Exploring the Possibilities of Gene Therapy for Marfan Syndrome by Analyzing Potential Interventions to Prevent Cardiac Manifestations

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ABSTRACT

Marfan Syndrome is an autosomal dominant genetic condition resulting from a mutation in the *FBN1* gene of Chromosome 15. Approximately 200,000 patients residing in the United States are affected by this condition, with about 50,000 new diagnoses each year [1]. Extreme cases of Marfan syndrome can lead to deadly outcomes from massive internal bleeding with predominant abnormal manifestations in the skeletal, cardiovascular, and ocular systems. Cardiac involvement is prominent and is the primary cause of sudden death. Aortic aneurysms can advance into aortic dissections or ruptures, which lead to complete tears of the aortic layers. As of now, there is no direct cure for Marfan syndrome but treatment typically involves symptom management, which ranges from medication to preventative surgery. Gene therapy targeting the *FBN1* gene can develop new, promising treatment options. In this review, we aim to discuss the usage of gene therapy for the treatment of Marfan Syndrome.

INTRODUCTION

Background

Marfan syndrome is an autosomal dominant genetic disorder due to a mutation in the *FBN1* gene of Chromosome 15, with a reported incidence of 1 in 3000 to 1 in 5000 [2]. The *FBN1* gene encodes the Fibrillin-1 protein, which controls the formation of elastic fibers in charge of assembling connective tissues in the human body. Connective tissues form the basis for organs and tissues by supporting, protecting, and providing an external structure [3]. A mutation in the FBN-1 gene results in decreased production levels of the protein Fibrillin-1, either by missense mutations or deletion of a base pair. Due to a minimal presence of Fibrillin-1, the transforming growth factor beta, or TGF- β, becomes abnormally active. In consequence, deterioration of elastic fibers is evident, resulting in improper connective tissue systems forming irregular growth of the upper and lower extremities.

Symptoms

Marfan syndrome is associated with a wide variety of other symptoms, as well. Patients with Marfan syndrome typically possess issues with their ocular, skeletal, and cardiovascular systems. Symptoms in the ocular system include near-sightedness as a result of partial lens dislocation.

Skeletal complications in Marfan Syndrome commonly include early-onset osteoarthritis and joint pain. Patients typically experience limited movement in their hips as one of the first signs of Marfan Syndrome. An abnormal curvature of the spine resulting in back and neck pain corresponds with the irregular presence of Fibrillin-1 and TGF- β proteins. Patients generally present with a tall stature. A tall stature can also be associated with poor blood flow to the extremities leading to a case known as cold fingers and toes. Patients may also experience speech issues due to prominent facial features such as high palates and small jaws that limit mobility. In cases of extreme Marfan Syndrome, individuals may undergo spontaneous pneumothorax, during which air escapes from the lung and occupies space between the chest wall and the lung. Without immediate treatment, this may result in death from lung failure. Much like other disorders, fatigue, shortness of breath, a rapid pulse, and chest pain are common symptoms.

The most prominent and severe abnormalities reside within the cardiovascular system, specifically the aorta. These conditions can be life-threatening and severely affect the daily lifestyle of the patient. Due to the intensity of the disorder, approximately 1 in 10 patients have a high risk of death from Marfan Syndrome [4]. In the human body, the aorta is the central artery that carries blood from the heart to other parts. Conditions such as an aortic aneurysm, dissection, rupture, and aortic valve regurgitation can cause great damage to the body.

Treatment Approaches

Experts presume that more than half of those who live with Marfan Syndrome are undiagnosed. Symptoms vary depending on the individual and can range from mild to extremely

serious. Currently, patients are only treated according to their symptoms. Due to the lack of a direct cure, doctors attempt to relieve pain and prevent further complications through medication and surgery. Doctors may also prescribe beta-blockers or angiotensin receptor blockers to help manage abnormal growth of the aorta [5]. Open-heart surgery is generally recommended for patients with aortic aneurysms. For those whose case has advanced to aortic dissection, surgeons may attempt to reconstruct the aorta with a graft, or synthetic tube, to prevent further leaking.

Gene replacement therapy has the potential to completely cure Marfan syndrome after repetitive administration. This involves delivering a normal copy of the FBN1 gene to target cells through Adeno-Associated Viruses to compensate for the defective gene. In addition, antisense oligonucleotides, or ASOs, can be designed to bind to pre-mRNA and aid in producing a functional Fibrillin-1 protein by blocking the translation of the mutated exon. In 2016, an FDA-approved scale of ASO treatment showed promising results in infants [6]. Further treatment options include siRNA therapy or gene editing through the Cas9 protein, which involves the use of CRISPR technology.

