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Analyze the Molecular Genetics of a Solitary Causative Genetic Material behind Muscular Dystrophy

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ABSTRACT

Muscular dystrophy is a genetic disorder characterized by progressive muscle weakness and degeneration. Duchenne muscular dystrophy (DMD) is the most common X-linked disorder muscular dystrophy. It is caused by an absence of the protein named as dystrophin, which helps to keep muscle cells intact. In my research on this topic, I will emphasis on a complete analysis of molecular gene behind muscular dystrophy. According to a survey with few hospitals/labs in my region with 40-50 patients, we had identified the ratio of around 90% of male and 10% of the female victim with the muscular dystrophy disorder. The lifespan of people having this disorder decreases unconditionally and it majorly effect the youngsters. Main concern for the treatment methodology or medical interventions are limited to treating symptoms.

KEYWORDS: *Muscular Dystrophy, Muscle weakness, Dystrophin*

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1. INRODUCTION

Muscular dystrophies are a clinically and heterogeneous group of disorders that all share characteristics of progressive muscular weakness. The clinical features includes muscular weakness, pain, cramps, stiffness and enlargement. Serum enzymes, electro diagnostic studies, DNA analysis and muscle biopsy are common methods of diagnosis.

The dystrophin gene is the largest one yet identified in humans and is located in the short arm of the X chromosome.

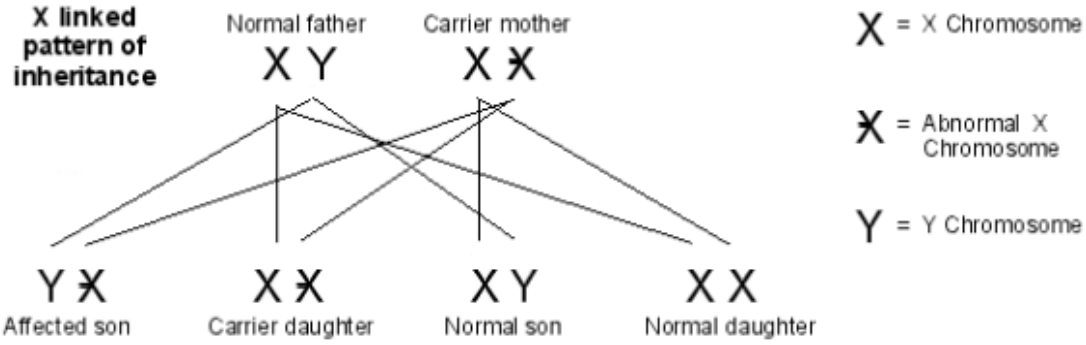

Figure 1: Possible Outcomes of children born to a mother who has carrier of abnormal gene

Most mutations are deletions of one or more parts of it. DMD occurs because the gene fails to produce virtually any dystrophin, which is a protein that transfers the force of muscle contraction from inside the muscle cell to outside the cell membrane.

2. Current Approach

Muscular dystrophy, categorized as disease in human body due to the absence of protein named dystrophin, which leads to insufficiency in respiratory system, progressive muscle related weakness and chronic disease of the heart muscle. Duchenne muscular dystrophy is present at birth but becomes apparent between the ages of 3 and 5 years. Cardiomyopathy is common as well as impaired brain function. Girls inherit two X chromosomes from their parents while boys inherit an X chromosome from the mother and Y chromosome from the father. A son born to a mother with the dystrophin gene mutation on either one of her

X chromosomes has a 50% chance of having DMD. While the daughter has a 50% chance of inheriting the mutation and being a carrier. Typically, women are carriers while the disease observed in males. A man suffering with DMD cannot pass the disease on to his son because he provides the Y chromosome that is free of the gene and the mutation but he can pass of the mutation to his daughter making her a carrier and putting her progeny (MALE) at risk.

At present corticosteroids are the only pharmacologic agents with proved benefits even though they are associated with several adverse effects like gastrointestinal symptoms and weight gain. Mutation suppression, cell therapy, gene therapy, utrophin modulation etc. offer potential solutions. Yet an absolute cure is yet to found. Serum aldolase test indicates muscle degeneration and a definite aid in distinguishing primary myopathy from secondary or neurogenic muscle atrophy.

