this knowledge gap. The diagnosis of various RDs is typically challenging due to

clinical heterogeneity of different RDs, which results in several subtypes with various clinical symptoms and courses. No diagnosis or incorrect diagnosis given to patients ultimately leads to no or insufficient patient registries, which ultimately results in a lack of precise epidemiologic studies on RDs (Zurynski et al., 2008). Unfortunately, only about 5,551 of the estimated 7,000 disorders classified as "single gene disorders" currently have detailed phenotypic information reported in the Online Mendelian Inheritance in Man® database (OMIM ®),1 while information on the remaining disorders has been sparse up to this point. Only 29% of the approximately 7,000 RDs have recorded epidemiology, according to the data from the Orphanet database2 that is currently available.

4.2 Issues that Patients and their Families Experience

RDs patients and their loved ones face a severe psycho-socio-economic burden. This is because RDs patients often experience social isolation, difficulty accessing critical healthcare treatments, a delay in diagnosis, uncertainty about the future, and financial difficulties. Most RDs severely limit patients' skills, significantly lower their quality of life, and drastically shorten their life expectancy. The majority of RDs develop in early childhood, which makes it difficult or impossible for young patients to enroll in school or college (Zurynski et al., 2008). The families of the patients must additionally deal with social prejudice in the form of prejudice and social exclusion. Many times, patients purposefully avoid receiving a physician consultation because of their fear of social stigma, which is exacerbated by their ignorance about their health problem (whether it be a hereditary or genetic ailment). Since these patients are not registered in hospitals, this has a direct impact on the accuracy of the patient registries. Patients frequently have difficulty locating specialists with solid clinical genetics training and experience. Clinicians who have extensive knowledge and experience in the management of RDs are primarily concentrated in geographically diverse specialized centers. These centers may be inaccessible to patients, or they may require the majority of patients to travel a

significant distance or relocate their residence in order to receive an assessment (Zurynski et al., 2008). Together, these issues cause diagnostic delays, incorrect diagnoses, or no diagnoses at all, which leaves patients without effective therapies. All of this complicates the patients' medical conditions as they are merely left with the choice of dealing with the early or late repercussions of their sickness (Yang et al., 2013). Additionally, the "diagnostic odyssey," a protracted effort to accurately diagnose RDs that frequently results in high medical costs, futile efforts, and the depletion of scarce resources, has its own set of financial repercussions for the patient's family because the cost of raising a child with a disability is higher than the cost of maintaining a child without a disability (Zurynski et al., 2008; Yang et al., 2013).

4.3 Diagnostic Challenges of Rare Diseases

It might be challenging to accurately diagnose RDs. Access to medical tests is crucial for their identification, which also necessitates identifying the underlying genetic reason (Boycott and Ardigo, 2018). A differential diagnosis of the condition that these people have been living with their entire lives is essential due to factors like clinical heterogeneity, and variable disease courses across various RDs patients. Establishing a differential diagnosis, however, is a laborious and time-consuming procedure that necessitates a diagnostic odyssey and typically depends on the clinical judgment of a concerned physician and the earlier mentioned diagnostic tests which a patient must undergo.

NGS has sped up the diagnosis of RDs. Despite the fact that NGS has considerably increased the rate of precise diagnosis in RDs patients, the diagnostic yield is still only 25–50%. (Li et al., 2018). Due to a number of technological restrictions, it is unable to produce a confirmed diagnosis for the large portion of patients still showing complicated phenotypes (Wenger et al., 2017). However, numerous methods for genetic diagnosis have just lately surfaced. NGS could be used in conjunction with a combination of comparison reanalysis of clinical and non-clinical

NGS data using a variety of recently developed data management pipelines and software, all while taking into consideration the most recent scientific literature. This would increase the diagnostic yield from NGS. For instance, a recent study that reanalyzed 40 unresolved exome reports eventually resulted in an accurate diagnosis in 10% of instances (Wenger et al., 2017).

4.4 Challenges in Therapeutics R&D and RD-Related R&D

The RDs-related R&D is extremely difficult and hampered by a number of difficult problems, such as a huge gap in knowledge about the underlying causes of various RDs, a lack of a global class code for their categorization, the need to assemble cohorts of patient populations for research studies because of their distinct infrequent prevalence, and a lack of funding opportunities for RDs-related research. These difficulties make it more difficult to choose the best therapeutic approaches and create specialized pharmacological molecules to address a certain clinical condition. An adequate estimate of the number of people affected for conducting broad translational and clinical research is not available from any one institution or country. This may be mostly due to the International Classification of Diseases (ICD) system, which is used by several nations to categorize diseases. Since the ICD is unsuitable for the majority of RDs, reliable epidemiological databases cannot be created using national and international patient registries, making it impossible to accurately estimate the economic and social burden of these conditions. A higher power study can only be produced by undertaking a worldwide population-based study because some RDs are so uncommon (1 in 1,000,000 people), making it impossible to gather enough patients from widely separated geographic areas for a clinical inquiry. The inability to enroll this many patients in a research study is further complicated by the absence of trustworthy patient registries, which results in inaccurate estimates of the severity of the disease, inaccurate cost estimates of the resources required for research, drug development, and clinical trials, and the loss of a potential funding possibility (Schieppati et al., 2008). Another significant barrier to developing the infrastructure needed to

maintain patient registries has been funding and policy-making (Forrest et al., 2011). Although national and/or worldwide patient registers have been consistently kept for specific disorders by various groups, there is no government recognition for the majority of these due to a lack of or restriction on documenting of RDs clients in the area hospitals (Schieppati et al., 2008).

4.5 Understanding Rare Genetic Disorders: Molecular Etiology Research Methodologies

In general, the first-line diagnostics consist of the following: hematological assessment, metabolic testing, radiographic examinations, illness-phenotype correlation, biochemical tests of the known disease biomarkers, and neonatal disease screening techniques. Once a tentative diagnosis has been made, the accuracy of the diagnosis is largely dependent on the ability to identify the molecular cause of the patient's ailment through genetic testing. Traditional and cutting-edge cytogenetic methods, single-gene scanning, and sequencing a panel of genes linked to particular disease types are some of the several techniques used for genetic screening. Although each of these approaches has its own limits, they are all often used to diagnose genetic disorders in many different nations and aid at-risk families in early disease identification, early intervention, and preventative and palliative treatment.

For many years prior, genetic epidemiology research based on linkage and association have been the main method used to understand the underlying genetic causes of a number of human disorders. Genetic epidemiological studies, however, have undergone a significant transition from traditional measuring, to population-based genome-wide association studies (GWAS), to studies based on the most cutting-edge Next-Generation DNA Sequencing (NGS) technologies as a result of further advancements in a number of biological techniques. On the one hand, while particular approaches to make it easier to identify the distinct molecular etiology of RDs exist, they are laborious and remain unhelpful in understanding RDs when compared to

community research design like GWAS and Twin-based Epidemiological Studies. The development of new, advancements has greatly enhanced these efforts. Below are quick descriptions of these study designs.