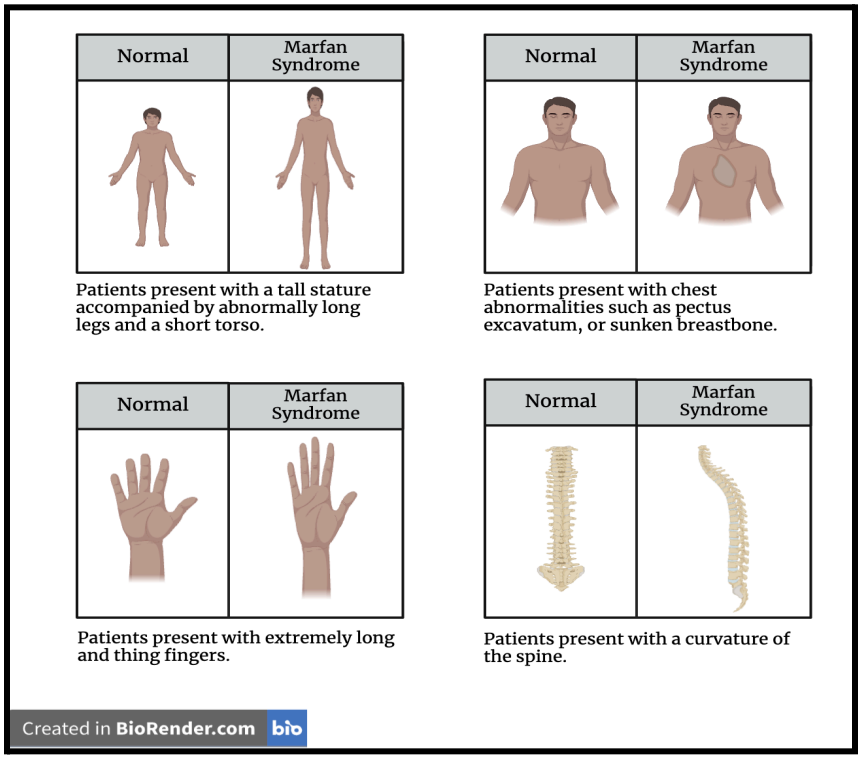

Figure 1. Manifestations of Marfan Syndrome. [7]

CONTENT

Aortic Aneurysms

Due to inadequate elastic fiber formation, an aortic aneurysm may arise. This results in the bulging and the separation of layers in the aorta. These balloon-like formations can weaken the aortic walls. Without treatment, only 20% of individuals survive after five years of formation [8]. Abnormal growth requires immediate surgery upon reaching a certain size. Eventually, this condition can progress into an aortic dissection. The stress formed by the high blood pressure can create a tear in a part of the aorta. In this case, blood pushes through the tear at a great volume. In consequence, the inner and middle layers of the aorta are dissected [9]. Without appropriate treatment, blood rushes through the outside aortic wall and an aortic rupture can form. Upon an aortic rupture, massive internal bleeding occurs and can lead to sudden death. Approximately 90% of patients die within 48 hours of rupture [10].