Demography		Levels of enzymes
Age	9 years	
Sex	Male	
CK		7476 U/L (NV= 150 to 499 U/L)
Aldolase		23.94 U/L (1 to 7.6 U/L)

Figure1: Levels of CK and Aldolase

Currently, treatment of Duchenne muscular dystrophy includes the use of corticosteroids that delay the progression of the disease; increase muscle strength and walking time as well reduce the progression of respiratory malfunction, cardiomyopathy and scoliosis. However, a cure has not found yet. A possible cure that explored by researchers is a gene therapy that would involve the insertion of the dystrophin gene through a vector. This has proven to be effective in animals like mice and dogs, but it's not tested on humans.

Working of CRISPR/CAS9 – This method of genetic therapy relies on bacteria's ability to defend itself against viral infection, which is similar to an immune system response. When the bacteria detects foreign DNA the CRISPR protein inserts a piece of this DNA into the bacteria's own genome. Therefore, the bacteria is able to recognize this DNA and protect itself from future viral attacks.

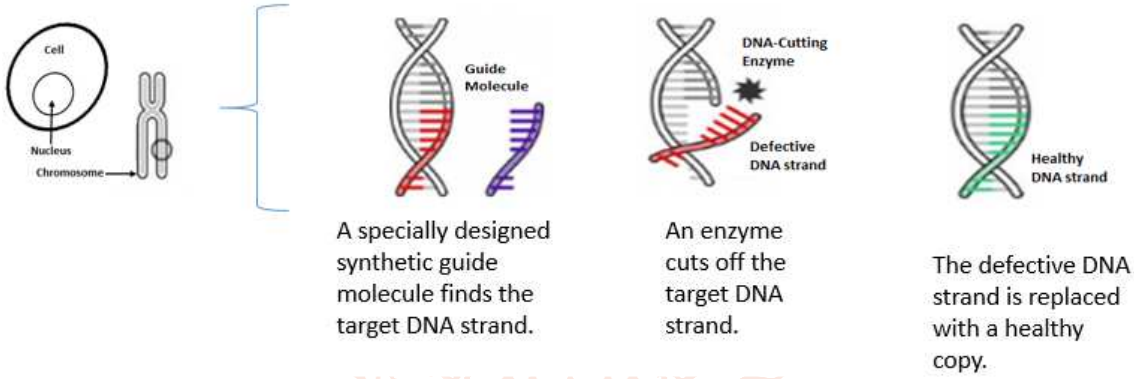

Figure 2: How CRISPR works

This technique is perform by producing antibodies that consist of two kinds of short RNA, one of which contains a sequence matching the viral DNA. These two short RNA form a complex with the protein called cas9, which is a nuclease capable of "cutting" DNA. The matching sequence, called as "guide RNA" and it leads the complex to the viral genome so the CAS9 can cut the viral DNA and prevent further replication and spread of the viral infection.

Using CRISPR to address Muscular Dystrophy – Duchenne muscular dystrophy is an X linked disorder, which caused due to various kinds of mutations in the DMD gene that enables cells to produce dystrophin that is a protein, which gives muscles strength and protects them from damage during contraction and relaxation. The technology uses CRISPR-CAS9 method to cut the mutated part of the DMD gene and allows to the cell's natural healing mechanism to repair and reform the healthy parts of the gene so a shortened but healthy version enabled to produce sufficient dystrophin protein. The mutation once corrected in muscle stem cells will ensure that the cells regenerated from cured cells will be healthy and free of the mutation. This gene-editing tool delivered to Mice through a viral vector known as AAV9. The transplanted muscle died first and then regenerated from it stem cells, which carried the edited gene. A clinical trial using the technology on dogs also provided promising results

3. Proposed Scope

Potential challenges – immune response against CAS9. The two most common sources of CAS9 are staphylococcus aureus and streptococcus pyogenes, which are common disease causing agents in humans. Approximately 80% humans proposed to exposed and thus, contain anti-CAS9 antibodies, which creates a threat of immune response against CAS9. The implications of this on gene therapy depends on the kind of editing. In ex vivo gene therapy cells treated in a dish prior to transplantation so immune response can prevented by using transient cas9 expression and waiting for the cas9 protein to clear before administering the corrected cells to patients. In vivo editing, on the other hand leads to long-term gene expression in the presence of a fully functional immune system. The most basic solution is to increase the efficiency of the viral vector used to administer the cas9 protein so that the amount of the administered vector can reduced. Other solutions include capsid modifications, improvement of transgene expression, avoiding expression in APCs, (sub) gene therapy in the brain etc.

