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Treatment of Duchenne muscular dystrophy (DMD) by gene therapy using adeno-associated viral vectors

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Abstract
Duchenne muscular dystrophy (DMD) is a severe type of muscular dystrophy. The symptom worsens quickly. The muscle loss occurs first in the thighs and pelvis followed by the arms. This can result in trouble standing up. Most of the patients are unable to walk by the age of 12. Affected muscles may look larger due to increased fat content. The disorder is the X-linked recessive. Where about two thirds of cases are inherited from a person's mother, and one third of cases are due to a new mutation by drugs. It is caused by a mutation in the gene for the protein dystrophin. Dystrophin is important to maintain the muscle fibre's cell membrane. Genetic testing can often make the diagnosis at birth. Medications used include steroids to slow muscle degeneration, anticonvulsants to control seizures and some muscle activity, and immunosuppressant's to delay damage to dying muscle cells. DMD affects about one in 5,000 males at birth It is the most common type of muscular dystrophy. The average life expectancy is 26 years Gene therapy can be used as treatment in early stages, DNA delivery anti-sense oligonucleotides and plasmid DNA, gene therapies and stem cell technologies. all show promise for being able to impact different types of muscular dystrophies. Focusing on developing direct gene replacement strategies to treat recessively inherited form of muscular dystrophy, particularly Duchenne muscular dystrophy (DMD) using adeno-associated viral vectors to deliver synthetic dystrophin genes for the purpose of developing gene therapy for DMD.

Keywords: Duchenne muscular dystrophy (DMD), protein dystrophin, genetic testing, gene therapy, anti-sense oligonucleotides, plasmid DNA, adeno-associated viral vectors

Introduction
Duchenne muscular dystrophy (DMD) is a severe progressive muscle degenerative disease caused by dystrophin mutations in childhood, occurring in about one of every 5000 male births. DMD is caused by the absence of dystrophin or due to mutation of *DMD* located on the X chromosome and thus primarily affects males. (DMD) is a rare neuromuscular disorder that causes progressive weakness and early death. Gene therapy is an area of new therapeutic development by using adeno associated viral vectors (AAV) vectors are the leading platform for gene delivery for the treatment of a variety of human diseases. Capsids such as AAV8 and AAV9 can target multiple muscle types throughout the body, enabling rAAV gene therapies to be developed for multiple muscle diseases, especially those afflicting muscles of the entire body, such as Duchenne muscular dystrophy (DMD). After the transfer of dystrophin protein muscle can serve as a bio-factory to produce secreted therapeutic dystrophin proteins for the treatment of muscle diseases

Symptoms: A progressive neuromuscular disorder, is muscle weakness associated with muscle wasting with the voluntary muscles being first affected, especially those of the hips, pelvis area, thighs, shoulders, and calves. Muscle weakness also occurs later, in the arms, neck, and other areas. Calves are often enlarged. Symptoms usually appear before age six and may appear in early infancy

Other physiological changes which occur are
1. Curved spine due to weak back muscles
2. Swollen calves due to fat and scar tissue build up
3. Arms held back for balance
4. Belly stick due to weak abdomen

Functions of dystrophin gene are

In skeletal muscle, dystrophin protein is located just beneath the sarcolemma connecting the cytoskeleton to the extracellular matrix via a dystrophin associated glycoprotein complex (DGC) and thus stabilizes the sarcolemma of the muscle fibre and protects it from damage during the repeated cycles of contraction and relaxation. Mutations in the dystrophin gene, resulting in decreased or lack of dystrophin expression, such as in DMD patients, results in destabilization of the DGC complex and muscle fibre degeneration.

Dystrophin is a support between actin binding end and myocyte membrane (fig1) the patient with DMD has a dystophin with shorter central rod (fig 2) so that it cannot provide support between actin binding end and myocyte membrane due to which cramps are formed in myocyte membrane (fig 3), as the crams are formed. The creatine kinase used for muscle strength is released out into the blood circulation (fig 4) as the result the muscles get weak and scar, fat tissue is formed in the damaged myocyte resulting in swollen and weak muscles