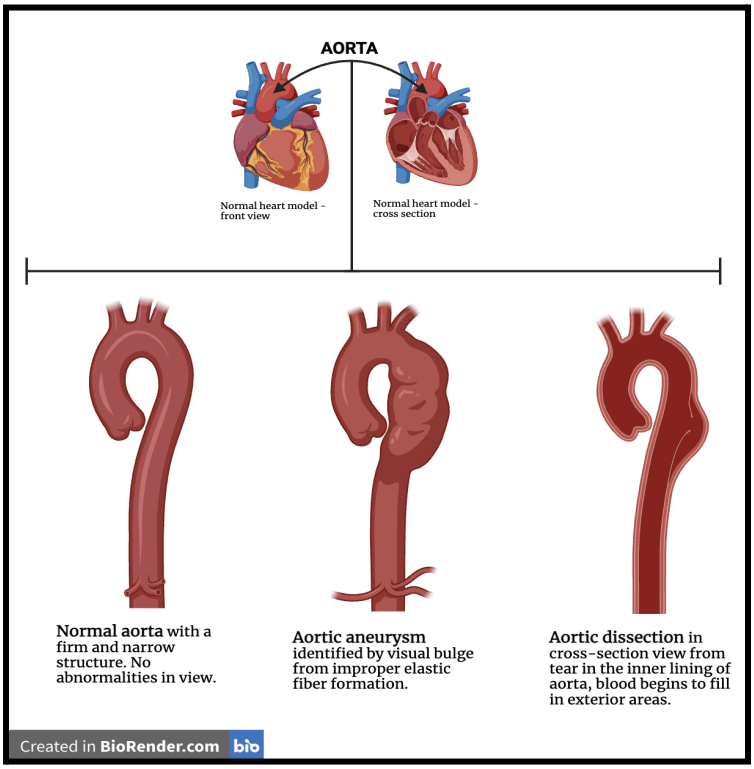

Figure 2. Cardiac Complications of Marfan Syndrome. [7]

Aortic Valve Regurgitation

Aortic valve regurgitation is a critical condition often seen in patients with Marfan Syndrome [11]. It involves the improper closing of the aortic valve, resulting in backward blood flow to the left ventricle. Due to a low level of Fibrilin-1 proteins, the connective tissue in the cardiac region is compromised. The elastic fibers forming the aortic valve function abnormally, often resulting in aortic valve regurgitation. Similar to aneurysms found elsewhere in the heart,

younger patients can present with aortic root dilation. Aortic root dilation (ARD) can develop into an aneurysm formed near the left ventricle, which can eventually progress into a dissection and sudden death [12].

The most common cause of aortic valve regurgitation begins with backflow of blood. This leads to increased left ventricular end-diastolic volume (LVEDV). Increased LVEDV is a state of volume overload that presents from a lack of proper exchange in blood flow between the left ventricle and the aorta. In consequence, the heart performs compensatory myocardial hypertrophy, which is an attempt to maintain a homeostatic balance of cardiac wall stress and oxygen consumption. In this state, the myocardium undergoes severe hypertrophic growth to maintain cardiac output. Eventually, this state progresses into severe lower ventricle enlargement. An increased lower ventricle size causes the ventricular apex to dilate closer to the chest wall, forming an uncomfortable pounding sensation and awareness of the heartbeat. Excessive lower ventricle stretching leads to a decreased stroke volume, which is the amount of blood pumped out of the lower ventricle during each systolic cardiac contraction, as a result of reduced forward blood flow. Shortly after this condition presents, patients experience systolic heart failure. The only treatment for aortic regurgitation is aortic valve repair or replacement. Both surgeries involve meticulous work and intense recuperation. Even after surgery, complete recovery is not promised and patients may require further treatment as time progresses.

Current Treatments

With no direct cure currently available, treatment for Marfan Syndrome primarily aims at managing symptoms and preventing serious complications [13]. Alleviating the pain caused by symptoms through medication and surgery remains the present choice for patients. The lack of a cure forces patients to undergo extreme damage to their body. Treatment options depend on which part of the body is affected and the severity. For cardiac-based symptoms, treatment options can be divided into medications and surgeries.

Conditions such as high blood cholesterol, coronary heart disease, and chronic kidney disease can induce an aortic dissection or rupture, which can be managed with medication. Beta-blockers are a class of drugs used to slow heart rate, lower blood pressure, and reduce the force of heart contractions. Angiotensin receptor blockers can also help manage high blood pressure. In severe cases, surgery can help repair or replace the affected section of the aorta. This may involve open repair, a stent, or thoracic endovascular aorta repair. Individuals with aortic valve regurgitation typically need surgery. The most common surgery options for such cases are aortic valve repairs and valve replacements. Aortic valve repairs are intensive, open-heart procedures that can burden a patient's health and financial composure. A valve replacement is an invasive and major open-heart surgery that can cause numerous complications.

Lifestyle changes may be implemented for patients with manageable Marfan Syndrome conditions. Regardless of the intensity, individuals with Marfan Syndrome are required to undergo constant monitoring and routine check-ups. For symptoms involving the external features, such as an abnormal curvature of the spine known as scoliosis, braces may be utilized to prevent worsening. Without treatment, patients may experience severe backaches and difficulty breathing. Conditions concerning the ocular region may require prescription glasses to correct myopia or astigmatism. Patients are also at a high risk of developing strabismus, retinal detachment, glaucoma, cataracts, and ectopia lentils. These ailments may require surgery.

