Treating Duchenne's Muscular Dystrophy by Using Gene Editing and Gene Therapy

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Abstract

Duchenne's Muscular Dystrophy, DMD, occurs mainly in boys aged two to four and is caused by a genetic mutation that provides no dystrophin, which is a protein that keeps the muscles intact. This paper explores and summarizes several successful gene editing and gene therapy clinical trials that have treated patients with DMD. Using a CRISPR-Cas9 method, it cuts out sections of the dystrophin gene allowing for the cell to make a shortened but functional version of the protein. FDA-approved treatments like Elevidys are a result of gene therapy and it delivers a shortened version of the dystrophin gene in an effort to restore its functionality. While gene editing and gene therapy are a great way to treat DMD and other diseases, there are still many safety and ethical concerns of using these technologies. Ranging from off-target effects, unwanted immune responses, and several ethical implications regarding these technologies. Addressing the many safety and ethical implications of gene editing and gene therapy is a crucial step towards advancing the potential of a future cure for DMD.

Introduction

This paper aims to explore how the use of gene editing and gene therapy can potentially cure Duchenne's Muscular Dystrophy. Gene editing is the process of adding, removing, or altering an organism's DNA. There are several technologies that allow these changes to happen such as, CRISPR-Cas9 which is the most popular. Cas9 is the most used protein that cuts DNA to alter a cell's genome. CRISPR-Cas9 works by using a guide RNA (gRNA) to direct an enzyme called Cas9 to a specific DNA sequence. Cas9 attaches itself to the DNA and cuts it, causing the targeted gene to shut off (Addgene, 2015). Gene therapy is not to be confused with gene editing. Gene therapy uses genes to prevent or treat a disease. Gene therapy works by adding new copies of a gene or replacing a defective or missing gene in a cell with a healthier version of that gene. Furthermore, the replacement of the gene to a healthier version can potentially prevent or cure the disease. Gene therapy has been used to treat many different diseases such as Duchenne Muscular Dystrophy (DMD). DMD occurs due to a faulty gene that lacks dystrophin, which is a protein that keeps the muscles intact (Taylor, 2023). Furthermore, common symptoms of the disease are muscle weakness such as, frequent falling, difficulty walking, etc. The common symptoms usually appear at ages two to four and primarily affect males because it is found on the X-chromosome. There is currently no cure to DMD; however, through the development of new technologies including gene editing and gene therapy, there may be a potential cure.

Mutations in Depth

DMD mainly affects males because it is found in an X-linked recessive pattern and if the X chromosome carries the mutated gene for DMD, the individual will likely be affected (Muscular

Dystrophy Association, 2024).

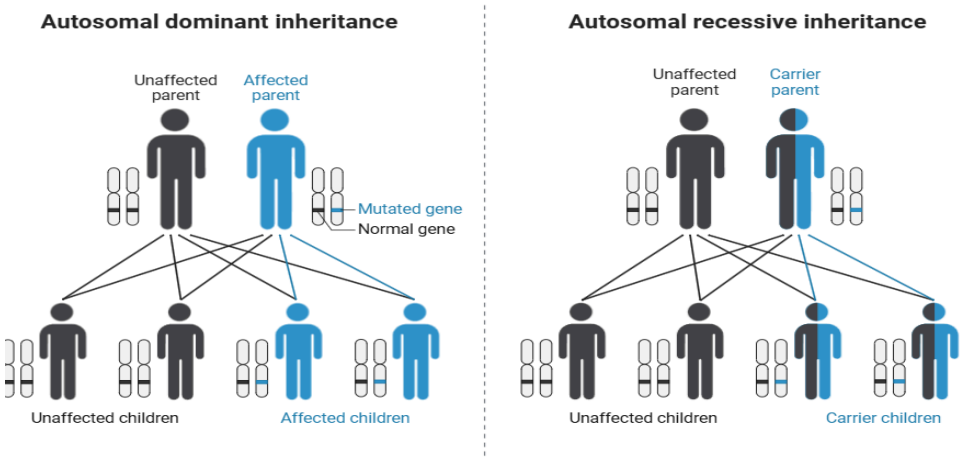

Figure 1: The relationship of dominant and recessive inheritance (Created using Bio render).

For this paper, we are primarily focused on exon deletion. Exons are parts of a gene that encode a protein and in a normal dystrophin gene these exons fit together perfectly like puzzle pieces. However, mutations in the gene prevent these exons from fitting together making it hard for the dystrophin protein to be present (UCLA Health, 2022).