	Age 6 Months		Age 12 Months	
	Affected range	Normal range	Affected range	Normal range
Tetanic flexion (Newton/kg)	0.438-0.794	1.23-1.48	0.402-1.08	1.26 -1.65
Tetanic extension (Newton/kg)	0.366-2.07	2.00-3.20	1.34-2.54	1.98 - 2.65
Tibiotarsal joint angle (degrees)	140-160	158-162	130-155	149 - 158
% Eccentric contraction decrement (@ 10 stims).	8.10-40.7	8.22-11.9	29.5-59.0	3.47 - 12.3
% Eccentric contraction decrement (@ 30 stims).	29.7-74.8	17.2-24.7	64.1-72.6	7.59 - 20.1
Maximum hip flexion angle	45-105	56-80	42-100	52 - 70
Pelvic angle	36-57	25-40	44-54	37 - 50
Cranial sartorius circumference (mm/kg)	3.00-5.10	2.16-2.74	4.62-4.62	2.17 - 2.34
Quadriceps femoris weight (g)	79.5-110	172-223	98.3-120.2	203 - 258
Quadriceps femoris weight (g/kg body weight)	6.65-8.27	9.20-12.7	8.19-8.86	8.99 - 10.8

Figure 3: Range for muscular dystrophy and its affected range in infants

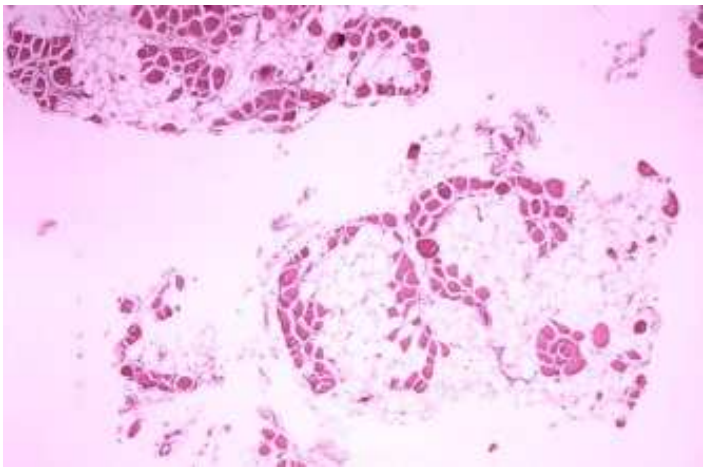

Figure 4: Histopathology of Duchenne muscular dystrophy (DMD)

A team led by Eric Olson at the University of Texas Southwestern Medical Center used CRISPR/cas9 technology to reverse the mutation responsible for Duchenne muscular dystrophy. DMD isn't the most obvious choice for CRISPR's find , cut and replace function because the dystrophin gene is the largest in the human genome, and the same disease can be caused by a multitude of different mutations. But Olson found a way to target a common error spot on exon 51, which he figured could, with a single slice, benefit approximately 13 percent of DMD patients.

This experiment followed previous work on mice and human heart cells. The CRISPR gene editing component was attached to a virus that has a strong affinity for muscle cells. This was then injected into community of four one month old beagles. One group of two dogs was given a shot directly in the lower leg and the second group was given an intravenous infusion. After eight weeks, CRISPR had restored dystrophin levels in the second group to more than 50 percent of normal in the legs, and more than 90 percent in the heart. According to the researchers, restoring even unto 15% of the dystrophin protein levels of the body could have a significant impact and could even prove to be curative. Another reason that this trial was a breakthrough because CRISPR was used body wide on a large mammal for the first time especially to address a fatal disorder of a large magnitude. "They showed obvious signs of behavioural improvement—running, jumping—it was quite dramatic," says Olson, but he did not make note of these qualitative observations on record because the sample size for the trial was extremely small.