Regardless of the treatment option used, patients with Marfan Syndrome undergo mental, physical, and financial stress. The current treatment choices are intense and expensive. Many families may not be able to consider treatment in any form an economical and probable choice. Considering the possibilities of gene therapies for Marfan syndrome is critical to promising patients an improved future.

Adeno-Associated Viruses

Despite the frequency of Marfan Syndrome diagnosis and the intensity of the illness, a direct cure has yet to be discovered. However, gene therapy provides hope for a future with a cure. One such hope lies within the possibilities presented by adeno-associated viruses, or AAVs. AAVs were first discovered in the 1960s during an adenovirus preparation, hence the name [14]. AAV vectors target specific cells to correct disease-causing genes. The process of adeno-associated virus therapy begins by replacing the vector genes with a therapeutic gene sequence. The therapeutic gene contains the necessary replacement for the infectious gene sequence. Once inside the body, the AAV targets a specific cell. Capsid-specific technology allows for the proper identification of target cells in the body. AAVs behave according to tropism, which is the ability to inject and thrive only in specific tissue types as determined by the capsid. Upon entering the cell, the therapeutic gene corrects and repairs the natural function of the disruptive protein through a series of steps. The capsid surrounding the AAV sheds in the nuclear membrane through endocytosis in the form of phagocytosis. The therapeutic gene, or vector genome, is then released. The therapeutic gene does not integrate with the nuclear DNA but instead assembles episomes. Episomes are forms of DNA that are not passed on in reproduction therefore, dividing cells will not see the effect of gene transfer in future cell generations. Afterward, using the body's natural design, mRNA attached to the episome allows for the correction of improper DNA sequences. In consequence, proteins form in their initial nature with the help of tRNA and subsequent processes.

Much like any other medical procedure, gene therapy through adeno-associated viruses offers its own advantages and disadvantages. AAVs infect both dividing and quiescent cells, which are non-dividing cells. This allows for genetic material delivery to a highly diverse range of cell types. In addition, AAVs cause long-term expression in non-dividing cells as AAVs are not diluted until the host cell divides. The most promising feature of adeno-associated viruses is the incredibly low pathogenicity and in turn, a similarly low immune response. However, the relatively small size of the vectors restricts the gene size to less than 4.5 kilobases in length. According to NIH, the *FBN1* gene consists of approximately 235 kb [15]. Future advancements in technology may allow for this gap to be overridden. With little to no negative impact on the human body, adeno-associated viruses offer hope to families of patients with Marfan Syndrome who are forced to suffer through the symptoms without a cure. A single administration of AAVs containing the *FBN1* gene of infected individuals could gradually cure the ailments associated with Marfan Syndrome. AAVs can be delivered to the body via an IV drip or a direct injection to the target tissue. The AAV6 and AAV4 variations have been hailed as excellent in transduction in cardiac cells. As a promising viral vector and candidate for gene editing processes, adeno-associated viruses may lead the future of a cure for Marfan Syndrome.

Antisense Oligonucleotides

Another promising treatment for Marfan syndrome is the use of antisense oligonucleotides or ASOs. ASOS are short synthetic structures similar in function to nucleic acids but with a more gene-based modality [16]. Antisense oligonucleotides can be used as drugs to target specific mRNA segments involved in the formation of disease-causing proteins. The process begins with the binding of ASOs to specific sequences of nucleotides in mRNA. The cleavage at the site of ASO binding allows for the destruction of target RNA, thereby silencing target gene expression and replacing it with a natural state of the gene sequence. This allows for modification in protein formation into a healthy condition. The binding of ASOs to mRNA can be separated into two different possibilities. The first possibility involves healing a broken gene by splicing around the errors. In the case of Marfan Syndrome, ASOs would deliberately remove the mutated section of the *FBN1* gene in Chromosome 15. The most promising possibility would be the usage of ASOs in increasing or boosting the production of a protein similar to the Fibrillin-1 protein. The compensating protein would pursue the same function but exist within a different location. In the human body, the structure with the most compatibility with the Fibrillin-1 protein would be the LTBP. Latent TGFβ binding proteins, or LTBPs, are a group of extracellular glycoproteins with a structure parallel to that of Fibrillin-1 proteins. Both proteins are rod-like extracellular matrix molecules that contain tandem EGF-like 6-cysteine repeats and 8-cysteine motifs [17]. By raising the amount of Latent TGFβ binding proteins in the body, an equivalent compensation for Fibrillin-1 proteins may suffice for the proper formation of connective tissues in areas like the aorta and retina. ASOs have allowed for hopeful outcomes in studies involving spinal muscular atrophy and frontotemporal dementia. In the near future, research may allow for positive results in patients with Marfan Syndrome, as well.