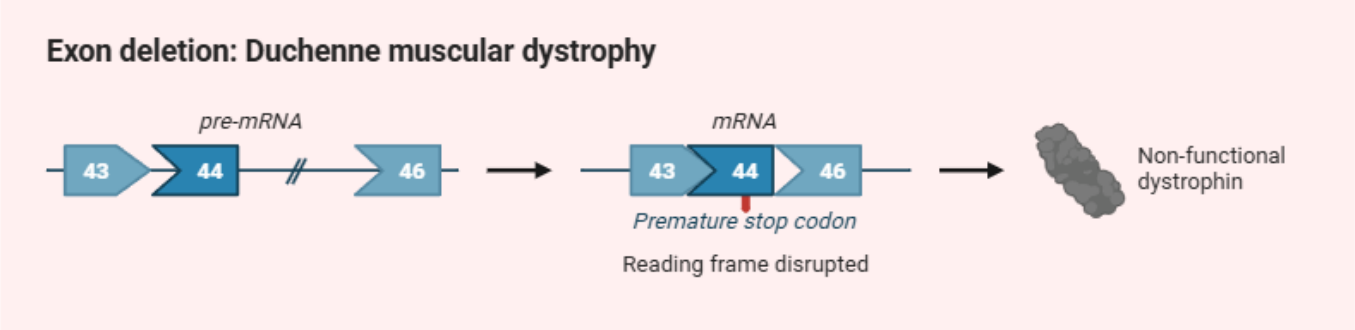

Figure 2: Visualization of exon deletion and how it disrupts the creation of dystrophin (Created using Bio render).

The figure shows a deleted exon in the dystrophin gene, this makes it difficult for the body to make dystrophin. There are in-frame deletions and out-of-frame deletions. An out-of-frame deletion occurs when there is no dystrophin protein being made which is what this paper is focused on (“Types of Genetic Variants”). An in-frame deletion is where a protein may be shorter than usual but is still functional and results in Becker Muscular Dystrophy (“Types of Genetic Variants”).

Application of Gene Editing

Given the new technology of CRISPR-Cas9, MyoGene Bio, a company co-founded by UCLA faculty members, utilizes a CRISPR-based treatment called MyoDys45-55. MyoGene Bio's proposed CRISPR-based treatment works by cutting out sections of the dystrophin gene allowing for the cell to make a shortened but functional version of the protein. (UCLA Health, 2022). Dystrophin contains a large number of repeated segments and sometimes these segments fold back together in a specific way forming a 3D structure. (UCLA Health, 2022). MyoDys45-55 creates a deletion that allows for this structure to be maintained. MyoDys45-55 only works on deleting exons 45-55 which is a "hotspot" where mutations occur most often.

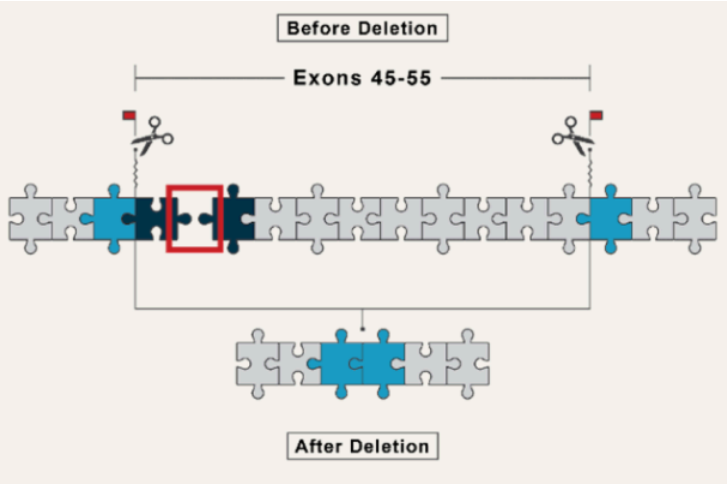

Figure 3: Delivering a short version of the dystrophin gene to the deleted exon (Created using Bio render).

Since MyoDys45-55 targets hotspots, it can impact around 10-15% of the patient population (UCLA Health, 2022). While MyoDys45-55 has not been used yet, because of its intended effectiveness it is set to be successful to many patients.

Application of Gene Therapy

Another way to treat DMD that is being researched is gene therapy. In 2023, the Federal Food and Drug Administration, FDA, approved Elevidys. Elevidys is an adeno-associated virus vector-based gene therapy treatment for patients aged four to five. Elevidys delivers a gene encoding micro-dystrophin,138 kDa, which is a shortened version of the protein dystrophin, 427 kDa. The FDA's approval was based on a Sarepta Therapeutics clinical trial. "In one study which involved two parts, individuals in part 1—which was randomized, double-blind, and placebo-controlled—were treated with either Elevidys or placebo and followed for 48 weeks. In part 2 of the study, individuals who received placebo during part 1 were treated with Elevidys, and individuals treated with Elevidys during part 1 received a placebo. All individuals were

followed for an additional 48 weeks" (FDA, 2023).

