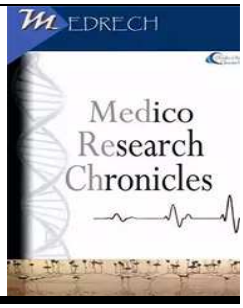
MEDICO RESEARCH CHRONICLES
ISSN NO. 2394-3971
DOI No. 10.26838/MEDRECH.2022.9.2.589
Contents available at www.medrech.com



CURRENT MUTATION-TARGETED DMD TREATMENTS AND THEIR THEORETICAL APPLICATION IN A SUB-GROUP OF ALBANIAN PATIENTS

Aferdita Tako Kumaraku, Aida Bushati, Armand Shehu, Renald Meçani, Paskal Cullufi
Child Neurologist, Mother Teresa University Hospital Centre, Tirana, Albania

ARTICLE INFO

Article History
Received: March 2022
Accepted: April 2022
Key Words: Duchenne muscular dystrophy, genetic diagnosis, DMD.

Corresponding author
A. T. Kumaraku*

ABSTRACT

Although the molecular origins of Duchenne muscular dystrophy have been known for several years, there is still no curative treatment for the disease. Exon skipping is a mutation-specific approach; which exon to skip depends on the size and location of the mutation. As such, having a genetic diagnosis of the disease is important. The genetic diagnosis mentioned in this paper was made privately in a sub-group of DMD patients from our clinic. Nine out of fourteen patients (64%) have mutations that are targeted by therapies that are currently licensed or undergoing Phase III trials.

ORIGINAL RESEARCH ARTICLE

2022, www.medrech.com

INTRODUCTION

Muscular dystrophies are inherited myogenic disorders characterized by progressive muscle wasting and weakness of variable distribution and severity. The commonest form of these inherited disorders—Duchenne muscular dystrophy—was originally described by Edward Meryon, an English doctor. At a meeting of the Royal Medical and Chirurgical Society in 1851, and later published in the transactions of the society,2 he described in detail the clinical presentation of this disorder, beginning in early childhood with progressive muscle wasting and weakness and leading to death in late adolescence. He showed that the disease was familial, and only affected boys. [1]

Patients with DMD present with muscle weakness at the age of 2 to 5 years lose the ability to walk at about the age of II years and do not usually survive beyond the early twenties. A consistent finding is the grossly elevated level of serum creatine kinase (CK), especially in the preclinical and early clinical stages of the disease. [2]

The Duchenne gene is located at Xp21 which affects the sarcolemmal protein dystrophin and is allelic with Becker muscular dystrophy. The protein product of the human Duchenne muscular dystrophy locus (DMD) and its mouse homolog (mDMD) have been identified by using polyclonal antibodies directed against fusion proteins containing two distinct regions of the mDMD cDNA. Dystrophin, the 427 kDa protein represents approximately 0.002% of total striated muscle protein. The gene is composed of 79 exons and 7 promoter regions. [1, 3, 4, 5]

The reported frequency of different mutations leading to DMD varies widely

duplications of one or several exons correspond to 7% of the mutations, point mutations account for 20%, while deletions are observed in 72% of the patients. Most deletions occur between the exons 44 and 55, corresponding to the dystrophin's rod domain. If these mutations alter the reading frame of dystrophin (out of frame-mutation), protein formation is truncated, no dystrophin is produced and the patient develops DMD. [5, 6, 7, 8]

Although the molecular origins of DMD have been known for several years, there is still no curative treatment for the disease. In DMD patients, as mentioned earlier, the mutated gene manifests deletions, duplications, and point mutations which interrupt the genetic information's reading frame.

Exon skipping is a mutation-specific approach; which exon to skip depends on the size and location of the mutation. Researchers are seeking to inject a molecule capable of interfering with the RNA splicing signals to omit an additional adjacent exon, thus restoring the reading frame and allowing for the expression of a protein that is smaller but partially functional, as with patients who have Becker muscular dystrophy. This synthetic, modified RNA molecule is referred to as an antisense oligonucleotide (AO) and is capable of binding with specific pre-RNA sites, masking and excluding this exon from the splicing. [12]

As such, having a genetic diagnosis of the disease is important. Most DMD patients have a deletion of one or more exons (~68%); the reading frame can be restored by single exon skipping for 70% of these deletions, while an additional 8% need skipping of two exons to restore the reading frame 12. Because deletions cluster between exon 45 and 55, the skipping of exons in this area apply to larger groups of patients, with exon 51 skipping applying to 14% of patients, exon 45 and exon 53 skipping both applying to an additional 8% of patients and exon 44 applying to an additional 6% 15. AON-induced single and double exon skipping leading to dystrophin restoration have both been shown feasible in patient-derived cell cultures and animal models. [5, 9]

Small mutations in in-frame exons can be bypassed by skipping the mutated exon, while mutations in out-of-frame exons generally can be restored by skipping both the mutated exon and one of the adjacent exons. As these mutations occur randomly throughout the coding sequence, the total number of patients benefiting from these skips is lower. The majority of these mutations (60%, 15% of all mutations) involve nonsense point mutations directly introducing translational truncation codons. "Read through" of a premature stop codon is a novel approach to treating genetic disorders due to a nonsense mutation. The development of a safe, orally bioavailable drug that has "read through" activity would be beneficial to Duchenne muscular dystrophy (DMD) patients with nonsense mutations in their dystrophin gene. To treat genetic disorders due to a nonsense mutation, ataluren (PTC124) has been developed as a first-in-class, investigational new drug designed to enable ribosomal read through of premature stop codons. [10, 11]

As of October 2020, there are currently four medications used to treat mutation-specific DMD approved in the US and/or EU: (1) Eteplirsen, an antisense oligonucleotide indicated for the treatment of. Duchenne muscular dystrophy (DMD) in patients who have a confirmed mutation of the DMD gene that is amenable to exon *51* skipping; (2, 3) Viltolarsen and Golodirsen for the treatment of patients with DMD amenable to exon 53 skipping; (4) Ataluren, indicated for the treatment of patients with genetic disorders due to a nonsense mutation.