4.5.1 Genetic Linkage Studies

In general, genetic linkage studies are performed to map or identify the most likely chromosomal or genomic loci that co-segregate with a disease phenotype. These studies are conducted on multiple individuals. These studies are either based on the knowledge of the disease's inheritance mode (dominant or recessive) in the parents' blood lines (parametric or model-based linkage analysis), or they are based on some genomic sequence polymorphic genetic markers, such as microsatellites, in the families that were chosen. These family-based research' basic tenet is that physically close genetic loci stay strongly linked during meiosis (i.e., the likelihood of recombination between them is less than 50%) and are independently passed down from parents to children. A population is said to be in linkage disequilibrium (LD) if a group of variants are linked together in the same haplotype. Genetic loci that are in LD are thought to be connected. Information on the co-segregation of genetic variations with the disease phenotype can be gleaned from studies based on linkage mapping in affected and unaffected siblings, affected people and their parents, in multi-case extended families, or pedigrees; these studies also reduce genotyping error and boost the assertiveness of their findings. Linkage studies are incredibly helpful in the identifier of a genetic locus that may be inhabiting an illness gene being chosen to share between affected individuals and only requires a very small number of markers to carry out the same in the initial stages of a genetic analysis of a clinical phenotype or trait for which the exact causal genetic component is unknown. Linkage analyses cannot, however, locate the causal gene by themselves. These mostly rely on "positional cloning" operations for this purpose. The efficacy of a linkage study may be

significantly diminished when the study participants exhibit both inadequate genetic basis and locus diversity, which is another drawback of linkage studies.

The most effective method for locating uncommon, highly penetrant variants that cause rare monogenic Mendelian illnesses and birth abnormalities is linkage analysis. The underlying genes for various RDs in big multi-case families have already been identified or mapped using a variety of linkage procedures (Barrett, 2014). It is important to note that nearly 3.5 hundreds known RDs' molecular causes have primarily been determined through traditional positional cloning techniques based on linkage analysis and "homozygosity mapping," in which the inheritance patterns of specific DNA markers, such as Single Nucleotide Variations (SNVs) and microsatellite repeats, were used to determine recombination events in lengthy multi-case pedigrees. However, the remaining disorders have been resistant to these conventional genetic screening techniques for a number of reasons. These reasons include locus heterogeneity, phenotypic heterogeneity, reduced penetrance, availability of a small number of patients or families that may not be sufficient to achieve a high-power study, and significantly reduced reproductive fitness in the patients as a result of early disease onset and severe effects. In addition, these remaining disorders have been resistant to these conventional genetic screening techniques for a number of reasons. These traditional methods are labor-intensive, costly, and time-consuming, and they remain ineffective in the case of spontaneously and quasi illnesses.

4.5.2 Next-Generation DNA Sequencing

Chip-based DNA arrays and multiple "massively parallel" or high throughput NGS technologies are two examples of the newly developed state-of-the-art molecular biological instruments that have helped to partly mitigate the limitations of prior investigations. The Human Genome Project, which utilized the "hierarchical shotgun approach" for the most part and was carried out by the classic Sanger biochemistry, was finally finished, and as a result,

the first information regarding the human genome became available. After two to three decades of incremental advancements in assembling technology and biochemistry, NGS technologies have substantially exceeded our capacity, allowing for the current multi-sample sequencing (multiplexing) of samples on one platform with improved accuracy and lower cost. A period of unprecedented productivity has since resulted from the gradual accumulation and accessibility of genomic data from individual people of biologically diverse populations. This has allowed for a thorough understanding of various common diseases and the susceptibility of various population groups to those diseases. NGS has become a key role in the quest to uncover the underlying etiology of numerous RDs over the past ten years. Through the considerably more affordable and accessible finding of the underlying genetic etiology of certain RDs, it has caused a paradigm change in clinical genetics and helped to precisely define many cases of RDs that had previously gone undiagnosed. The advancement of disease-specific diagnostic methods and therapeutics has resulted from the effective resuscitation of our understanding of the etiologies of several genetic disorders, particularly those of rare hereditary diseases, made possible by the discovery of new illness genes and innovative genetic variations through various NGS platforms.

Since the preceding decade, NGS has aided in overcoming obstacles related to conventional gene finding methods, made it simpler to determine the genetic etiology of a number of RDs, and given insights into the unique biological mechanisms underlying each of these disorders. Whole Genome Sequencing (WES) has comparatively grown more common in the detection of RDs genetics since 2010 since WGS has a wider range, inherent complexity, and higher cost. It is based on the sequencing analysis of the exome, which makes up around 1% of the human genome and contains 85% of the genetic variants known to have a significant impact on human physiology and lead to illness phenotype (Choi et al., 2009). WGS has emerged as a remarkable scientific achievement that has increased our understanding of the molecular

pathways driving human development and disease biology and the structure and function of the human genome. More than five hundred and fifty previously unknown genes associated with disease have been uncovered with the help of WES (Boycott et al., 2013).

The discovery of the genes causing Miller syndrome and Kabuki disorder (OMIM: 147920) provided the first evidence of the possible use of WES in RDs diagnoses and research (OMIM: 263750). The majority of those with Kabuki disorder and Miller syndrome, respectively, possessed different MLL2 gene and DHODH genomic variations, which the University of Washington researchers had discovered and published using WES and targeted sequencing. Later, using WES, protein truncating PRRT2 variants in a family case of Paroxysmal Kinetogenesis dyskinesia were discovered (Chen et al., 2011). WES was used to find variations in the genes SMARCA4, SMARCB1, SMARCA2, ARID1A, SMARCE1, and ARID1B that code for proteins involved in the SWI/SWF chromatin-remodeling complex and linked to the rare congenital defect known as Coffin-Siris syndrome (OMIM: 135900) (Tsurusaki et al., 2012). Whole exome sequencing (WES) was used to detect ectrodactyly and fatal pulmonary acinar dysplasia in a 5-day-old infant born to healthy consanguineous parents (Barnett et al., 2016). There has been a flurry of studies on the discovery of genes and new variants associated with RDs since the launch of WES in 2010, which has likely accelerated the precise identification of a number of suspected described and uncharacterized RDs. In between 30 and 50 percent of RDs cases treated in clinical settings, WES has been successful in generating accurate diagnoses (Fresard and Montgomery, 2018). Despite this, it is still unclear and undiscovered what the molecular causes of over one-third of RDs are (Boycott and Ardigo, 2018). WES is still not the gold standard preventative for clinically uncharacterized diseases because of technical limitations that prevent the dedication of variations in non-coding and/or, structural variations, regulatory genomic region, complex genetic mechanisms like somatic mosaicism and gene imprinting that underlie a significant portion of RDs (Fresard and

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Montgomery, 2018). Another NGS technology, the WGS, is here helping to rescue from the constraints of WES. WGS is able to identify SNVs as well as structural and epigenetic alterations in the genome in addition to identifying SNVs (Wang et al., 2015).