Time to rise (TTR)	Change vs Placebo LSM* Diff in Seconds
Overall (n=124)	-0.64 (p=0.0025)
Ages 4-5 (n=59)	-0.50 (p=0.0177)
Ages 6-7 (n=65)	-0.78 (p=0.0291)
10-meter walk test	**Change vs Placebo LSM Diff in Seconds**
Overall (n=124)	-0.42 (p=0.0048)
Ages 4-5 (n=59)	-0.33 (p=0.0319)
Ages 6-7 (n=65)	-0.52 (p=0.0363)

Figure 4: Results of clinical trial by Sarepta Therapeutics (Sarepta Therapeutics, 2023).

The trial results favored Elevidys by improving the time to rise of 5 seconds by 90% percent. Additionally, those who got Elevidys improved their score on the North Star Ambulatory Assessment, a test used to measure motor abilities in children with DMD, by 2.6 points. Because of its approval through the Accelerated Approval Pathway which allows the FDA to approve drugs for serious life-threatening diseases, the approval of Elevidys is not just any approval but a transformative and life-changing approval for many patients with DMD and will potentially be successful in the future per the FDA's approval.

Ethical Concerns with Gene Editing and Gene Therapy

While gene editing and gene therapy are a great way to treat DMD and other diseases, there are still many safety and ethical concerns of using these technologies. One of many are off-target effects; CRISPR and other gene editing tools sometimes target unwanted parts of the genome. This can further lead to potential mutations that cause more health issues and are a major concern when treating patients using CRISPR. CRISPR off-targeting occurs when the gRNA binds to regions in the genome with a similar sequence that is off-target to the intended one. Additionally, the design of the gRNA can be a factor as well. Immune responses can also trigger unwanted consequences. In a gene therapy trial, a 27-year-old patient was set to receive an alternative form of DMD protein. However, that patient later died due to injuries in their lungs caused by an unwanted immune reaction to the adeno-associated vector which was given in a high dosage (Taylor, 2023). The dosage that was tested was similar to other clinical trials but in this case it went wrong because of the patient's lean muscle mass of 45% (Taylor, 2023).

Another concern is the diversity of patients undergoing trials or receiving gene editing or therapy treatments. People in more densely populated areas may have more accessibility to treatments whereas people in more rural areas have limited or lack access (Bateman-House, Alison, 2024). Additionally, these treatments are expensive to develop and administer, which makes them inaccessible to people in low-income areas greatly (Bateman-House, Alison, 2024). Addressing these safety and ethical concerns is important to treat DMD effectively and safely.

Conclusion

This paper discussed different successful clinical trials that used gene editing and gene therapy to treat DMD. The advancements of gene editing and gene therapy hold promise to change the lives of patients with DMD; ranging from the MyoDys45-55 CRISPR-based treatment to the gene therapy route of the FDA approved Elevidys. However, there are many safety and ethical concerns with this new form of treatment. Gene editing and gene therapy poses concerns for off-target effects, unwanted immune responses, and limited accessibility. To combat off-target effects, they can be predicted by an open source online software that can be accessed through the internet (Congting Gou, 2023). “The prediction algorithms of these software are primarily based on sgRNA sequences, thus the outputs of these methods are usually biased toward sgRNA-dependent off-target effects” (Congting Gou, 2023). These tools can help minimize the risk of CRISPR off-targeting. As for unwanted immune responses, they are caused when the body thinks the virus is a foreign substance and attacks the virus. To potentially mitigate unwanted immune responses one way is to simply reduce the amount of the virus being given to the patient (Sack, Brandon K., et al, 2009). This method has the potential of producing a more beneficial and therapeutic protein, which is helpful for tolerance (Sack, Brandon K., et al, 2009). Limited accessibility is addressed through implementing policies that provide equal access to treatments, creating special access programs to address patient affordability concerns, price regulations, government funding for research, etc (Lee, Tsung-Ling, et al, 2024). Additionally, these concerns also pose the need for more research before beginning trials, numerous testing, and following healthcare policies. Ultimately, the future of gene editing and gene therapy is dependent on the transformative science technologies and ethical applications coexisting. To ensure they can coexist with each other we first must address patient accessibility and safety concerns with these new technologies.

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