CAS9 And CRISPR

Clustered Regularly Interspaced Short Palindromic Repeats technology, also known as CRISPR, is a popular and relatively developing form of gene therapy used in treating genetically inherited illnesses [18]. This system consists of two major components, a Cas9 protein and a guide RNA. In short, the guide RNA serves to recognize the DNA sequence requiring correction, and the Cas9 proteins cut the identified section. For an individual with Marfan Syndrome, this treatment option would involve creating a guide RNA to recognize the mutation in the *FBN1* gene involving the deletion of a nucleotide. Afterward, the Cas9 protein and guide RNA must be attached to form a complex that is then introduced into the target cell. Upon locating the disease-causing region, the Cas9 protein will cut the DNA. To modify the irregular sequence, a replacement with a healthy sequence should be inserted in place of the problematic gene. The use of CRISPR technology in this situation would be limited after this stage. As human biological processes continue, the Fibrillinn-1 proteins should appropriately form. CRISPR-Cas9 technology has been making rapid advancements in the field of medicine, bearing favorable results for numerous hereditary illnesses. Certain studies have been published promising a beneficial connection between the usage of CRISPR technology in individuals with Marfan Syndrome.

RECENT ADVANCES

This portion of the review will discuss relevant studies on applications and cases regarding the prospective treatment options for Marfan Syndrome, including CRISPR/Cas9 technology, adeno-associated viruses, and antisense oligonucleotides. Information concerning Marfan Syndrome-related FBN1 genes, as well as other associated genes, will be included in Table 1.

REFERENCES	STUDY	TECHNOLOGY	MODEL	MUTATION GENE	RESULTS
CRISPR/Cas9 in zebrafish: An attractive model for FBN1 genetic defects in humans [19]	Yin July 2021	CRISPR	Zebrafish	*FBN1*	Generated mutant zebrafish with a frameshift mutation in the *FBN1* gene, showing similar traits of Marfan Syndrome
Correction of the Marfan Syndrome Pathogenic FBN1 Mutation by Base Editing in Human Cells and Heterozygous Embryos [20]	Zeng November 2018	Base-Editing	Human Embryos	*FBN1-T7498C*	Achieved successful genetic correction of the *FBN1-T7498C gene* in human embryos
Cardiac Gene Therapy with Adeno-Associated Virus-Based Vectors [21]	Chamberlain May 2017	AAV	Human Cardiac System	--	The AAV delivery system must be further refined but it shows promising results.
The Antisense Oligonucleotide Nusinersen for Treatment of Spinal Muscular Atrophy [6]	Edinoff June 2021	ASO	Human Infant	*SMN1*	Treatment approved by the FDA in 2016 with promising results in infants.
Nusinersen versus Sham Control in Later-Onset Spinal Muscular Atrophy [21]	Mercuri February 2018	ASO	Human Infant	*SMN1*	Tests prove the drug Nusinersen for symptomatic infants helps improve at least 2 motor skills with consistent treatment.

Table 1. Recent Advances Involving Genetic Therapies

CRISPR/Cas9 in Zebrafish

Nearly 90% of cases with Marfan Syndrome are caused by mutations in the *FBN1* gene. This study conducted by Yin et al. considers the possibility of treating Marfan Syndrome by inducing the mutation in zebrafish [19]. The DNA of zebrafish shares up to 70% similarity, in terms of genetic function and structural resemblance, with humans. This trait allows zebrafish to be an accurate model for considering the use of CRISPR in humans. As an ideal model for genetic research of human diseases, this study attempts to characterize and generate *FBN1* gene mutant zebrafish with the aid of CRISPR/Cas9 gene-editing technology. CRISPR/Cas9 was applied in zebrafish to develop the frameshift mutation. The offspring of the mutant zebrafish were compared with wild-type zebrafish and observed for any abnormalities in the developmental stage. Similar to individuals with Marfan syndrome, the offspring zebrafish were noted to have slender body structures and irregular blood flow throughout the heart. The production of a fish with such traits allows for the presentation of a stage to consider treatment through CRISPR/Cas9. Additional studies in the future will be able to use the mutant zebrafish as a test subject for possible cures for Marfan Syndrome. This zebrafish model of Marfan Syndrome provides a source for further understanding of the disorder, as well as the relative science of it.