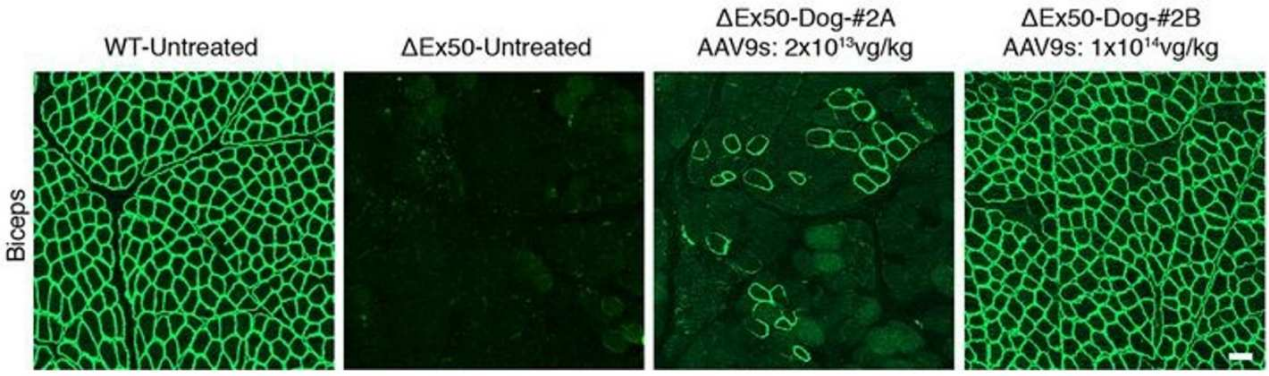

Fig 5: Experimental analysis of bicep muscles to check restored dystrophin level

The restored dystrophin levels (green) in the bicep muscles of a healthy dog (first panel fro left), an untreated dog (second panel from left), and dogs treated with low (third panel from left) and high (fourth panel from left) intravenous dose of CRISPR gene editing technology.

CRISPR technology had been used before to restore dystrophin expression in mice suffering from Duchenne muscular dystrophy. In order to test it further a larger mammal had to be used. Dogs with DMD offer something mouse models can't – they display many similar symptoms to humans affected by DMD, such as muscle weakness and wasting.

Although the results of the study were extremely promising to the effect that this technology can be used as a potential cure for humans it has to be taken into account that the sample size was extremely small (4 dogs) and the duration of observation was also very limited. In order to fully understand the implications of using the CRISPR gene editing tool a study of a longer observation time and larger sample must be conducted.

"Viruses are promising and effective tools for gene therapy, but there are potential dangers. Using even a harmless virus for gene therapy could result in a life-threatening immune response against the virus," said Jason Nomburg, a virology graduate student at Harvard University. "This is especially true when the virus is administered systemically to the blood at high concentration, like in this study. A second injection can be especially dangerous because it has even greater potential to provoke an immune response. Without knowing how durable the treatment is long-term, this might be a one-time shot."

Another major concern is safety because the gene editing technology may have " off target effects" in that it may affect unwanted sites. fortunately, that didn't happen in this particular study.

4. CONCLUSION

Duchenne muscular dystrophy is a genetic disorder that is characterised by progressive muscular weakness due to disrupted production of the protein dystrophin that is responsible for keeping muscle cells intact and ensures their smooth functioning. The symptoms are usually observed in early childhood, around 2-3 years, and is generally observed in boys because its an X-linked disease. Currently, corticosteroid therapy is the only pharmacological method to address the problem, however, there is only an impact on symptoms and it is not curative. CRISPR/cas9 gene editing technology is an interesting avenue in this regard because of its potential for permanent exon skipping leading to the capacity for dystrophin restoration which might even lead to normal muscle functioning. The aim of gene replacement is to substitute the diseased dystrophin creating gene with a healthy one. The primary challenge is to determine the safe and effective methods to deliver this healthy replacement dystrophin or dystrophin - like gene to muscles body wide. This is because currently viral vector mediated gene replacement is being explore which poses the threat of an immune response by the body of the patient. Research needs to be focused mainly on targeted delivery and long term expression of the healthy gene. But the relatively successful clinical trials with dogs and mice do provide hope that gene replacement and editing may actually hold a permanent cure to this fatal disease.

Few questions framed by Nishtaa Modi as part of her market research with medical practitioners. Below responses she got while interviwing the same.

[Nishtaa]: Have you ever seen patients having muscular dystrophy symptoms?
[Doctor]: Yes, we had seen many patients with muscular dystrophy symptom
[Nishtaa]: What was the age group of people
[Doctor]: From 6 months old kid to 60+ years old citizen.
[Nishtaa]: Which enzyme is responsible for this?
[Doctor]: It is caused because of lack of dystrophin protein in body
[Nishtaa]: Any specific symptoms?
[Doctor]: Progressive weakness, loss of muscle mass, difficulty in walking and later on swallowing
[Nishtaa]: Is that curable, if yes explanations
[Doctor]: Yes, with proper medications as well as exercise.
[Nishtaa]: If not curable, any manual exercise suggested
[Doctor]: Genetic therapy can have scope. And, Physical theraphy can improve quality of life
[Nishtaa]: Drug suggested for improvement of disease?
[Doctor]: Corticosteroids like Prednisone

DECLARATION OF PATIENT CONSENT

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Nishtaa Modi is a student of grade 11 studying in the Shri Ram School Aravali. She intends to pursue higher studies in biochemistry and genetics. She plays the Sitar and is an avid debater. She hopes that some day she can make her passion for biology and chemistry her profession.