The Impact of Gene Editing and Gene Therapy on the Healthcare of Today

Aarush Gupta

Abstract

Advancements in gene editing and gene therapy have introduced a new era of possibilities in modern medicine. There are many gene editing methods, with CRISPR receiving most of the spotlight. Genetic correction shows promise for many different diseases and individuals and serves as a bridge between the medicine of today and the medicine of tomorrow. With rapid progress, the lengthy clinical trial process and the emphasis on ethics ensure the safety of treatments and uphold the value of benefiting patients. The original thoughts on many diseases are evolving as these techniques advance. We are currently witnessing change in the field of medicine through the lens of genetics.

Introduction

The field of genetics has grown tremendously in recent decades, and with it, many new technologies have emerged. Among these advancements, two systems of genetic correction have become prominent: gene therapy and gene editing. These technologies began to develop in the late 1900s, leading to the invention of three methods for genome editing: zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and clustered regularly interspaced palindromic repeats (CRISPR). Among these, CRISPR has completely revolutionized the field of gene editing. In particular, a majority of the success in genome editing has occurred in the twenty-first century, with two major milestones. One of these was the creation of the first genome-edited organism in 2002, using zinc-finger nucleases on *Drosophila melanogaster*, commonly referred to as the fruit fly (Carroll, 2021). The second milestone was the publication of a groundbreaking paper in 2012 that explained how CRISPR-Cas9 could be used for gene editing (Jinek et al., 2012). These technologies offer the potential to cure numerous diseases, particularly hereditary and genetic disorders such as cystic fibrosis, sickle cell disease, and Huntington's Disease (Johns Hopkins Medicine, n.d.). Furthermore, with new CRISPR-based technologies emerging, including base editing, created in 2016, and prime editing, created in 2019, concerns about the ethics and safety of gene therapy and gene editing have increased. Major reservations regarding the safety of genome editing include off-target effects and mosaicism. Furthermore, gene therapy and editing raise questions about who gets access to treatments, the ethics of germline editing, and the delineation of the boundary between treating diseases and enhancing the human species (MedlinePlus, 2022; National Human Genome Research Institute [NHGRI], 2019). Hence, when editing an individual’s genome, the safety and ethics of the edits must be considered to prevent negative consequences and uphold moral principles. The recent advancements in gene therapy and editing are very promising for the future of disease treatment. However, with the risks associated with changing the genome, it is essential to be cautious when considering these powerful technologies. Analyzing disorders such as cystic fibrosis and Huntington’s Disease and examining how CRISPR might affect them, further exploring the trial processes for the two major genome-altering mechanisms, and evaluating the moral implications and safety standards of gene editing and gene therapy can provide insight into how genome editing can be conducted in a way that benefits society.

An Examination of the Core Aspects of CRISPR-Cas9

CRISPR-Cas9 has transformed the field of genome editing by enabling precise alterations to DNA sequences. Its affordability and user-friendly nature have propelled it to the forefront of gene editing techniques. Beyond healthcare, CRISPR-Cas9 applications extend into biotechnology and agriculture. CRISPR consists of the Cas9 protein and a guide RNA (gRNA) (Aljabali et al., 2024). The discovery of CRISPR dates back to 1987 when repeated sequences interspersed with unique sequences in the genome of *Escherichia coli*. In 2012, researchers modified the CRISPR-Cas9 system from *Streptococcus pyogenes* for genome editing, demonstrating its ability to make precise DNA modifications (Konstantakos et al., 2022). The first step in gene editing is identifying the target DNA sequence. A synthetic gRNA, consisting of 20 nucleotides, is then designed to match this sequence. This gRNA binds to the DNA at the protospacer adjacent motif (PAM) site and directs the Cas9 enzyme to induce a double-strand break just before the PAM sequence. This break triggers the cell's natural repair mechanisms, including non-homologous end joining (NHEJ) and homology-directed repair (HDR) (Aljabali et al., 2024). HDR uses the sister chromatid as a template for DNA repair, ensuring precise gene editing outcomes. NHEJ, conversely, repairs double-strand breaks without a homologous template, often resulting in random insertions or deletions at the repair site. HDR's use of an external DNA template enables almost any desired DNA alteration, which is advantageous in clinical settings that require high accuracy. However, HDR is less frequently employed by mammalian cells, which typically favor NHEJ. NHEJ operates throughout the cell cycle except during mitosis, whereas HDR is confined to the S and G2 phases. Additionally, NHEJ is faster than HDR and can inhibit HDR through various cellular mechanisms (Yang et al., 2020).

