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Biotechnological Approaches to Develop Personalized Medicines for Rare Genetic Disorders

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Abstract

Less than 5 out of 10,000 people have a rare condition. However, many find them harmful; the World Health Organisation (WHO) estimates that 7,000 uncommon diseases impact 7% of the world's population. This viewpoint discusses advancements in diagnosis, treatment, and prevention with an emphasis on hereditary metabolic disorders. There is a discussion of the obstacles to the discovery, creation, and application of treatments unique to uncommon diseases. Utilising innovative strategies, care networks, and collaborative frameworks is advised in order to fully realise the potential of personalised genomic medicine in order to lower morbidity and improve the standard of living for these vulnerable patients. Through the use of techniques like gene expression, CRISPR-Cas technologies, and genetic engineering of cell models and model organisms, it is possible to identify biomarkers for disease activity and diagnosis, develop therapeutic strategies, and uncover disease mechanisms through a process known as "translational medicine."

Keywords: Rare Disease, Drugs, Gene Therapy, Nucleic Acid Drugs, Stem Cell Therapy.

1 INTRODUCTION

Despite the fact that 95% of rare diseases lack a suitable therapy, private foundations, the pharmaceutical industry, and national governments are investing a lot of money on rare disease research and medication development. Thanks to developments in functional proteomics and genomics, personalised medicine a new medical revolution that has just begun—is defined by the creation of gene, cell, and molecular therapies that produce treatments unique to individual patients (Finkel et al., 2021). This method is justified by the fact that distinct mutations and genetic variability across individuals can have a substantial impact on illness susceptibility as well as the way a given drug reacts to it. Hospitals and healthcare systems have several difficulties in treating and caring for people with uncommon diseases. Hospital budgets and resources are typically strained by high costs, and patients with rare diseases generally require complicated, comprehensive, and intense care, which can be difficult for hospitals to deliver due to a lack of experience and a low patient volume.

Hospital resources are greatly burdened by rare and ultra-rare diseases, which have complex symptoms and low prevalence, making diagnosis extremely challenging (Brunetti et al., 2020). Low patient numbers and a lack of financial incentives may make access to specialised care less favourable. It is very difficult for hospitals to plan and oversee the care of patients with uncommon disorders since

they frequently need to see a range of medical specialists, including neonatal intensivists (Bernuy-Guevara et al., 2020). An increasing number of people are becoming aware of rare genetic disorders, which place a heavy strain on the health care system due to their high rates of death, disability, years lost from illness, hospital admission and re-admission rates, and medical expenses. There are various obstacles involved in carrying out clinical studies for uncommon genetic disorders.

- The small patient population and potential geographic dispersion of rare genetic illnesses can make it challenging to enrol a sufficient number of participants in therapeutic studies.
- Since various genetic mutations can cause a variety of uncommon genetic disorders, it can be challenging to find a patient population that is homogeneous enough to take part in clinical studies.
- Vulnerable groups, such youngsters or those with serious life-limiting illnesses, are frequently impacted by rare genetic abnormalities. Concerns about clinical trial conduct and the use of experimental therapies in these populations may arise as a result of this.
- Because the majority of orphan medications are quite expensive, accessing the drug and the reimbursement process can be difficult even if it is approved.
- The genetic disease causes ongoing damage to the fetus that can be prevented by earlier treatment.
- The disease will require hematopoietic stem cell transplantation and this may be improved when performed before birth.

