An Overview of Gene Editing and Genetic Diseases

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I. Introduction

In recent times, our society has witnessed many diseases that have caused a decline in the population. With the emergence of gene editing and gene therapy however, many of these illnesses can be combated. These advancements can introduce a new and potentially permanent form of treatment to ailments that were previously deemed incurable. Gene editing utilizes specific technologies to alter the genome of an organism by disconnecting the DNA at a specific area and replacing or adding a gene. Meanwhile Gene therapy attempts to remediate a faulty gene by introducing new genes to counter its effect. Many forms of diseases have shown signs of dissipating after involving gene editing or gene therapy in clinical trials. However, within the scientific community, many ethical considerations arise based on the viability of this solution and potential risks. On top of this, the federal government has been questioned on whether there should be further measures in place to reduce the risk of clinical trials.

II. Gene editing

Gene editing allows scientists to make direct changes to the human genome. The first technologies began in the early 1990s with newer advancements such as CRISPR in 2009. Within the medical industry, gene editing is being tested to treat diseases including Cancer, Sickle Cell Disease, High Cholesterol, Diabetes, Parkinson's disease and more. In most cases, the gene editing technology targets the mutated gene responsible for the disease and replaces it with its
healthy form. To conduct a precise change to the genome, scientists begin by identifying a target or a specific gene/section of DNA they want to alter. Based on the requirements of the task, the tool is designed to edit the exact gene. These "programmed" tools are delivered to the cell through viral delivery systems, non-viral methods, direct delivery of pre-assembled complexes, and Ex Vivo delivery. Viral delivery systems (AAV & Lentiviruses) are modified to remove their pathogenic capabilities and are inserted with a payload consisting of the technology and the required DNA/RNA. The virus then "infects" the cell and releases its components within the cell's cytoplasm/nucleus. Non Viral methods include Electroporation, Lipid Nanoparticles, and Chemical Transfection. The technologies are packaged within a carrier system. This system uses physical forces (ex. Producing pores with electrical signals) , chemical interactions (ex. Lipid-lipid membrane fusion) or biological mechanisms (ex. Cell penetrating peptides) to insert the technology within the cell. These physical, chemical and biological processes are otherwise known as the direct delivery of preassembled complexes. Ex vivo delivery utilizes an already modified cell which is then cultured and then infused back into the patient. This method minimizes the risks associated with In vivo editing which mainly happens in the body. In order to achieve the modifications to the genome, gene editing uses

DNA repair pathways. These repair mechanisms fix the double or single-strand break caused by the gene editing tool. The two most common forms are Non-Homologous End Joining (NHEJ) and Homology-Directed Repair (HDR). In NHEJ, the two DNA strands are fused without the need for a homologous template. Although this process is fast and efficient, it is prone to causing errors such as insertions, deletions, substitutions, and other mutations. 90% of the time, Non-Homologous End Joining is performed to repair a break in the DNA since it can operate throughout the cell cycle. On the other hand, HDR uses a template to precisely join the two strands of DNA, minimizing the number of mutations compared to NHEJ. However, this process is slower and inefficient, occurring only during the S phase and G2 phase of the cell cycle (mitosis), and there is only a 10% probability of two strands being repaired via this strategy. After the strands are fused, the gene is replaced with another.

III. Gene Editing Technology: CRISPR/Cas9:

CRISPR (clustered interspaced short palindromic repeats) is an immune system response that is utilized by microbes to recognize and remove invaders. In the first compromise with the virus, the human body generates multiple types of antibodies to produce an immune memory. In the second iteration of facing the virus, we are successfully able to recognize and eliminate it. CRISPR establishes a genetic memory using a guide RNA that has a complementary sequence to the virus's section of RNA. After it recognizes the virus, the guide RNA can bind to the virus and destroy it. There are multiple ways that gene therapy and cell therapy can influence the usage of CRISPR. It can either be used to fix a mutated gene or change the expression of a gene and the proteins it produces. For genetic diseases caused by a single mutation, it is much easier to solve compared to a more complex/detrimental disease. Cells can also be removed from a patient and engineered to better recognize foreign invaders/diseases (cell therapy). Unlike many other forms of gene editing, CRISPR can be mutated more easily compared to single protein tools. For those tools, it was required to redesign the entire protein sequence to test and see whether it would work. With the use of an RNA strand instead of a protein, it is much easier to redesign the target recognition sequence. Cas9 is an endonuclease protein which can cut both of the DNA strands. Combined with the guidance of the sgRNA, the Cas9 protein can cut out the mutated or targeted part of the DNA. From there, the swap’s precision and chance of mutation is dependent on whether the ends are joined through Homology-Directed Repair or via Non-Homologous End Joining.