Advancing Cystic Fibrosis Treatment with CRISPR

An estimated 90,000 individuals globally are affected by the most prevalent genetic disorder that significantly shortens life expectancy, cystic fibrosis (CF). It is an autosomal recessive condition that necessitates mutations in both CF gene alleles. It primarily affects the digestive and respiratory systems (Bierlaagh et al., 2021). This gene encodes the cystic fibrosis transmembrane conductance regulator (CFTR), a chloride channel protein belonging to the adenosine triphosphate (ATP)-binding cassette (ABC) transporter family. The CFTR protein contains two membrane-spanning domains (MSD1, MSD2), two nucleotide-binding domains (NBD1, NBD2), and a regulatory domain (R) with multiple phosphorylation sites. Upon phosphorylation, the regulatory domain undergoes a conformational change, resulting in the opening of the chloride channel (Fu et al., 2001). A major challenge in using CRISPR or other gene editing techniques for CF is the vast number of mutations in the CFTR gene (Bierlaagh et al., 2021). Despite the challenges, significant progress has been made. Researchers are using lipid nanoparticles containing CRISPR-Cas9 to replace mutated DNA. Daniel Siegwart and his colleagues from the University of Texas Southwestern Medical Center successfully delivered these nanoparticles to defective lungs in mice. Previously, these nanoparticles only targeted the liver, but this new approach allowed for the correction of the CFTR gene in the lungs, a major organ affected by the disease (Lesté-Lasserre, 2024).

Gene Therapy

Gene therapy is another method for altering the genome. However, it does not receive as much attention as gene editing. The key difference is that gene therapy uses a viral vector to

insert a normal gene into the genome, whereas gene editing involves directly modifying the DNA. They share many of the same characteristics, which explains why gene editing is often classified as a type of gene therapy (American Society of Gene & Cell Therapy [ASGCT], 2021). In gene therapy, two types of vectors can be used: viral and non-viral. Viral vectors account for approximately 70 percent of gene therapy trials, including adenoviruses, retroviruses, and lentiviruses. While these vectors are commonly used, they still raise safety concerns as they have the potential to provoke immune responses that could lead to unintended genetic mutations during the insertion of the therapeutic gene into the patient's genome. Additionally, these vectors have practical limitations, such as their ability to carry only a small amount of genetic material, making them unsuitable for delivering large genes. Their preparation is also complex, and they cannot be used repeatedly in the same patient because the body's immune system recognizes them after the first administration. This has led to the emerging use of non-viral vectors, such as cationic polymers and lipid nanoparticles, as a solution. These non-viral vectors do not face the same problems as viral vectors (Wang et al., 2023). Two approaches, ex vivo and in vivo gene therapy, have emerged from the considerable advancements in gene therapy. Ex vivo gene therapy involves collecting a patient's cells, genetically altering them in a lab, and then reintroducing them into the patient. This approach is especially useful for targeting particular organs. One of its main benefits is typically a low immune response since the cells used in ex vivo treatment come from the patient. However, logistical difficulties limit its use when aiming for internal organs such as the lungs, heart, or brain. To overcome these restrictions, in vivo gene therapy was developed. This method alters the target cells' genetic composition by directly injecting genetic material, such as DNA, into the patient's body. Clinically tested for hereditary diseases, in vivo gene therapy is also being evaluated for acquired conditions, including atherosclerotic arterial disease, restenosis, and cardiac transplant rejection. Both approaches represent significant achievements in gene therapy, providing new hope for previously untreatable diseases and paving the way for radical changes in medicine (Soofiyani et al., 2013).