The effectiveness of data mining methods used for the processing of sequencing data is crucial to the success of NGS. Tools for processing raw NGS data and calling variants are widely available. A number of initial stages are involved in processing raw NGS data, including quality assurance, post-trimming quality assurance, adapter trimming, PCR duplicate elimination, alignment of sequencing runs to the reference genome, and variant calling. FastQC is a tool for examining the quality of pre- and post-trimming assembling data quality reports. Initially, high-quality trimming of the reads, Trimmomatic is utilized. PCR duplicates are removed using the Picard tool's MarkDuplicates feature. The reads are aligned to the reference genomes using Bowtie and the BWA program. The GATK for SNVs, DISCOVAR, and other tools are used for variants calling from NGS data. For structural variations, there are several tools available, including DELLY, LUMPY, GASV, CoNIFER, CONTRA, Platypus, XHMM, and SAM. These techniques can more accurately mine different kinds of variants from NGS data.

4.6 Examples of Rare Genetic Disorders

I. Severe combined immune deficiency (SCID): ADA Deficiency is a hereditary disorder that weakens the immune system and is a prevalent result in SCID. The majority of infections, such as bacterial, viral, and fungal infections, cannot be fought off by people with SCID caused by ADA deficiency. Most ADA deficient sufferers experience symptoms before the age of six months. The first signs of an ADA deficit are pneumonia, persistent diarrhea, extensive skin rashes, delayed growth, and/or developmental problems. Some ADA-deficient individuals experience symptoms later in life. The autosomal recessive inheritance pattern of ADA

deficiency results from mutations in the ADA gene. SCID refers to a group of extremely rare conditions that are brought on by abnormalities in numerous genes that play a role in the development and operation of disease-fighting immune cells. This condition affects a very small percentage of the population. Although SCID babies first seem healthy, they are prone to serious illnesses. The disease is fatal to newborns if they do not undergo immune-restoring therapies such as blood-forming stem cell transplants, gene therapy, or enzyme therapy, and it often takes the first year or two of a child's existence to take their lives. There is no previous history of sickle cell disease in the families of more than eighty percent of infants diagnosed with SCID. However, the creation of a prenatal diagnostic test has enabled it to find SCID before symptoms show, ensuring that affected infants get life-saving care (*Severe Combined Immunodeficiency (SCID), 2019*)

II. Inherited retinal dystrophy (IRD): IRDs are a collection of uncommon eye conditions brought on by gene abnormalities that cause the degeneration of cone and rod photoreceptors or the retinal pigment epithelium. The progression of retinal degeneration is frequently irreversible, and clinical symptoms can include color or impaired vision, peripheral vision abnormalities, and eventually vision loss. Gene therapies that either supply unmutated genes or truncate mutant genes in order to restore functioning retinal proteins are therefore required. Coincidentally, the immune-privileged state of the eye, its accessibility, and significant developments in genetic identifying and gene delivery methods heralded the advent of gene therapy for IRDs (Chiu et al., 2021).

III. Hemophilia: It is a rare disease that results from a variation or change in a gene that codes for the coagulation factors proteins required to create a blood clot. As a consequence of this modification or mutation, the clotting protein might not exist any longer or might act in an abnormal manner. These genes can be found on the chromosomes.

IV. Cystic fibrosis (CF): It is a prevalent, life-decreasing autosomal recessive genetic disorder that manifests as chronic and progressive obstructive lung disease, malabsorption, sinusitis caused by pancreatic exocrine inadequacies leading to malnutrition, liver disease, and diabetes mellitus associated with CF (CFRD). Significant advancements in therapies to improve nutritional status and lung health, as well as assertive treatment of long-term respiratory tract infections and circulatory diseases for end-stage lung disease, have resulted in earlier diagnosis and via newborn screening and significant increases in survival. At the time of its onset in 1938, CF was a childhood disease that was always fatal, but today the estimated median survival is 44.4 years. Since more than 50% of CF patients are now adults (18 years of age or older), the illness has evolved from being a condition that only affects children to one that affects patients as they enter maturity and become caregivers for adults.

V. Huntington's disease: An uncommon, genetic condition known as "Huntington's disease" results in the progressive degradation of brain nerve cells. Movement, thinking (cognitive), and psychological issues are frequently symptoms of Huntington's disease, which has a significant influence on a person's functioning abilities. Although the hereditary nature and progressive history of HD were initially characterized in the 19th century, there is still no disease-modifying therapy for this debilitating neurogenetic ailment. HD has served as a model disease for the use of human molecular and genetic approaches to identify gene mutations, comprehend pathogenesis, and propose logical targets for the creation of therapeutics thanks to the active involvement of HD families (Gusella, Lee, & Macdonald, 2021).

VI. Duchenne Muscular Dystrophy: One in 5000 males have Duchenne muscular dystrophy (DMD), an X-linked, muscle-wasting disease. By the time they are twelve years old, those who are affected are wheelchair-bound and eventually pass away from respiratory and cardiac issues. Mutations in the dystrophin-coding DMD gene are the root cause of the disease. A structural protein called dystrophin keeps muscle fibers intact and guards them against harm

brought on by contractions. The integrity and performance of the muscle fibers are compromised when dystrophin is absent, eventually resulting in muscular deterioration. There is currently no cure for DMD sufferers' declining muscular function. Gene transfer to reestablish dystrophin production utilizing the secure, non-pathogenic AAV vector is a potential method for curing this fatal condition.

VII. Parkinson's Disease: With a frequency of much more than 6 million people worldwide, Parkinson's is the second most prevalent neurological condition. According to this figure, the prevalence of Parkinson's disease has increased by 2.5 times in the previous generation, making it one of the main causes of neurological dysfunction. The substantia nigra and other brain regions exhibit cell loss along with neuronal inclusions inside the sort of Lewy bodies and Lewy neurites, which are the pathologic hallmark of Parkinson's disease. Parkinson's disease is categorized as a synucleinopathy because Lewy bodies are primarily made up of aggregated and misfolded-synuclein species (Tolosa et al., 2022).

4.7 Diagnosis of Rare Genetic Disorders

The ADA-diagnosis may be suspected based on birth screening or symptoms, and confirmed based on the findings of blood and genetic tests.

The diagnosis of mild ADA deficiency in children and adults is made on the basis of symptoms such as numerous atypical infections, low WBC levels in the blood, absence of tonsils or lymph, and low rates of the adenosine deaminase enzymes. The identification of the mild version of ADA deficiency can be confirmed through genetic testing for ADA gene variants.

Parkinson's disorder: Making an actual diagnosis as quickly as possible is crucial because numerous other diseases share similarities with cancer but call for different therapies.

There is currently no blood test or laboratory test that can be used to diagnose Parkinson's disease in instances that are not inherited. A neurological examination and a people's health history are used to make the diagnosis. Another crucial indicator of this disease is improvement after starting treatment.