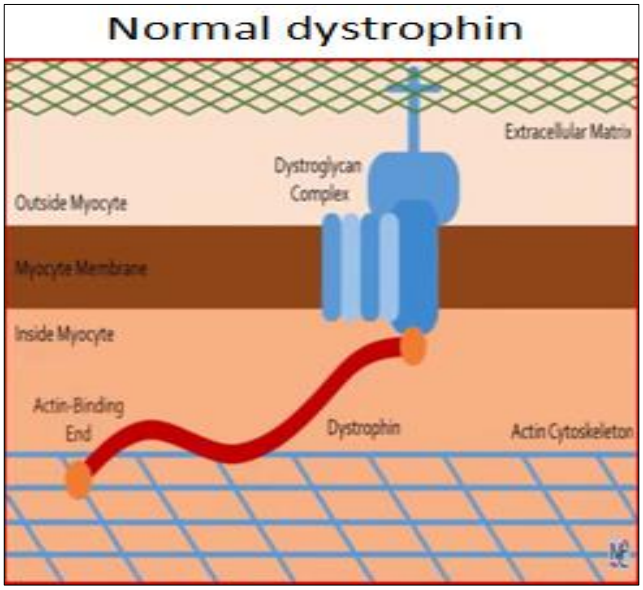

Fig 1: shows normal dystrophin function

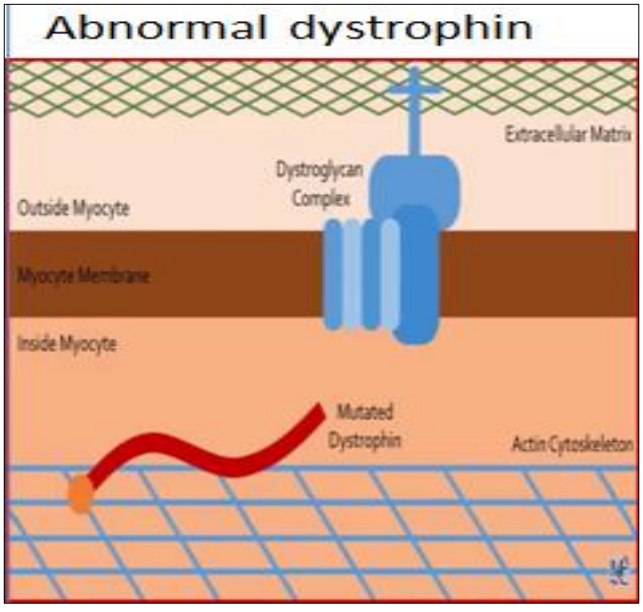

Fig 2: shows the abnormal function of mutated dystrophin

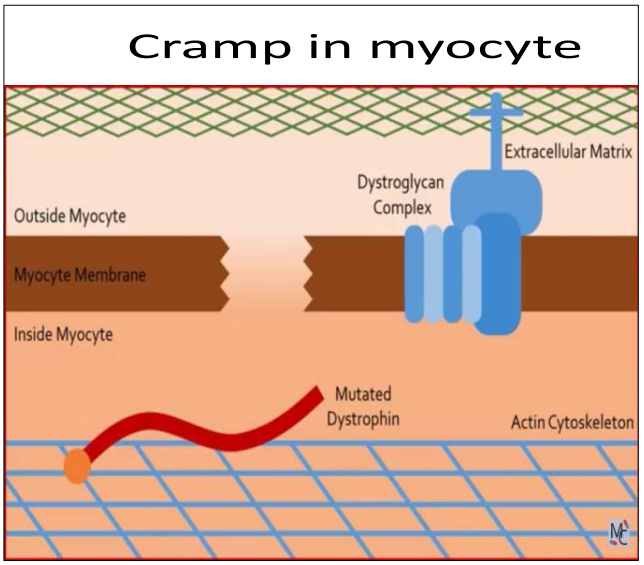

Fig 3: shows the cramp formed in myocyte due to abnormal function of mutated dystrophin.

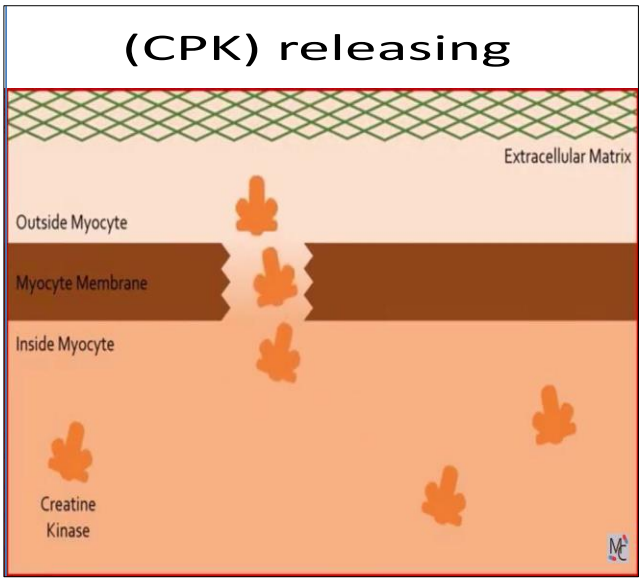

Fig 4: Shows the release of creatine kinase from the cramp in myocyte

AAV over adenovirus

The adeno-associated viruses are small and relatively simple viruses that have greater potential as vectors for gene therapy.

It is a single-stranded, non-enveloped DNA virus of 4.5. kb in size.

The viruses' apparent lack of pathogenicity.

It has the ability to stably integrate into the host cell genome at a specific site.