Since drug safety and efficacy have previously been demonstrated, the drug repositioning strategy may be a more economical and effective means of developing therapeutic solutions for uncommon genetic illnesses (Raghu et al., 2024). Additionally, the process of developing new drugs may be sped up (Long et al., 2022). Various estimates indicate that a growing proportion of repurposed pharmaceuticals are making their way through the regulatory approval process, making up between 20% and 30% of all drugs licensed annually. The transport and preservation of the new genetic information are the primary biological obstacles to all genetic medications. To overcome these obstacles, one must comprehend the following: the disorder's molecular foundation, its mode of inheritance, the variety of mutations and genotype-phenotype relationships that give rise to the disease phenotype, the ways in which alternative genes affect the phenotype, and the how, where, and when the disease manifests itself. Maximising the chance of treatment success and reducing the danger of drug toxicity for each patient is the aim of personalised medicine (South et al., 2019). Depending on the type of causal defect(s), a rescue plan must be developed when the biological flawed mechanism has been identified. There are several methods available, ranging from artificial intelligence-based systems to high-throughput chemical screening to high-content gene screening for therapeutic target discovery.

2 RELATED WORK

Nearly all tissues express the GNE gene, which genes for a bifunctional enzyme that is necessary to catalyse the rate-limiting step in the sialic acid biosynthesis pathway. But mutations in this gene mostly affect skeletal muscle, sparing other tissues from harm (Stockton et al., 2020). Understanding the links between genotype and phenotype in the majority of diseases is a barrier to drug discovery. Therefore, it's critical to comprehend how basic biological processes are impacted by genetic abnormalities that cause disease. Model systems are crucial for understanding the biology of uncommon genetic illnesses because human individuals are challenging to investigate. Numerous disease pathways have been studied using mice, zebrafish, Drosophila, and cell-based in vitro systems (Tang et al., 2015). Potential therapeutic compounds have also been screened using these systems. Numerous essays in this volume are devoted to this significant field of study. It's interesting to note that early disease mechanism discoveries were led by Indian researchers. One of the first "lysosomal storage diseases" to be identified was metachromatic leukodystrophy, which occurred not long after lysosomes were discovered in 1955. A study (Bhattacharya et al., 2018) tells the tale of this ground-breaking discovery, which was made in 1963 by the late Professor Bimal Bachhawat of Christian Medical College, Vellore, in association with American scientist James Austin. Sen et al.'s paper from 2024 (Patel et al., 2024; Mashangva et al., 2024) discusses mitochondrial depletion disorders as well as a few potential molecular routes that could result in disease. Comprehending the mechanistic aspects will facilitate the creation of therapies tailored to individual organelles. These patients are currently currently given only symptomatic treatments.

Gupta & Bachhawat, (2023) explains in depth the state of therapeutic development and availability for patients with Gaucher disease (one of the main lysosomal storage disorders) in India. The authors come to the conclusion that developing and producing treatments in India is crucial to guaranteeing that patients have access to medications. A multifaceted strategy is needed to create medications for rare diseases, including patient registries, natural history models of nearby patients, technology platform development, and the design of innovative therapeutic compounds that are free of intellectual property rights. India has to increase its ability to manufacture locally produced medications for rare diseases and sell them at competitive pricing. It also needs a regulatory framework that makes performing clinical studies for medication approval possible. Every rare disease will also need a clinical centre where trials may be carried out in accordance with globally recognised guidelines. It is difficult to integrate several facets of the rare disease treatment process in India, partly because uncommon diseases present difficulties not found in common diseases. This special issue has several articles that address the regulatory environment, as well as the creation of different therapeutic approaches and possible medications for a range of uncommon genetic illnesses.

Gupta & Bachhawat, (2023); Maffioletti et al., (2018) describe the development of an antisense oligonucleotide (ASO) platform for exon-skipping therapy. By modifying mRNA splicing through exon skipping techniques, stop codon-containing exons can be removed, producing a nearly fully

functional protein. Protein synthesis may terminate prematurely due to a variety of mutations that vary throughout people. Consequently, platform technologies are required to develop medications that can be administered to individuals with various types of nonsense mutations. Many of the current therapies for Duchenne muscular dystrophy (DMD) and SMA are delivered using this approach. Chen et al., (2017); Bernuy-Guevara et al., (2020) have devised a patented process for producing morpholino-based ASOs and a plan for ASOs to efficiently infiltrate cells. The promise and difficulties of ASO therapy for patients are covered in their paper.