Since 2010, the number of new genes and mutations found each year has dramatically increased as a result of technological breakthroughs in DNA sequencing as well as developments in the analysis of large amounts of data. Currently, 3573 genes linked to rare disorders are listed on Orphanet (May 2017). Gene-sequencing panels with a wide range of commercial options are readily available for the relatively inexpensive examination of genes associated with certain diseases or groups of diseases, and websites like Orphanet include directories of diagnostic labs and the genetic testing they provide. Exome sequencing is becoming technically viable and more cost-effective for a more thorough study, opening up new possibilities for Mendelian illness diagnosis and study. Exome sequencing restricts sequencing mostly to the human genome's protein-coding regions. Despite making up only 2% of the entire genome, these 180 000 exons are thought to contain 85% of the mutations that cause heritable Mendelian illness. However, it must be kept in mind that some cases of rare conditions may be brought on by variations affecting regulation and noncoding DNA regions. Exome sequencing currently costs around US$1000 in a therapeutic context. Studies claim that this approach can detect mutations that have already been detected with a sensitivity of 98.3%. The assessment and validation of potential mutations known as VUS—which have not yet been reported or validated—remains a key difficulty. Exome data analytics algorithms provide statistical likelihoods of a variation being pathogenic; nevertheless, unless the identical variant is found in separate patients with comparable clinical characteristics and/or functional testing are carried out, ambiguity remains. Databases like ClinVar, which list pathogenic and normal community variants, are crucial resources. The detection of cytogenetic variations, however, isn't well suited for exome

analysis. The definitive confirmation of the diagnosis of single gene disorders through the definitive diagnosis of the causative variant is a crucial step for the patient. It also enables more accurate genetic counseling and provides historical data for prenatal investigations of pregnant patients within the family. Due to the frequent difficulties in determining the clinical importance of deletion and amplifications, known as CNVs, the number of genetic illnesses linked to chromosomal aberrations is more difficult to determine. The human genome is substantially more diverse on a structural basis than previously believed, with the majority of these structural variations being discovered through the advent of molecular cytogenetic techniques, particularly CMA.

The topic of therapy for rare diseases is closely related to that of diagnosis because, in the end, any patient undergoing genetic testing (or any healthcare provider advising such testing) wants to know what therapies the results may suggest. Since more people are aware of the existence of genetic tests, there is a rising expectation for efficient treatments. However, diagnosis frequently does not result in disease-modifying therapy, despite the possibility that it will enhance illness management. Because metabolic disorders are usually susceptible to treatment that targets pathophysiological aspects, such as enzyme substitution and/or dietary therapy, successful illness management results in a significant increase in life quality. For instance, there is an enzyme replacement therapy for the inborn metabolic mistake known as hypophosphatasia. Since 2012, clinical data supporting effective illness management have been made available thanks to asfotasealfa, a chimeric synthetic protein with just an alkaline phosphatase ectodomain. Asfotasealfa is currently accepted for usage in the USA, Canada, Japan, the EU, and Canada. Patient care switches back to supportive measures when there is no asfotasealfa present. In 2016, Tarailo-Graovac et al. presented proof that patients with intelligent developmental problems and unexplained metabolic phenotype experienced a 44% rate of improvement in treatment beyond genetic counseling, at least for neurometabolic

disorders, as a result of whole-exome sequencing in conjunction with deep clinical phenotyping. To put it another way, the integration of genetic and clinical data has allowed for the personalization of patient therapy, which has included, among other things, dietary adjustments for at least 5 probands. According to estimates, these changes improved people's quality of lives. Options for gene and cell therapy are also getting more and more available, in addition to nutrition therapy and enzyme replacement. Cellular treatment based on a bone marrow transplant has been around for a while and is enough to cure patients of disorders like SCID. But the underlying genetic flaw is not corrected. Therefore, the main cause of rare conditions, the genetic mutation, is typically not addressed by currently available therapies. A number of nations have enacted orphan laws that provide incentives for the development and improvement of access to effective therapies for some individuals with rare diseases. High expenses and a lack of accountability continue to be problems, though, an overview of rare genetic illnesses, their estimated literature incidence, genes involved, and contemporary therapies (and estimated costs).

Gene treatments are methods for treating genetic illnesses permanently. By replacing the damaged gene or introducing healthy copies of these genes into the genome, gene treatments aim to address the DNA abnormalities that cause any specific genetic disorder. Despite the seeming simplicity, effective gene therapy needs the introduction of new genetic data into a sufficient number of target cells. These cells then need to persist and reproduce in the host in order to maintain the therapeutic benefits without triggering adverse reactions. Gene therapy was initially created with the goal of treating cancer, but it has now broadened its use to treat monogenic, rare disorders in an effort to lower the likelihood of developing illnesses with very clear hereditary causes. Another significant attractive factor is the potential for orphan status. Consequently, the first gene therapy clinical studies were conducted in the field of uncommon diseases. The field is now exploring T cell and oncogenic viral therapy targeted at various

locations as a result of the success of previous clinical trials. Due to the simplicity of cell isolation, *ex vivo* transfected was the primary method employed in the early stages of gene therapy, which initially concentrated on blood diseases. After a patient died in 1999 during a trial using an adenovirus to treat ornithine transcarbamylase deficiency, subsequent in vivo studies had minimal effectiveness. Additionally, the ineffectiveness of previous experiments contributed to a gradual loss of interest. Adenosine deaminase deficiency, SCID, b-thalassemia, X-linked adrenoleukodystrophy, and Wiskott-Aldrich syndrome are only a few of the conditions that can be treated with viruses like lentiviruses and g-retroviruses that have recently improved genotoxicity and integration. AAV has also been successful in treating hereditary lipoprotein lipase deficiency, an uncommon condition that causes severe hypertriglyceridemia to appear in childhood. Leber's congenital amaurosis type 2 (NCT00999609), cystic fibrosis (NCT00004533), choroideremia (NCT01461213), muscular dystrophy (NCT00428935), and a1-antitrypsin deficiency are further clinical trials that are in various stages of development (NCT00377416). Gene editing techniques like ZFNs, TALENs, and most recently CRISPR-Cas have emerged in recent breakthroughs in gene therapy, putting the field on the verge of a revolution. The ability to specifically target and fix mutations that cause disease is these tools' principal benefit. Since the late 2000s, ZFNs have been examined clinically after multiple proof-of-concept assays (NCT00842634, NCT02388594, NCT02500849, NCT01252641, NCT01044654, NCT02702115, NCT02225665, NCT02695160, NCT02800369). Additionally, CRISPR is being used in the first clinical trials to remove PD-1 in cancer patients (NCT02793856, NCT02867345, NCT02863913, NCT02867332). These trials will be crucial in the future for uncommon diseases even though they are not yet recruiting. Clinical trials of RNAi to quiet faulty genes through target mRNA knockdown have recently been conducted. 35 trials were listed as registered at clinicaltrials.gov as of September 2016. In some of these trials, therapeutic results have been noted despite the limited amount of available data.