Correction By Base Editing

A study by Zeng et al 2018 discusses the urgent demands for treating genetic disorders, such as Marfan Syndrome, through the use of treatments involving genetic therapies [20]. The focus of this experiment was to correct an *FBN1* frameshift mutation in a human embryo, thereby producing an efficient non-Marfan Syndrome-associated response. Base editing technology is a growingly popular option for clinical trials attempting to cure a genetic disorder. This technology was used in this study and showed promising results. The *FBN1-T7498C* pathogenic mutation causing Marfan Syndrome was corrected in mutant cells. After reviewing a low risk for negative impact, the team attempted genetic correction of the *FBN1-T7498C* gene. The technology allowed for nearly 89% efficiency of mediated perfect correction. No off-target results, including additional mutations in the form of insertion or deletion, were detected from post-experiment testing. A whole-genome sequencing analysis prior to and after the experiment depicted a positive correlation between the correction of the *FBN1-T7498C* gene and base editing technology. The results derived from this study suggest and promote the initiation of CRISPR and base editing technology in platforms involving genetic disorders.

Adeno-Associated Viruses

The cardiac-based system plays a prominent role in the damage caused by Marfan Syndrome. The vast majority of patients with Marfan Syndrome are treated for abnormalities within the heart, or more specifically the aorta. Adeno-associated virus treatment is emerging as a newly developed source of treatment for issues pertaining to the heart [22]. This study focuses on addressing the complications presented by heart failure. The result of this study concludes that cardiac gene therapy with the usage of Adeno-Associated Viruses holds significant promise for a direct cure for all inherited cardiac disorders. Through efficient delivery of the therapeutic gene to cardiomyocytes, heart conditions may be relieved. Although promising results were

seen, they can only be guaranteed with further experimentation and analysis of future studies. The team involved in this study faced minute difficulty with delivering the therapeutic gene to the site of correction. This formidable roadblock can only be overcome with advanced technology and scientific methods for proper and efficient delivery.

Nusinersen- ASOs

Similar to Marfan Syndrome, Spinal Muscular Atrophy can be caused by a deletion mutation. The concept of muscle weakening is prevalent in both disorders. The implementation of ASO treatment in SMA showcased promising results that could be transferred to the treatment of Marfan Syndrome [6]. Spinal muscular atrophy (SMA) is an autosomal, recessive disorder. A mutation in gene SMN1 of Chromosome 5 caused by a deletion causes a disruption of survival motor neuron production. In this case, both copies of the SMN1 gene are missing. SMN2 occupies the same function as SMN1 with a narrow capacity system, lowering the presence of functional SMN protein within motor neurons. SMA is categorized by intense muscle weakness. Limited mobility from a loss of spinal cord motor neurons can progress into complete paralysis and eventually death. Nusinersen was approved by the FDA for the treatment of Spinal Muscular Atrophy in December 2016. As an anti-sense oligonucleotide, this drug acts as a mechanism to restore proper protein production. Deletion or frameshift mutations can be corrected using mRNA modification. ASOs in their natural state are short, single-stranded agents that target a certain section of mRNA to modify protein expression. Used for the treatment of SMA, Nusinersen was presented with four initial intrathecal injections, followed by three yearly maintenance doses. Studies proved improved motor function for early and late-onset patients. Recent advances testing the appropriate and most efficient delivery time of Nusinersen indicated beneficial outcomes for patients treated during early ages, prior to any motor function loss. The risk of ventilator application for symptomatic infants significantly decreased.

CHERISH Study- ASO

In a clinical trial conducted by individuals of the CHERISH group, Mercuri et al. identified improvement in motor skills through treatment by Nusinersen [21]. This phase 3 trial was characterized by a double-blind and sham-controlled foundation. 126 children with onset symptoms of Spinal Muscular Atrophy after 6 months of age were randomly assigned to a 2:1 ratio of intrathecal administration of Nusinersen or a sham control group procedure. Initial, primary points were taken using a Hammersmith Functional Motor Scale–Expanded test at baseline, with no months of treatment. Final score points were taken using the same test at 15 months of treatment. A statistically significant increase from baseline to 15 months of treatment was seen. The average score improved by more than 3 points, indicating the development of at least 2 motor skills. The least-square mean increase in the HFMSE score for the treatment group taking Nusinersen was 4.0 points. The least-square mean increase in the HFMSE score for the sham control group was -1.9 points, indicating decreasing motor function capabilities. A notable difference in group comparison prompted the conclusion of the study. Children treated with the Nusinersen Anti-Sense Oligonucleotide drug had clinically meaningful growth in motor function compared to those in the control group, which consisted of individuals with a natural deterioration from SMA.