IV. Gene Editing Technology: Talens

Transcription Activator-Like Effector Nucleases(Talens) are engineered enzymes that contain two parts in gene editing. They have a DNA-binding domain which recognizes and binds to a specific DNA sequence and a Nuclease Domain which cuts the DNA near the binding site. The Transcription Activator-Like effector is found in plant-pathogenic bacteria like Xanthomonas

spp. These TALEs represent the DNA-binding domains and can recognize DNA sequences through their repeating modules corresponding to each nucleotide base. The models are about 34 amino acids long, and their most crucial parts are the RVDs(Repeat Variable Disresidues), which determine the DNA base each module will bind to. In gene editing, this amino acid sequence is reconstructed, corresponding to the target DNA. The FokI Nuclease, which is in charge of cutting the DNA, is fused to the TALE protein. Then, two TALENS bind on opposite sides of the target site to dimerize and cleave the strand on both sides. Contrasting with CRISPR, the main takeaway from Talens is the complexity involved in their design and assembly. There are current therapeutic applications such as Immunotherapy and the treatment of genetic disorders (DMD, ADA-SCID), as well as in stem cell and regenerative medicines (PSCs).

V. Gene Editing Technology: Zinc Finger Nucleases

Zinc Finger Nucleases are a type of artificial restriction enzymes which are composed of a separate Zinc Finger DNA-binding zone and a DNA-cleavage domain. A ZF domain holds stabilizing folds which are formed with the help of Zinc ions that bond to specific amino acids (Cysteine, Histidine, etc.). This contributes to the structure, which consists of a β-sheet and an ***α***-helix. The ***α***-helix is the part of the Zinc Finger that is able to interact with the DNA. Because of the amino acid residues on the ***α***-helix, the Zinc Finger can make contact with the bases of the DNA and recognize a 3 base-pair sequence. By modifying the sequence of the Amino Acids, it can be customized to pair with any sequence. To improve specificity, multiple Zinc Fingers can be linked together in tandem. These arrays generally contain between 3 - 6 Zinc Finger Repeats and, based on efficacy, can recognize 9 to 18 base pairs. In comparison, the purpose of the nuclease domain is to initiate double-stranded breaks. These are derived from the FokI enzyme-- Type II restrictive endonuclease. In contrast to other enzymes, the FokI enzyme has both a DNA-binding domain and a nuclease domain. Essentially, allowing it to do 2 jobs at once. These domains must be oriented correctly on their respective DNA sequences in order to form a functional dimer. Compared to other technologies, ZFNs are highly researched and proven effective in therapeutic and editing applications. They also come with the advantage of having a high specificity, although they are difficult to modify.

VI. Genetic Disease: Down Syndrome

Down Syndrome is a genetic disease caused by a trisomy of the 21st autosomal chromosome. This means that instead of containing 46 chromosomes (23 pairs), a diagnosed patient will have an odd 47 chromosomes. People who are born with this genetic mutation experience abnormal brain and body development. The chance of being affected by Down Syndrome is approximately 1 in every 700 newborns. In general, the cases are usually

distributed sporadically through a nondisjunction mutation during meiosis, where nobody commonly receives an autosomal dominant or recessive gene. Although 95% of cases are affected by trisomy 21, 4% of cases are caused by the translocation of the 21st chromosome. This is when the chromosome relocates to another chromosome. Mosaic Down syndrome is when some cells contain 46 chromosomes while others contain 47 chromosomes, with a high rarity of only 1-2% of the cases. Those with Down syndrome have distinct physical characteristics, including a flattened facial profile with small ears and mouth, as well as a short stature, short neck, and small body features. They also suffer from cognitive disabilities and behavioral/mental health issues. Currently, research is being conducted to find the most viable method to prevent Down Syndrome in the future whether it be silencing the 21st chromosome with the XIST gene, by individually targeting specific genes on the chromosome or, by repressing the gene expression with epigenetic modifiers or with prenatal gene editing.