However, RNAi use is only relevant for disorders where partial gene knockdown is interesting. With the development of the CRISPR-Cas9 system, it is now theoretically possible to alter the DNA sequence of people who have hereditary diseases. Since its discovery, this process has been utilized by many organisms, including humans, to modify DNA. It was first used by bacteria as an adaptable immunological defense. The most current generations of the technology enable exact DNA sequence cutting and replacement, providing the possibility of mutation repair. The first CRISPR clinical experiment was given provisional permission in the USA in June 2016. The University of Pennsylvania developed experiment, which focuses on cancer immunotherapy, will target tumors with CRISPR-modified T cells taken from cancer patients. In China, a new experiment is being conducted with the aim of deleting the PD-1 gene from the T cells of patients who have non-small-cell lung cancer. Although no clinical studies for a particular rare genetic condition have yet begun, we think that this technique could revolutionize the study of rare diseases.

Chapter 5

Gene Therapy for Rare Genetic Disorders

2017, the FDA authorized the first gene treatments for use in the US following substantial study in laboratories and in clinical trials with humans around the globe.

FDA-approved gene treatments for the following rare genetic disorders are discussed below.

5.1 Gene Therapy for Severe Combined Immunodeficiency

Severe combined immunodeficiency (SCID) is caused due to mutation in the *ADA* gene and *IL2RG* gene (γc gene) responsible for expressing proteins involved in T cell survival. The absence of the ADA enzyme leads to an accumulation of toxic metabolic by-products deoxyadenosine and dATP within the T lymphocytes that cause the cells to die resulting in ADA-SCID. The deficiency of common cytokine receptor γ chain (receptor for several interleukins) encoded by *IL2RG* gene blocks the differentiation of lymphocytes and natural killer cells leading to X-linked SCID. These deficiencies disrupt the immune system and makes the patients highly vulnerable to infectious diseases.

Matching hematopoietic stem cell (HSC) transplants from siblings or parent or unrelated donor has been the standard treatment for ADA-SCID and X-linked SCID (SCID-X1). However, this type of treatment only restores the immune function partially. Gene transfer to human cells are considered as a potential approach in the treatment of both congenital and acquired genetic disorders. Several studies have shown that gene therapy can be used as an effective treatment strategy for SCID. In the year 2000, the first clinical trial was performed by Cavazzana-Calvo et al on infants with SCID-X1 where the *ex vivo* delivery of the functional *γc* gene to the CD34+ haematopoietic stem and progenitor cells via retroviral vector was found to restore the immune

function in 8 out of 10 patients after a follow up period of 10 months. In another study, the observations were found to be similar when bone marrow CD43+ cells of two patients with ADA-SCID were transduced with the normal *ADA* gene(Kohn, Sadelain, & Glorioso, 2003). While most of the children with SCID were largely cured with gene therapy, few of them developed leukemia because the viral enhancer induced transactivation of oncogenes. However, elimination of this element and use of a promoter instead led to safer but efficacious gene therapy(Fischer & Hacein-Bey-Abina, 2020). With further advances in gene transfer technology, viral vectors with high transduction efficiency and safety have been developed. While approved in Europe, gene therapy for SCID caused by ADA deficiency is still in the preliminary stage of study in US.

5.2 Gene Therapy for Inherited Retinal Dystrophy

Inherited Retinal Diseases (IRDs), often known as inherited retinal dystrophies or degenerations, are a category of disorders that can vary greatly in both their clinical presentation and their genetic make-up. IRDs afflict approximately one in two thousand people all over the world, and they are the major cause of blindness in Western populations of working age, underscoring the socioeconomic significance of these conditions.

Even among patients who have the same IRD subtype, patients can experience vastly different rates of advancement and degrees of severity in the course of their illness from the time they are born until adulthood.

Visual impairment caused by loss of photoreceptor function is characteristic of type 2 IRDs. This loss of function might be the result of primary degeneration of rods or cones, or it can be the subsequent result of degeneration of the retinal pigment epithelium (RPE) or the choroid. IRDs can be difficult to diagnose because mutations in different genes can cause similar clinical phenotypes (due to the limited phenotypic repertoire of the retina), and individuals with the

same mutation can demonstrate varied phenotypic variation (variable expressivity), even within families. This can make it difficult to determine the cause of an individual's symptoms. Patients who have a clinical presentation of an IRD should be offered a referral to a multi-disciplinary IRD specialist center for assessment, genetic testing, and genetic testing if clinically indicated to determine the genetic diagnosis. Current testing strategies are able to detect an illness mutation in about 75% of cases. A genetic diagnosis can help in determining the IRD subtype even when there are multiple clinical differential diagnoses, can clarify the risk to other members of the family, and can inform decisions concerning pre-implantation genetic condition for family planning that involves in vitro fertilization. According to the findings of previously published clinical cohorts, a genetic identification may also make it easier to customize a clinical management regimen and follow-up care plan that are both suited to the causal gene (Hu et al., 2021).

The U.S. Food and Drug Administration approved the use of a gene therapy called Luxturna in 2017 for use in adults and children with retinal degeneration caused by two mutations in the RPE65 gene. Numerous genes have been linked to retinal dystrophies, as was previously indicated, however as of right now, only RPE65-related diseases are clinically treatable.

Gene therapy may be an option for adults and children (over the age of 12 months) who have genetically proven mutations both in sets of the RPE65 gene. If there are sufficient surviving retinal cells to administer the treatment, patients must undergo a thorough clinical evaluation and testing *("Gene Therapy for Inherited Retinal Dystrophy (Luxturna®)," n.d.).*

Hereditary retinal dystrophies are a large class of genetic retinal illnesses brought on by mutations in any of more than 220 different genes. They are linked to progressive visual impairment. Affected individuals with biallelic RPE65 mutation-associated retinal degeneration range in number from 1,000 to 2,000 in the United States. A given gene's two

copies each contain a mutation (which need not be the same mutation in biallelic mutation carriers). Normal vision depends on the production of an enzyme, a protein that speeds up chemical reactions, which is controlled by the RPE65 gene. RPE65 activity is decreased or eliminated as a result of mutations in the RPE65 gene, interrupting the visual cycle and causing vision impairment. Biallelic RPE65 mutation-associated retinal dystrophy patients' vision gradually deteriorates over time. This visual loss, which frequently occurs during adolescence or childhood eventually leads to total blindness.

A healthy copy of the RPE65 gene is directly delivered to retinal cells by the drug Luxturna. In order to repair the patient's eyesight loss, these retinal cells then generate the typical protein that causes light to be converted into an electric signals in the retina. The normal human RPE65 gene is delivered to the retinal cells by Luxturna utilizing a naturally existing adeno-associated virus that has been altered using recombinant DNA techniques. Only patients with live retinal cells, as assessed by the treating physician, should get Luxturna(s). Each eye must get Luxturna treatment individually on consecutive days, with a minimum of six days between each surgical surgery. A surgeon with skill in intraocular surgery administers it through subretinal injection. Prednisone should be administered orally for a brief period of time during treatment to prevent an immunological response to Luxturna.