3 MATERIALS AND METHODS

An ultrasonic processor (sonicator) was used to fragment 10 µg of genomic DNA in 200 µl of MQ water. In our lab, DNA was sheared using a previously refined sonication process that used a 2 mm sonicator shaft and cycles of 15 seconds "ON" at 40 amplitude and 15 seconds "OFF" for 15 minutes. Ice was subjected to sonication, which reduced foaming. After everything was finished, a 1.5% agarose gel was loaded with 5 µl of sheared DNA to confirm that it fell within the 200–600 bp size range. After adding 3M sodium acetate and 100% ethanol, DNA was precipitated and stored at -80°C for the entire night. After air drying and washing the pellet with 70% ethanol, it was dissolved in 50 µl of sterile MQ water. Finally, the concentration and purity of DNA was verified both on agarose gel and Nanodrop spectrophotometer.

Cell Therapy

In several rare genetic illnesses, haematopoietic stem cell transplantation has shown promising results. These include Gaucher disease, thalassaemia, Canavan disease, Hurler syndrome, adrenoleukodystrophy, and severe combined immunodeficiency. Cell-based gene therapy, using chimeric antigen receptor T cells that express specific proteins, has been used recently to treat a range of lymphomas and haematological malignancies. Moreover, a number of inherited metabolic disorders, including Wilson's disease and anomalies in the urea cycle, that are marked by deficiencies in liver-specific enzymes have demonstrated potential in response to liver organ and cell donation. Haematopoietic stem cells have the ability to develop into all other blood cell types through the process of haematopoiesis.

Red bone marrow is home to haematopoietic stem cells, which derive from the mesoderm. In addition to bone marrow, umbilical cord blood is a rich source of haematopoietic stem cells and can be used as a second source of cells for transplanting these cells (Bahnson et al., 1994). The placenta is used to harvest umbilical cord blood after the cord is cut and the baby is delivered. Following that, the cord blood cells are collected, sanitised, and stored for future use in a cord blood bank. Treatments for lysosomal storage disorders and other genetic ailments include haematopoietic stem cell transplantation. The FDA has approved HPC (umbilical cord blood) as the first-ever approved umbilical cord haematopoietic stem cell (HPC-C) cell treatment for patients with disorders of the haematopoietic system. Donors at a higher risk of developing certain illnesses are prohibited from

donating umbilical cord blood (HCB) when it is "tested to exclude donors with sickle cell anaemia or anaemia due to abnormalities of haemoglobin C, D, or E." This puts the donor at risk of infection or inherited hemoglobinopathy from the recipient, who may be a gullible beneficiary.

General Molecular Biology Methods

The process for extracting DNA from fully developed cells was comparable. The cells were collected using trypsinization or a cell scraper, and then they were placed in an Eppendorf tube. To remove the pellets, the cells were centrifuged at 1500 g for 10 minutes following two PBS washes. The cells were suspended in 1 millilitre of DNA extraction buffer that had been carefully mixed with 10 Âµl of Proteinase K (10 mg/ml). For the whole night, the tube was maintained at 37 °C in a water bath. The next day, 5 Âµl of RNAse (10 mg/ml) was added, and the incubation was continued for another day. The protocol for phenol-chloroform extraction was followed. The protocol for extracting phenol and chloroform was adhered to. DNA was precipitated by combining 1/5th volume of cold absolute ethanol with 3M ammonium acetate. The pellet of DNA was cleaned using 70% ethanol, semi-dried, and then dissolved in 50 µl of TE buffer before being kept in storage at -20°C.

In the last twenty years, high-throughput molecular target screening has supplanted low-throughput animal model investigations, resulting in a fundamental shift in the methods used to find new medications. The steps involved in modern drug discovery and development include target identification, assay development, preclinical development, clinical trial development, throughput screening of small molecule libraries for hit identification, lead optimisation and discovery, and applications for FDA approval. The FDA may put the sponsor of the IND application on a clinical hold if it finds that testing an experimental medication on humans would be dangerous. Before the investigational drug's Phase I clinical trials can begin, the FDA must approve the IND. Before the medication is authorised for sale, phase II and phase III trials must be completed.