VII. Genetic Disease: Severe Combined Immunodeficiency

SCID (Severe Combined Immunodeficiency) is a group of mutations that compromises a patient's immune system by mutating genes correlated to infection fighting and development of immune cells (specifically T and B cells). Infants usually don't have a family history of the disease and it can be fatal without transplants, gene therapy or enzyme therapy. In most cases it is inherited through an autosomal recessive pattern making it undetectable when being passed down through generations. Adenosine deaminase deficiency (ADA-SCID) is the most common form where there is an absence of the ADA enzyme. The ADA enzyme is a vital part in the purine salvage pathways for converting adenosine and deoxyadenosine (byproducts of DNA and RNA) into inosine and deoxyinosine. The absence of this enzyme can result in toxic levels of deoxyadenosine build up. This deoxyadenosine then phosphorylates intracellularly forming dATP which inhibits the ribonucleotide reductase. The ribonucleotide reductase enzyme plays a role in converting ribonucleotides into deoxyribonucleotides which are necessary for DNA synthesis and repair. The lack of the reductase enzyme impacts Lymphatic DNA which rely on rapid proliferation to reinstate immune function. This results in impaired lymphocyte development and function, suggesting why patients carry a lower number of T and B cells. Besides T-B-NK- SCID (ADA-SCID) another form is T^- B^- NK^+ SCID where a RAG 1/2 Deficiency causes a lack of RAG enzymes preventing TCR and BCR gene(VDJ) rearrangement. Because of this, T and B cells aren't able to form functional receptors and mature. In the same phenotype, the artemis deficiency causes cells to die from broken DNA because they aren't able to repair it after the RAG enzymes cut it. In a T^- B^+ NK^- SCID, a γ-chain deficiency effects cytokine receptors. Signals needed for T & NK development fail, resulting in an absence of T and NK cells or, through a JAK3 deficiency, even with a present receptor, signals aren't able to be transmitted within the cell causing the same issue. Modernly, scientists are experimenting to collect a patient's hematopoietic stem cells, genetically edit them and reinfuse them back into the body. However this process is still in the preclinical stages.

VIII. Conclusion

In conclusion the future has opportunities open for advancements in gene editing technologies. CRISPR allows for easy design and implementation of the technology allowing companies to efficiently develop treatments without incurring high costs. However, other technologies like Zinc Fingers have a higher specificity and are more effective. Talens on the other hand have already been proven to work in stem cell treatment and regenerative medicine applications. With a combination of these technologies, companies will be able to establish successful treatments for common genetic diseases like Down Syndrome and Severe Combined Immunodeficiency. Despite the hints of its upside, there are many controversies over gene editing and its possible impact within healthcare and the clinical trial space. It will take years of collaboration to make it a usable form of treatment to eradicate these genetic diseases.

References

[1] - Behind the Bench Staff. "Unlocking the Future: Exploring Viral and Non-Viral Delivery Methods for Gene Therapy Manufacturing." *Thermo Fisher Scientific*, 21 Mar. 2025, www.thermofisher.com/blog/behindthebench/unlocking-the-future-exploring-viral-and-non-viral-delivery-methods-for-gene-therapy-manufacturing/. Accessed 2 June 2025.

[2] - Chae, Keun et al. "CRISPR-based gene editing of non-homologous end joining factors biases DNA repair pathway choice toward single-strand annealing in *Aedes aegypti*." *Current research in biotechnology* vol. 5 (2023): 100133. doi:10.1016/j.crbiot.2023.100133

[3] - Dr. Lei Chen. "Lipid Nanoparticles - The Vanguard of CRISPR Delivery Systems." *GenScript*, 15 Oct. 2024, www.genscript.com/lipid-nanoparticles-the-vanguard-of-crispr-delivery-systems.html? Accessed 2 June 2025.