In a clinical development program including a total of 41 patients ranging in age from of 4 and 44, the safety and efficacy of Luxturna were determined. Biallelic RPE65 mutations were seen in all of the patients. Based on a Phase 3 clinical trial with 31 participants, the ability of a person to successfully complete an obstacle course at varied light levels was used as the major indicator of Luxturna's effectiveness. In comparison to the control group, the group of individuals who got Luxturna showed considerable gains in their capacity to finish the obstacle course in low light.The most frequent side effects from using Luxturna were cataract, increased

intraocular pressure, retinal tear, and eye redness (conjunctival hyperemia) *(FDA Approves Novel Gene Therapy to Treat Patients With a Rare Form of Inherited Vision Loss, 2017)*

5.3 Gene Therapy for Hemophilia

Although there are already a number of different methods for editing genes, the utilization of zinc finger protein nucleases is currently the most advanced in terms of their development in the treatment of hemophilia. Sangamo Bio Sciences has developed a method that targets an albumin locus in hepatocytes in order to replace it with an F9 gene construct (SB-FIX), which has the potential to provide long-term expression of FIX (FIX is a known clotting factor). Through the use of AAV2/6, hepatocyte-specific integration can be achieved through a single IV injection. An investigation into the efficacy, safety, and tolerability of the treatment in its initial phase (NCT02695160) is currently under way. It is conceivable that other technologies, such as CRISPR-Cas9, will also be pursued for the treatment of hemophilia; however, none of these technologies have yet surfaced in any phase of human clinical development (Croteau et al., 2021).

5.4 Gene Therapy for Cystic Fibrosis

This treatment method offers the opportunity for a cure that is not only long-lasting but also permanent by removing the genetic mutation that causes cystic fibrosis and replacing it with the "right version" of the gene that codes for the transmembrane conductance regulator (CFTR). In point of fact, ever since the CF gene was discovered, a great number of research have attempted to rectify the CFTR mutations through the use of gene therapy techniques. Although gene repair had some level of success in cell and animal models, developing a therapeutic that was effective for patients had proven to be more challenging. According to findings from research conducted *in vitro*, not all cells are required to express appropriate CFTR for proper epithelial functions to take place. In an experiment in which normal cells and CF mutant cells

were mixed together, it was shown that just 6–10% of the epithelium required to have epithelial cells expressing normal CFTR in order to restore chloride transport in a manner that was comparable to that of normal epithelia. In contrast, a study that targeted genes found that homozygous F508del human airway epithelial cells might have their mucus transport fully restored with as little as 25% gene repair. It is currently unknown how many cells must contain wild-type CFTR in order for there to be a therapeutic benefit for the patient. However, in theory, correcting a stem cell population within the airways could provide a renewable and long-term source of endogenous cells that are capable of renewing the damaged epithelia with cells that express wild-type CFTR. This possibility is based on the fact that stem cells are found throughout the airways. Surprisingly, however, there are no other clinical trials being conducted for CF gene therapy. The only exception to this is the RESTORE-CF study, which is a Phase I and II clinical trial for MRT5005, a medication that delivers CFTR-encoded mRNA to the lungs. This could be mostly attributable to a few different reasons- 1) the requirement for repeated delivery since it is unable to target the stem cells and progenitor cells of the airways in order to maintain expression while cell turnover occurs, 2) due to the highly inflammatory milieu, suboptimal delivery or limited effectiveness of targeting of the donor plasmid or gene to the CF airways, 3) the inability to efficiently deliver large DNA pieces of the CFTR gene using the delivery techniques that are currently available, 4) concerns over off-target safety, which can lead to insertional mutagenesis, 5) immune barriers, which limit the effective administration of viral vectors (Lee et al., 2021).

5.5 Gene therapy for Duchenne Muscular Dystrophy

For the treatment of infrequent, monogenetic illnesses like DMD, gene therapy offers fresh hope. DMD is a disorder caused by a mutation of a specific gene, DMD, making it a good candidate for gene transfer. A single treatment may also have long-term and possibly even life-long advantages as muscle tissue are non-dividing and have a lengthy lifespan. In actuality,

investigations in both preclinical and clinical settings on neuromuscular illnesses show no reduction in durability to far. The goal of DMD gene therapy is to systemically distribute a gene encoding a conditions prevail of dystrophin to all target organs implicated in DMD pathology in order to slow the disease's progression.

The choice of an AAV vector with strong tropism for the skeletal, diaphragm, and heart muscles is necessary for the most effective treatment of DMD. The vector has to be delivered systemically in order to reach all of these muscles. Due of the enormous doses needed, the long - term safety of the chosen vector becomes even more crucial.

AAV vectors have many benefits, but they have a significant drawback when it involves treating DMD. AAVs are relatively tiny and only have a 5 kilobyte (kb) maximum DNA storage capacity. This is not a problem for many genes, but DMD has a unique problem because of it. The cDNA for the DMD gene is about 14 kb in size, which is far larger than the packaging capacity of AAV vectors. The dystrophin genome is one of the biggest in the genetic code, spanning 2.3 megabases. Therefore, the dystrophin transgene's design needs to be carefully considered. The transgene must also fit into AAV and produce a protein that is stable, functional, correctly targeted to the sarcolemma, successful in restoring the DAPC, successful in maintaining cell integrity, and ultimately capable of enhancing clinical function (Asher et al., 2020).

Here, we go over the justification behind the planning of our clinical trial to evaluate a novel gene transplantation therapy in DMD patients. Our design (SRP-9001 micro-dystrophin) includes a micro-dystrophin transgene with spectrin repeats 1-3 and 24, as well as hinges 1, 2, and 4 and a muscular MHCK7 promoter with a cardio promoter region.

AAVrh74, which was isolated from rhesus monkeys at Nationwide Children's Hospital, was selected as the transportation vector for DMD gene therapy. AAVrh74 differs from the more

well-known numbered AAV strains 1–9 in that it has a higher degree of similarity to AAV8, another virus belonging to the same phylogenetic group (i.e., common ancestry). The use of a non-human origin vector that avoided previous human infection was meant to reduce the possibility of preexisting immunity. Indeed, first clinical trials targeting the DMD patient population indicated that the seroprevalence of AAVrh74 was quite low (15–20%). (boys 4–7 years of age). AAVrh74 was one of the AAV serotypes with the lowest costs of preexisting immunity, according to a study that looked at the frequencies of antibodies against such a variety of AAV serotypes. AAVrh74 may be used in more patients because of its low seroprevalence.