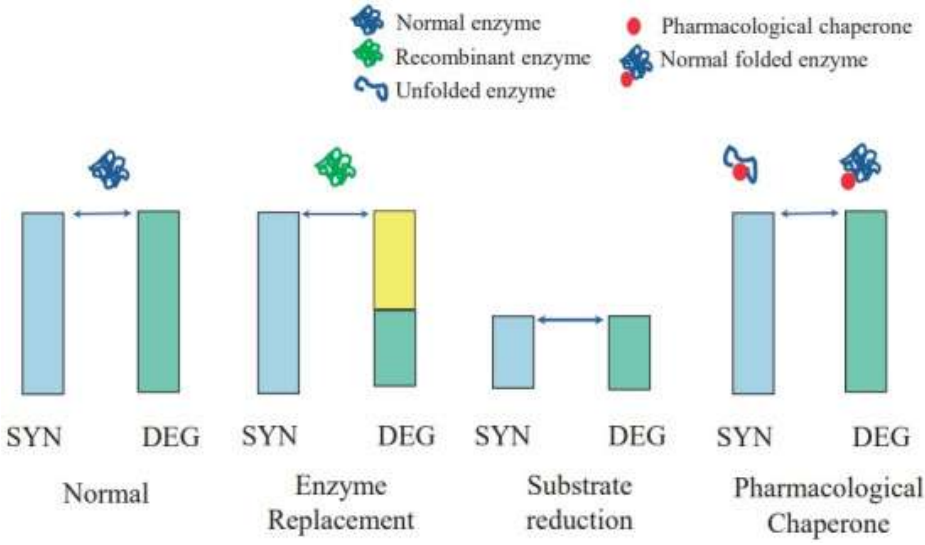

Figure 1: Small-molecule Treatment for Lysosomal Storage Diseases: Its Molecular Mechanism. Synthesis is SYN, and Degradation is DEG

Figure 1 displays Small-molecule treatments come with a number of benefits and drawbacks. Small molecules are easily synthesised in a laboratory and are generally smaller than biologics. This lowers the cost of small-molecule treatment and facilitates its commercialisation through easy scaling up. Small molecules can easily cross cell membranes, including the blood-brain barrier, and attack intracellular proteins and enzymes, making them useful for treating diseases caused by abnormalities in intracellular signalling pathways. Oral administration of small molecules is another practical option that helps with patient adherence. Nevertheless, tiny compounds may have off-target interactions that result in side effects, and they are frequently less selective in their targeting than biologics. Small compounds need frequent dosage due to their rapid metabolism or excretion. This limits their effectiveness.