(AAV) Adeno-Associated Viruses AAV belongs to the genus Depend parvovirus within the family Parvoviridae. the life cycle is depends on the presence of a helper virus, such as AdV. AAV is found in multiple vertebrate species, including human and non-human primates. AAV does not cause any human diseases. The genome is flanked by two T-shaped inverted terminal repeats at the ends that largely serve as the viral origins of replication. The rep gene encodes four proteins required for viral replication; they are named after their molecular masses: Rep78, Rep68, Rep52 and Rep40. Capsids AAV8 and AAV9 targets the muscle types

throughout the body, rAAV gene therapy is developed for multiple muscle diseases, especially those afflicting muscles of the entire body, such as Duchene muscular dystrophy (DMD).The treatment method is by using viral vectors some viruses are known to incorporate their own DNA into human cells, we have taken advantage of the skills making viruses carrying modified Dystrophin Gene instead of viral genes(fig 6) now the incorporated dystrophin gene which get incorporated into DNA and Myocytes and allowing them to produce modified Dystrophin protein (fig 7) in the individuals body and the modified dystrophin gene will function in the place of mutated dystophin gene thus provide the support to the myocyte during the contraction and relaxation so there will be no further damage to the myocyte and in growing children the myocyte will be repaired hence there will be no release of CPK into the blood hence there is enough CPK for contractions and relaxation of muscle

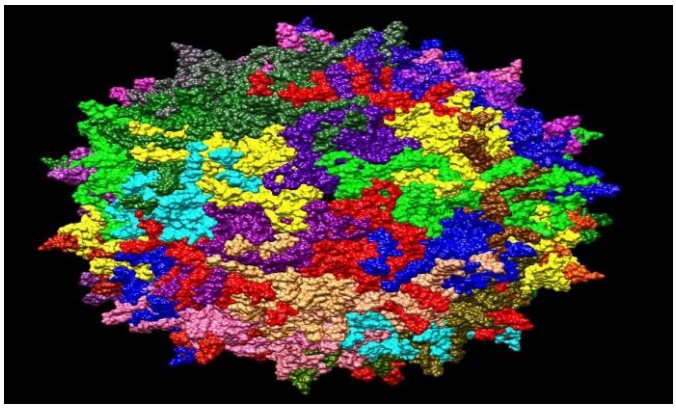

Fig 5: Adeno-associated virus structure

Example: Adeno-Associated Virus-5, Avian Adeno Associated Virus, Adeno-Associated Virus-3

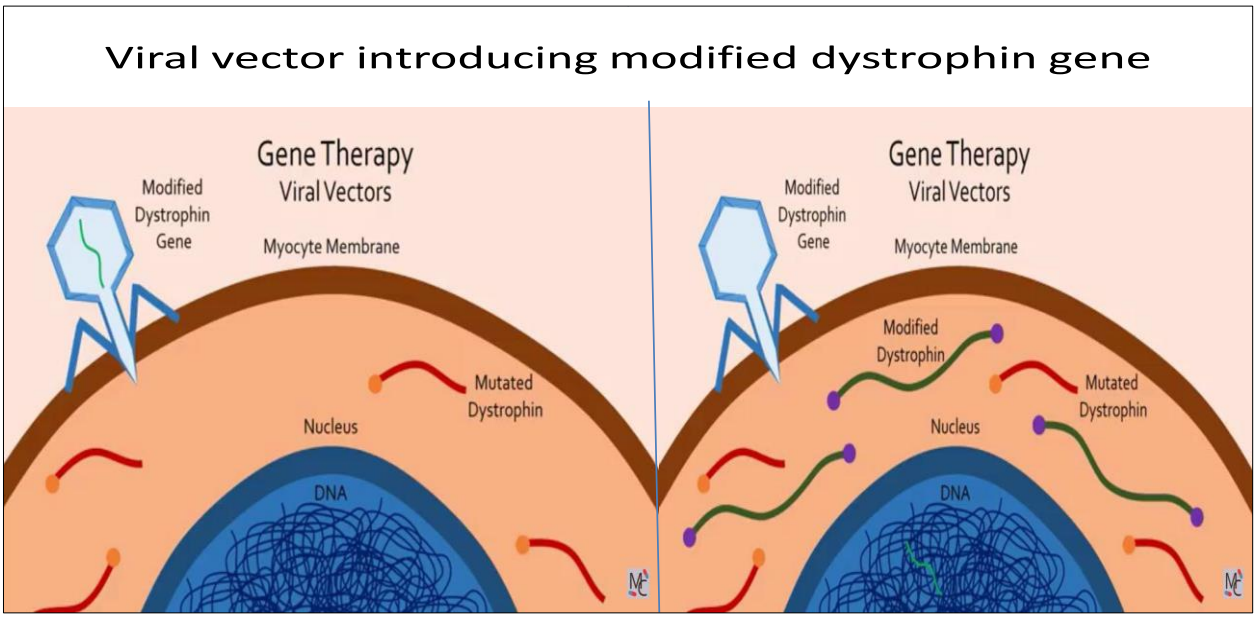

Fig 6: shows mutated dystrophin gene

Fig 7: shows the modified dystrophin gene introduced in body by AAV

Conclusion: Treatment of Duchenne muscular dystrophy (DMD) by gene therapy using adeno-associated viral vectors. The method was found to be highly reproducible. Our aim is to apply this technique to treat DMD patients from this promising therapy.

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