SRP-9001 micro-dystrophin expression is driven by the MHCK7 promoter. Muscle creatine kinase (MCK) transcription factor region and myosin heavy chain (MHC) enhancer make up the regulatory element. Given that the MHCK7 promoter causes significant specific gene expression in all muscle tissue, particularly the diaphragm, as well as heart muscle with little expression in off-target tissues, it was decided that this promoter would be the most appropriate for DMD gene therapy. Additionally, the regulatory element's -MHC enhancer component significantly increases transgene expression in the cardiac while simultaneously stimulating enhanced expression in skeletal muscles. The MHCK7 promoter is also space-efficient, including only the essential components, which is crucial considering the restrictions placed on AAVs' packaging size.

The naturally occurring DMD gene is extremely big, therefore the transgenic has to be carefully and efficiently designed to fit inside the AAVrh74 vector. The approach adopted was to create a micro-dystrophin, which is a condensed form of the dystrophin gene. Only the most essential components of the dystrophin protein are encoded by the SRP-9001 micro-dystrophin transgene.

Gene therapy was applied to DMD patients of 4- to 7-year-olds (ClinicalTrials.gov NCT03375164 and NCT03769116). This cohort represents the first chance to treat because it falls within the age range where the majority of individuals are typically diagnosed with DMD. Furthermore, there is significantly more tissue to preserve with treatment because muscle degeneration is mostly in its initial stages at around this age range. Although most likely at a slower rate, some boys in this age group may be making gains on functional measures; deterioration in function is typical around age 7 and higher, and some may go through that transition during the period of the study. To detect therapeutic efficacy across various trajectories, thorough outcome measurement and cohort size determination are necessary due to the potential variation in functional status.

Another important inclusion condition for the research was that participants had to have had consistent corticosteroid medication for at least 30 days before beginning gene transfer therapy (ClinicalTrials.gov NCT03375164 and NCT03769116). Steroids may potentially provide some functional benefit in addition to reducing the immunological response to the treatment. Therefore, it's critical to standardize corticosteroid administration throughout all study arms and patient groups.

Future studies intend to broaden the age range and types of DMD gene mutations included in the inclusion criteria. Given that micro-dystrophin gene delivery therapy has the potential to protect cardiac, pulmonary, and muscular functioning at all stages of DMD, we also intend to include non-ambulatory patients(Asher et al., 2020).

5.6 Gene Therapy for Huntington's Disease

Huntington's disease (HD) is a well-known condition that has the potential to serve as an excellent model due to the fact that it is an autosomal dominant progressive neurological movement illness that only affects a single genetic target.

Because huntingtin (Htt) contains repeating lengths of the amino acid glutamine, it is prone to misfolding, which can eventually lead to the aggregation of the protein, which results in the loss of the protein's original function. Chorea, dystonia, incoordination, cognitive impairment, and behavioral issues are some of the clinical manifestations that can be caused by this mutant protein.

The cellular functions of Htt are not yet understood to their full potential. The Htt protein can enter the nucleus, but it is most active in the cytoplasm, where it participates in the transport of vesicles, where it also has the ability to influence gene expression.

The genetic underpinnings of the vast majority of neurodegenerative diseases are not yet recognized to their full extent. On the other hand, given that HD is caused by a single genetic target, this would attach to the Htt gene, thereby lowering the amount of expression it produces. As a result, a large number of the treatments that were investigated for HD tried to target this particular gene through a variety of techniques of gene therapy. The discovery of RNA interference in 1998 led to the development of gene therapy as a viable treatment option. Because this method functioned to silence genes at the posttranscriptional level, it transformed the field of gene therapy. According to the findings of the study, it is possible to develop small interfering RNA, short hairpin RNA, and microRNA sequences that can attach to the Htt gene and inhibit its production. In most cases, delivery is accomplished by the use of viral vectors, more specifically AAV vectors. It was successfully delivered the RNA to the mice, which contributed to the suppression of the production of mutant Htt aggregation. Another study used methods that were very similar to the ones used in the first study in order to treat HD. This study was based on the utilization of recombinant AAV serotype-5 vectors in the delivery of the anti-mutant huntingtin protein (mHTT) small interfering RNA molecule into a transgenesis HD mouse model(Byun et al., 2022).

Mouse models

The creation of genetic mice models that take advantage of the mutation underlying HD and imitate a number of the clinical and neurodegenerative abnormalities seen in HD patients represents a significant advancement in the research of HD. To hasten the illness manifestation, these model systems frequently include significantly extended CAG repeats. Nevertheless, the human disease often has fewer repetitions and a more gradual beginning with more neurodegeneration than the rodent models do. However, these models offer a chance to evaluate HD gene therapy strategies. HD treatments have been explored to use both viral and non-viral gene transfer. Several strategies have been tried, including allele-specific blocking of transcription elongation and the vector delivery of genes encoding neurotrophic factors to improve interneuron survival and muHTT mRNA (SNP)-specific microRNAs and standard hairpin RNAs to prevent the formation of the mutant protein. A number of viral vectors, primarily those derived from adenovirus, adeno-associated virus, and lentivirus, have been employed to deliver genes. Additionally, numerous experiments have been carried out using small interfering RNAs, aggregate-specific antibodies, and directly administered antisense oligonucleotides (ASOs) (Glorioso et al., 2015).

While gene therapy has been utilized to treat HD in other studies, not all of the therapies directly targeted the Htt gene. The PA28 gene was administered via LVs in various research since it is thought that this gene transfer enhances motor behavior and proteasome function in HD mice models. Their findings demonstrated that this gene transfer did in fact aid in the improvement of motor function in HD animal models.

Clinical trials

In addition to vector selection, pharmacodynamic and illness progression biomarkers will be necessary for clinical trial design to gauge disease improvement. A lot of progress has been

made in this area, giving rise to confidence that blood and cerebrospinal fluid analyses, such as the quantification of spinal fluid biological markers of axonal transport action and magnetic resonance spectroscopy to assess metabolic markers of neurodegeneration, can be used to monitor the effectiveness of therapy. ISIS Pharmaceuticals, Carlsbad, California, USA, is now conducting the very first clinical gene therapy trial using chemically altered antisense oligonucleotide (ASOs) that do not differentiate amongst mutant and wt HTT mRNA. These substances must be given chronic, which necessitates frequent hospital visits, and they must be supplied intrathecally, which restricts their dispersion. However, this development signifies a critical first step in the conversion of laboratory results into clinical practice. The development of permanent muHTT gene knockout and substitution therapies will probably be made possible by new vector designs, opening the door for their widespread use to other polyQ-related NDs. In fact, it's possible to predict that at least one of these emerging techniques will be used in the upcoming generation of medicines(Glorioso et al., 2015). A recent study is going on where, 26 patients will undergo treatment through brain surgery and will be followed up at specialized HD centres around the world while enrolment is still open in the US(Byun et al., 2022).