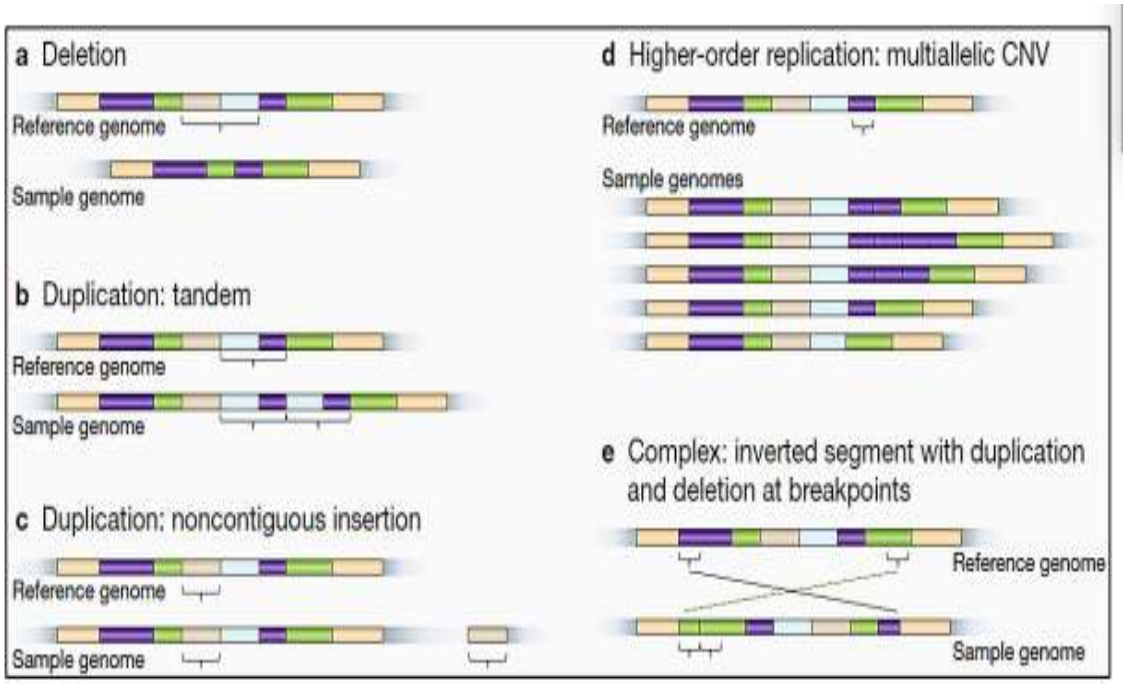

Figure 2: Types of Genomic Copy Number Variations

Depending on how they originated, CNVs can be of several forms, ranging from straightforward sequence duplication or deletion to intricate inversions and translocations (Figure 2). Beyond simple effects on gene dosage, CNVs can modify the function of enhancer and regulatory elements, alter the physical proximity of genes and promoters, change the architecture of the chromatin, and have long-term effects on the expression of genes worldwide.

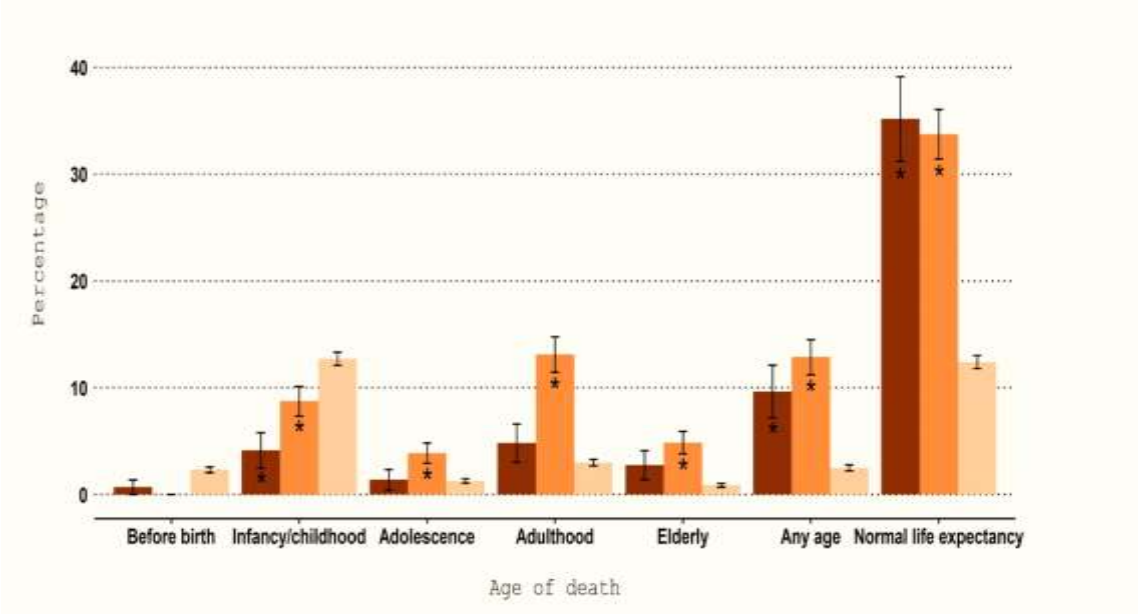

Figure 3: For RDs within the Spectrum, the Interval Average Age of Onset and Death were shown as Proportions and Standard Errors