[4] - Fridovich-Keil, Judith L.. "gene editing." Encyclopedia Britannica, 4 May. 2025, https://www.britannica.com/science/gene-editing. Accessed 2 June 2025.

[5] - Tsuchida, C. A., K. M. Wasko, J. R. Hamilton, and Jennifer A. Doudna. "Targeted Nonviral Delivery of Genome Editors In Vivo." *Proceedings of the National Academy of Sciences of the United States of America*, vol. 121, no. 11, 2024, e2307796121. https://doi.org/10.1073/pnas.2307796121.

[6] - Vectors: Tools for Gene Delivery." *Genehome*, www.thegenehome.com/how-does-gene-therapy-work/vectors? Accessed 2 June 2025.

[7] - Xue, Chaoyou, and Eric C Greene. "DNA Repair Pathway Choices in CRISPR-Cas9-Mediated Genome Editing." *Trends in genetics : TIG* vol. 37,7 (2021): 639-656. doi:10.1016/j.tig.2021.02.008

[8] - Justiz Vaillant, Antonio A., and Mitra Mohseni. *Severe Combined Immunodeficiency*. StatPearls, updated 8 Aug. 2023, StatPearls Publishing, Jan. 2025, https://www.ncbi.nlm.nih.gov/books/NBK539762/.

[9] - "Severe Combined Immunodeficiency (SCID)." *National Institute of Allergy and Infectious Diseases*, www.niaid.nih.gov/diseases-conditions/severe-combined-immunodeficiency-scid. Accessed 2 June 2025.

[10] - "CRISPR-Cas9: Mechanisms, Types, and Biological Applications." *Biology Insights*, biologyinsights.com/crispr-cas9-mechanisms-types-and-biological-applications/. Accessed 2 June 2025.

[11] - Redman, Melody et al. "What is CRISPR/Cas9?." *Archives of disease in childhood. Education and practice edition* vol. 101,4 (2016): 213-5. doi:10.1136/archdischild-2016-310459

[12] - Becker, Sebastian, and Jens Boch. "TALE and TALEN Genome Editing Technologies." *Gene and Genome Editing*, vol. 2, Dec. 2021, p. 100007. *Science Direct*, https://doi.org/10.1016/j.ggedit.2021.100007.

[13] - Joung, J Keith, and Jeffry D Sander. "TALENs: a widely applicable technology for targeted genome editing." *Nature reviews. Molecular cell biology* vol. 14,1 (2013): 49-55. doi:10.1038/nrm3486

[14] - "Transcription Activator-Like Effector Nucleases (TALENs) Explained." *The Scientific World*, 12 Feb. 2025, www.scientificworldinfo.com/2025/02/transcription-activator-like-effector-nucleases-explained.html. Accessed 2 June 2025.

[15] - Carroll, Dana. "Genome engineering with zinc-finger nucleases." *Genetics* vol. 188,4 (2011): 773-82. doi:10.1534/genetics.111.131433

[16] - Urnov, Fyodor, Edward Rebar, Michael Holmes, et al. "Genome Editing with Engineered Zinc Finger Nucleases." *Nature Reviews Genetics*, vol. 11, no. 9, 2010, pp. 636–646. https://doi.org/10.1038/nrg2842.

[17] - "Zinc Finger Nucleases." *Gene Therapy Net*, www.genetherapynet.com/gene-editing-tools/zinc-finger-nuclease.html. Accessed 2 June 2025.

[18] - Akhtar, Fatima, and Saira R. A. Bokhari. "Down Syndrome." *StatPearls*, StatPearls Publishing, Jan. 2025, https://www.ncbi.nlm.nih.gov/books/NBK526016/. Accessed 2 June 2025.

[19] - "Down Syndrome." *CDC*, 26 Dec. 2024, www.cdc.gov/birth-defects/about/down-syndrome.html. Accessed 2 June 2025.