Gene Editing and Huntington’s Disease

Huntington’s Disease (HD) is a genetic neurodegenerative disorder often associated with motor dysfunction and neuron loss. Symptoms include choreoic movements, cognitive and behavioral disorders, psychiatric issues, motor disruptions, dementia, weight loss, and sleep disturbances. It is a progressive disease, with symptoms varying at its onset. Genetically, HD results from the expansion of the trinucleotide cytosine-adenine-guanine (CAG) repeats in the HTT gene. Gene editing has been used to solve this problem. Zinc finger proteins found in ZFNs work by reducing the mutant Huntingtin (mHTT) protein without affecting wild-type HTT expression. In contrast, CRISPR excises the CAG repeats to silence mHTT expression. Due to this approach’s effectiveness, a majority of the new research into gene editing for HD has focused on CRISPR (Alkanli et al., 2023).

Bioethical and Safety Concerns

There are four main principles of bioethics: justice, autonomy, beneficence, and nonmaleficence (Varkey, 2021). For justice, it is essential to consider access to editing resources and ensure that treatments are available to the general population, not just a small group of individuals. Gene editing and therapy are often expensive, which can make it difficult for individuals in low and middle-income brackets to afford treatment. This challenges the

principle of justice, as only a small, wealthier group can afford treatment. Additionally, access becomes an issue for less developed nations, where these treatments may not be available, further limiting the benefits to a small segment of the population (Hildebrandt & Marron, 2018). This also requires a clear separation between eliminating disease and eugenics to ensure we do not create “designer babies” with an enhanced genome instead of the wild type (Sufian & Garland-Thompson, 2024). In terms of autonomy, informed consent from the patient is essential (Varkey, 2021). Closely related to the autonomy principle is the issue of germline editing, as future generations cannot decide whether they want edited genes before they are born (Schleidgen et al., 2020). Regarding beneficence and nonmaleficence, it is necessary to consider the potential benefits of the treatment against the side effects, including the risk of mosaicism and off-target effects from gene editing tools, particularly CRISPR (Ayanoğlu et al. 2020).

Conclusion

Gene editing and gene therapy have revolutionized modern medicine. However, as with any rapid advancement, it is crucial to ensure ethical considerations are also prioritized. With CRISPR’s flexibility and lower price, gene editing finally appears to be a solution within the public’s reach. Ongoing trials for changing the genome to eliminate major diseases offer many families hope for treatment. Gene editing holds immense potential to cure diseases once thought incurable, making it one of the most vital fields to advance in the future. As we navigate this transformative era, it is our collective responsibility to harness this potential for the greater good, shaping a future where the unimaginable becomes reality.

References

Aljabali, A. A. A., El-Tanani, M., & Tambuwala, M. M. (2024). Principles of CRISPR-Cas9 technology: Advancements in genome editing and emerging trends in Drug Delivery. Journal of Drug Delivery Science and Technology, 92, 105338. https://doi.org/10.1016/j.jddst.2024.105338

Alkanli, S. S., Alkanli, N., Ay, A., & Albeniz, I. (2023). CRISPR/Cas9 Mediated Therapeutic Approach in Huntington's Disease. Molecular neurobiology, 60(3), 1486–1498. https://doi.org/10.1007/s12035-022-03150-5

American Society of Gene & Cell Therapy [ASGCT]. (2021, November 2). Gene editing. https://patienteducation.asgct.org/gene-therapy-101/gene-editing#:~:text=What's%20the%20Difference%3A%20Gene%20Therapy,to%20treat%20or%20prevent%20disease.