For RDs across the spectrum, the mean age of onset and death were shown as percentages and standard errors. For a total of 96.3% and 64.2% of RDs, respectively, information was provided about the mean age of onset and death. (Show Figure 3) Keep in mind that percentages were determined using the total number of RDs in each category of condition. We observed significant results (*) when we looked at whether the proportions in the uncommon and borderline common disease categories differed considerably from the extremely rare disease category.

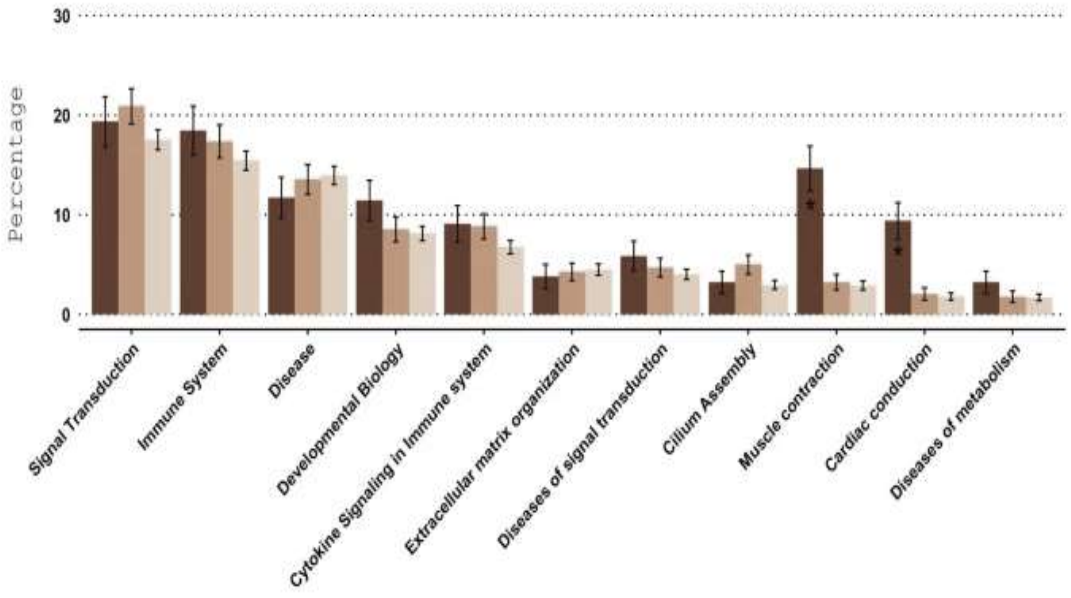

Figure 4: Summary of the Ratio (and Standard Error) of Genes Linked to Disorders

An overview of the fraction (and standard error) of disease-related genes linked to enriched Reactome pathways divided by the total number of genes associated with the sickness category under

examination (Figure 4). There were eleven improved Reactome pathways found for each type of disease.

4 CONCLUSION

Gene, stem cell, and small nucleic acid therapies are the main targets of new drug research efforts; mounting data indicates that these therapies are also vital for treating uncommon disorders. Although there have been significant advancements in the field of gene therapy using viral vectors, information regarding the treatment's long-term effectiveness (more than 10–20 years of observations) is still lacking. Gene editing holds great potential, and non-viral delivery systems will probably get safer and more effective as well. As a result, current methods for nucleofection (e.g., delivering CRISPR-CAs9 riboproteins) or transfection of plasmids or RNA into CD34+ cells are still linked to considerable toxicity in the target. Different kinds of liposomes are also still being developed. Advancements in non-viral delivery would facilitate the challenging task of circumventing the GMP manufacturing of highly costly viral vectors.

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