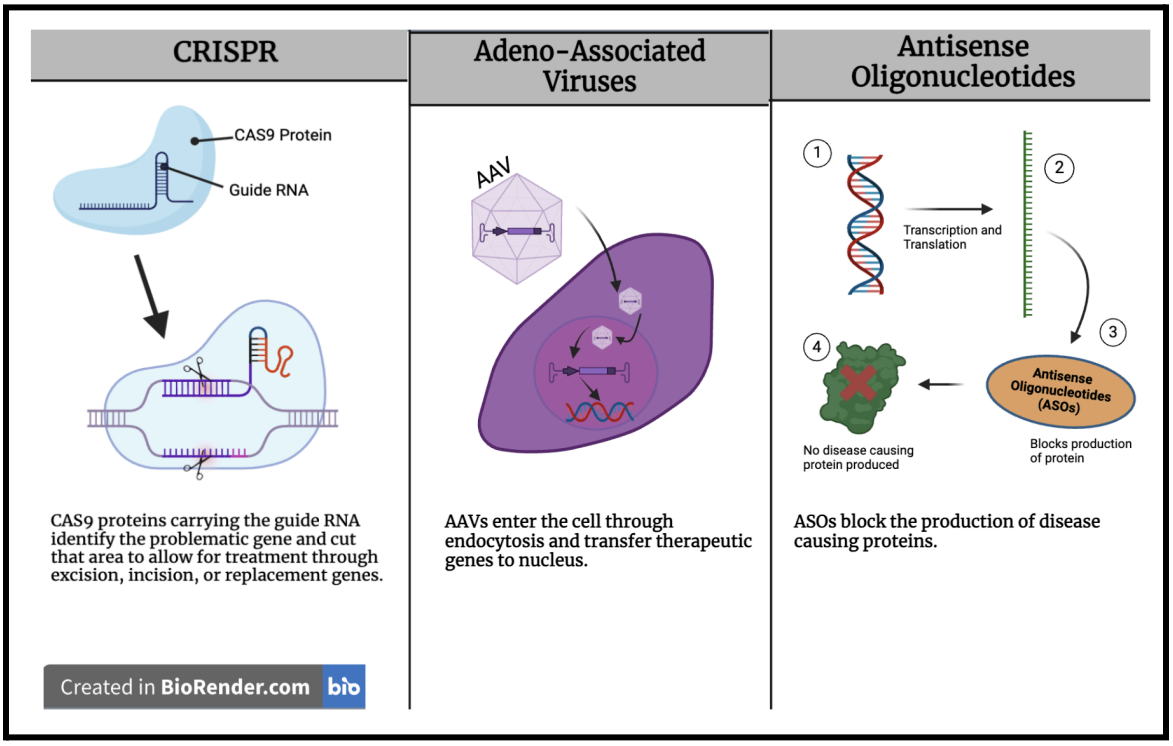

Figure 3. Main Gene Therapy Options for Marfan Syndrome. [7]

CONCLUSION

Marfan Syndrome is a genetic disorder with an extensive patient population size. The severe damage and deterioration to the body parts, as a result of this disorder, affect every inch of an individual's life, from their food consumption and lifestyle choices to their daily activities. Conditions associated with the cardiac system, such as an aortic aneurysm, aortic dissection, and aortic valve regurgitation, severely burden a patient and their family, with regard to their mental, physical, and financial well-being. The current treatment resorts to alleviating symptoms caused by Marfan Syndrome, as there is no present cure. The possibilities offered by genetic therapies including CRISPR, adeno-associated viruses, and antisense oligonucleotides, may guide the path to a better future, one with a cure for Marfan Syndrome. Although the collected information provided by this study suggests a positive use of gene therapy in Marfan Syndrome, some limitations preside over this paper. Factors such as subject size in studies, translation errors, and varying interpretations can introduce bias and inaccuracies which must be considered for a complete understanding of the true applicability of the findings. This paper has provided a thorough examination of several genetic therapies and associated clinical trials with promising results. Further research must be conducted in order to prove sustainable cures for individuals with Marfan Syndrome.

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