Ayanoğlu, F. B., Elçin, A. E., & Elçin, Y. M. (2020). Bioethical issues in genome editing by CRISPR-Cas9 technology. Turkish journal of biology = Turk biyoloji dergisi, 44(2), 110–120. https://doi.org/10.3906/biy-1912-52

Bierlaagh, M. C., Muilwijk, D., Beekman, J. M., & van der Ent, C. K. (2021). A new era for people with cystic fibrosis. European journal of pediatrics, 180(9), 2731–2739. https://doi.org/10.1007/s00431-021-04168-y

Carroll, D. (2021). A short, idiosyncratic history of genome editing. Gene and Genome Editing, 1, 100002. https://doi.org/10.1016/j.ggedit.2021.100002

Fletcher, L. (2024, July 26). Back to basics - base & prime editing. Front Line Genomics. https://frontlinegenomics.com/back-to-basics-base-and-prime-editing/

Fu, J., Ji, H. L., Naren, A. P., & Kirk, K. L. (2001). A cluster of negative charges at the amino terminal tail of CFTR regulates ATP-dependent channel gating. The Journal of Physiology, 536(Pt 2), 459–470. https://doi.org/10.1111/j.1469-7793.2001.0459c.xd

Hildebrandt, C. C., & Marron, J. M. (2018). Justice in CRISPR/Cas9 Research and Clinical Applications. AMA journal of ethics, 20(9), E826–E833. https://doi.org/10.1001/amajethics.2018.826

Jinek, M., Chylinski, K., Fonfara, I., Hauer, M., Doudna, J. A., & Charpentier, E. (2012). A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. Science (New York, N.Y.), 337(6096), 816–821. https://doi.org/10.1126/science.1225829

Johns Hopkins Medicine. (n.d.). Genetic disorders. https://www.hopkinsmedicine.org/health/genetic-disorders

Konstantakos, V., Nentidis, A., Krithara, A., & Paliouras, G. (2022). CRISPR-Cas9 gRNA efficiency prediction: an overview of predictive tools and the role of deep learning. Nucleic acids research, 50(7), 3616–3637. https://doi.org/10.1093/nar/gkac192

Lesté-Lasserre, C. (2024, June 13). Lung-targeted CRISPR therapy offers hope for cystic fibrosis. New Scientist. https://www.newscientist.com/article/2435568-lung-targeted-crispr-therapy-offers-hope-for-cystic-fibrosis/

MedlinePlus. (2022, February 28). What are the ethical issues surrounding gene therapy? https://medlineplus.gov/genetics/understanding/therapy/ethics/

National Human Genome Research Institute [NHGRI]. (2019, March 13). What are the ethical concerns of genome editing? https://www.genome.gov/about-genomics/policy-issues/Genome-Editing/ethical-concerns

Razi Soofiyani, S., Baradaran, B., Lotfipour, F., Kazemi, T., & Mohammadnejad, L. (2013). Gene therapy, early promises, subsequent problems, and recent breakthroughs. Advanced Pharmaceutical Bulletin, 3(2), 249–255. https://doi.org/10.5681/apb.2013.041

Schleidgen, S., Dederer, H. G., Sgodda, S., Cravcisin, S., Lüneburg, L., Cantz, T., & Heinemann, T. (2020). Human germline editing in the era of CRISPR-Cas: risk and uncertainty, inter-generational responsibility, therapeutic legitimacy. BMC medical ethics, 21(1), 87. https://doi.org/10.1186/s12910-020-00487-1

Sufian, S., & Garland-Thomson, R. (2024, February 20). The Dark Side of CRISPR. Scientific American. https://www.scientificamerican.com/article/the-dark-side-of-crispr/

Varkey B. (2021). Principles of Clinical Ethics and Their Application to Practice. Medical principles and practice: international journal of the Kuwait University, Health Science Centre, 30(1), 17–28. https://doi.org/10.1159/000509119

Wang, C., Pan, C., Yong, H., Wang, F., Bo, T., Zhao, Y., Ma, B., He, W., & Li, M. (2023). Emerging non-viral vectors for gene delivery. Journal of Nanobiotechnology, 21(1), 272. https://doi.org/10.1186/s12951-023-02044-5

Yang, H., Ren, S., Yu, S., Pan, H., Li, T., Ge, S., Zhang, J., & Xia, N. (2020). Methods Favoring Homology-Directed Repair Choice in Response to CRISPR/Cas9 Induced-Double Strand Breaks. International journal of molecular sciences, 21(18), 6461. https://doi.org/10.3390/ijms21186461