5.7 Gene Therapy for Parkinson's Disease

It has recently come to light that there are multiple potential targets for the genetic treatment of Parkinson's disease. These targets can be placed in either the "disease modifying" or "non-disease modifying" category, depending on their effects on the disease. The non-disease-modifying treatments are targeted at alleviating the symptoms of Parkinson's disease by aiming to normalize abnormal firing in the basal ganglia. This can be done by the production of dopaminergic or GABAgenic enzymes. These treatments are symptomatic, and they do not affect the pathophysiological process that is the underlying cause (Axelsen & Woldbye, 2018).

<u>Clinical trials</u>

Genetically modified therapeutic genes are used in gene therapy to treat Parkinson's disease (PD) patients by actively replacing, eliminating or correcting their defective genes. Gene therapy has made use of several serotypes of non-replicating genetically designed viral vectors, such as lentiviruses and adeno- associated viruses (AAV). Gene therapy can stop the death of dopaminergic brain neurons. Gene treatments for Parkinson's disease (PD) patients primarily aim to boost the brain's neurotrophic activity so that patients' motor balance is improved. In addition, gene therapy is another potential therapeutic strategy for treating PD. It controls the levels of the enzyme glucocerebrosidase.

In six clinical trials, including AAV2-GDNF, AAV-GAD, CERE-120 (2 trials), PR001A, and GZ/SAR402671, gene therapy is being used to treat Parkinson's disease (PD). Adeno-associated virus serotype 2 carrying glial cell line-derived neurotrophic factor (AAV2-GDNF) is now being tested for PD by Brain Neurotherapy Bio, Inc. in a non-randomized open-label safety trial. In this Phase I trial, putamen is transfected with AAV2-GDNF; the trial status indicates that subject recruitment has not yet begun, and no published data are available (NCT04167540). Twelve advanced PD patients participated in Phase I trial that found that subthalamic nucleus gene transfer using the AAV-GAD (adeno-associated virus carrying glutamic acid decarboxylase) was safe, well-tolerated, and significantly improved motor unified Parkinson's disease rating scale (UPDRS) scores (NCT00195143).

Sangamo Therapeutics, Inc., an American biotechnology company, developed CERE-120 (AAV2-neurturin) for Phase I/II clinical trial (NCT00985517). Bilateral stereotactic administration of CERE-120 into the substantia nigra was safe and well-tolerated. Additionally, a news report on the study revealed that the treatment did not make the patients' challenges any better when compared to the control group. There was no significant difference between the

treatment and control groups in another Phase II research with CERE-120 (NCT00400634). Out of a total of 38 patients, 13 patients experienced side effects with CERE-120 treatment. In addition, three of the patients who had administered CERE-120 experienced tumor growth. Overall, the study's data revealed no appreciable advancements above the control group.

Glucocerebrosidase activity is the aim of gene therapy employing PR001A. Preclinical results of PR001A treatment also shown significantly reduced levels of Lewy bodies and -synuclein aggregation. The GBA1 gene, which codes for glucocerebrosidase, is administered once to the cisterna magna as part of the treatment, according to Prevail Therapeutics. The PR001A Phase I/IIa trial is currently recruiting subjects and has no accessible data; it is anticipated to be finished by June 2027 (NCT04127578). Genzyme, an American biotechnology company, is working on a tiny molecular treatment for early Parkinson's disease called GZ/SAR402671. In comparison to placebo controls, a Phase II clinical trial evaluates the safety and tolerability of oral GZ/SAR402671 for four weeks (NCT02906020). Additionally, preclinical research showed that taking GZ/SAR402671 by mouth reduced lipid buildup, slowed tau phosphorylation, and advanced -synuclein, ubiquitin, and ubiquitin phosphorylation, all of which increased cognitive impairments. Administration of GZ/SAR402671 was well-tolerated and safe in the Phase I study, but the trial was stopped owing to failure to achieve the primary and secondary endpoints on June 4, 2021 (NCT02906020). In conclusion, future success of gene treatments focusing on the GBA1 gene (PR001A) or neurotrophic factors (AAV-GAD) is possible.

Delivering the intended gene via an appropriate vector into the targeted brain region is a delicate and challenging task in gene therapy. For instance, the integration of numerous genes is not possible using AAV-based vectors because of their restricted capacity of 4.7 kb. Impaired gait, dorsal root ganglia dysfunction, ataxia, and elevated transaminases are a few risk factors

for gene therapy. In some instances, the targeted organs or tissues may experience significant toxicity as a result of the transgene's overexpression (Maruthi Prasad & Hung, 2021).

Chapter 6

Challenges and Future Directions

6.1 Challenges

Delivering the gene to the right place and switching it on

It is essential that the genetic mutation enters the appropriate cell because delivering a gene to the inaccurate cell would be ineffective and might potentially harm the patient.

Avoiding the immunological response

Gene therapy can occasionally introduce new genes that are thought to be potentially dangerous intrusions. The patient may experience an adverse immunological reaction as a result, which could be hazardous. Therefore, it is a challenge for scientists to discover a means to transfer DNA without the immune response "noticing". Typically, to achieve this, vectors that are not as likely to provoke an immunological response are used.

Ensuring the new gene does not interfere with the operation of other genes

The goal of gene therapy is to introduce new genes that will eventually integrate into the patient's genome and function normally for the remainder of their life.

The possibility exists that the genetic mutation will interfere with the action of another gene by inserting itself into its pathway. This can have negative consequences; for instance, cancer might develop if it interacts with a crucial gene involved in controlling cell division.

6.2 Future Directions

Gene therapy might offer potential cures for genetic illnesses. However, further research is required to fully comprehend the cellular and genetic pathophysiology of each disease. It is also necessary to make technical advancements (particularly to deliver goods to non-dividing cells) and to increase safety. There are always risks involved in developing novel therapies, but if the possible advantage turns out to be great, some risks are tolerable.

There are still unresolved issues. By 2022, the FDA was expected to have authorized about 40 additional gene treatments, according to a prediction made by the Massachusetts Institute of Technology in 2017. Europe might anticipate a similar scenario. If we want to include these cutting-edge medicines into standard medical procedures, this may call for changes in the current social and economic institutions. Governments, medical professionals, and scientists should concentrate on educating and assisting society in assimilating emerging revolutionary treatments. To guarantee proficiency and application, health-care systems need sufficient hospital architecture and educated employees. Financial toxicity continues to jeopardize accessibility and availability even as scientific research examines therapies for a variety of uncommon disorders (Maldonado et al., 2021).

Chapter 7

Conclusion

In conclusion, we have discussed some information regarding the discovery process, as well as the process of developing treatments for rare genetic disorders. Despite the fact that there are a few additional approaches, such as recombinant proteins, antibiotics, and stem cells, it is hoped that they will bring major advancements in the treatment of genetic illnesses. In the not too distant future, societies and other international authorities will assess and upgrade the existing legislation in accordance with the development of therapies. In order to make this innovative step available to people all around the world, it will be necessary to enlist the assistance of collaborative efforts from teams comprised of experts from a variety of fields, including governments.

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