METHODOLOGY

Genetic analyses were performed by Centogene laboratories in a sub-group of

patients. The genetic diagnosis was then confronted against current approved and Phase III therapies.

RESULTS AND DISCUSSION

Patient Nr.	Location	Variant Coordinates	Mutation Type
Patient 1	Intron 62	DMD, c.9225-647A>G	splice site disruption
Patient 2	Exon 12	DMD, c.1382_1383del p.(Asn461Argfs*21)	premature stop codon; point non-sense deletion
Patient 3	Exon 37	DMD, c.5175del p.(Asn1725Lysfs*17)	premature stop codon; point non-sense deletion
Patient 4	Exon 46-51		multiple exon deletions
Patient 5	Exon 45-49		multiple exon deletions
Patient 6	Exon 46-47		multiple exon deletions
Patient 7	Exon 12	DMD, c.1382_1383del p.(Asn461Argfs*21)	premature stop codon; point non-sense deletion
Patient 8	Exon 46-51		multiple exon deletion
Patient 9	Intron 50	DMD, c.7309+2_7309+3insTT	insertion disrupts splice site
Patient 10	Exon 12-13		multiple exon duplications
Patient 11	Exon 22	DMD, c.2869C>T p.(Gln957*)	premature stop codon; point non-sense substitution
Patient 12	Exon 46-51		multiple exon duplications
Patient 13	Exon 45		single exon duplication
Patient 14	Intron 20	DMD, c.2623-2A>C	premature stop codon; point non-sense substitution

The genetic testing results showed that 7 out of 14 patients (50%) have mutations caused by single/multiple exon deletion/duplication.

Four out of seven (29% of the group) have theoretically exon 45 skipping-amenable mutations and one has an exon 44 skipping-amenable mutation. Currently, Casimersen (SRP-4045, Sareptra Therapeuticals) is undergoing Phase III trials for mutations amenable by exon 45 skipping, offering a potential therapy to DMD patients with this specific genetic mutation. Two others have mutations caused by exon duplication/deletion theoretically not amenable by exon skipping therapy.

Five out of fifteen patients (36%) have point non-sense mutations that result in a premature stop codon. These patients are candidates for ataluren therapy, licensed in the EU for DMD caused by a nonsense mutation. Ataluren has completed Phase III trials in the US.

Two patients have mutation intron located mutations that disrupt the function of splice sites.

To conclude, nine out of fourteen patients (64%) have mutations that are targeted by therapies that are currently licensed or undergoing Phase III trials. This offers promise regarding the limited therapeutic options and poor prognosis of DMD.

Promising universal therapies for DMD unrelated to mutation type include gene therapy. PF-06939926 is an investigational, recombinant adeno-associated virus, serotype 9 (AAV9) carrying a shortened version of the dystrophin gene (mini-dystrophin) currently under FDA Phase III trial.

REFERENCES

1. Emery, A. E. (2002). The muscular dystrophies. *The Lancet, 359*(9307), 687-695. doi:10.1016/s0140-6736(02)07815-7
2. Worton, R. G., & Thompson, M. W. (1988). Genetics of Duchenne Muscular Dystrophy. *Annual Review of Genetics, 22*(1), 601-629. doi:10.1146/annurev.ge.22.120188.003125
3. Hoffman EP, Brown RH Jr, Kunkel LM. Dystrophin: the protein product of the Duchenne muscular dystrophy locus. Cell. 1987 Dec 24;51(6):919-28. DOI: 10.1016/0092-8674(87)90579-4. PMID: 3319190.
4. Cohn, R. D., & Campbell, K. P. (2000). Molecular basis of muscular dystrophies. *Muscle & Nerve, 23*(10), 1456-1471. doi:10.1002/1097-4598(200010)23:103.0.co;2-t
5. Beytía Mde L, Vry J, Kirschner J. Drug treatment of Duchenne muscular dystrophy: available evidence and perspectives. *Acta Myol*. 2012;31(1):4-8.
6. Manzur AY, Kinali M, Muntoni F. Update on the management of Duchenne muscular dystrophy. Arch Dis Child. 2008 Nov;93(11):986-90. DOI: 10.1136/adc.2007.118141. Epub 2008 Jul 30. PMID: 18667451.
7. Muntoni F, Torelli S, Ferlini A. Dystrophin and mutations: one gene, several proteins, multiple phenotypes. *Lancet Neurol*. 2003;2(12):731-740. doi:10.1016/s1474-4422(03)00585-4
8. Pichavant C, Aartsma-Rus A, Clemens PR, et al. Current status of pharmaceutical and genetic therapeutic approaches to treat DMD. *Mol Ther*. 2011;19(5):830-840. doi:10.1038/mt.2011.59
9. Erik H. Niks & Annemieke Aartsma-Rus (2017) Exon skipping: a first in class strategy for Duchenne muscular dystrophy, Expert Opinion on Biological Therapy, 17:2, 225-236, DOI: 10.1080/14712598.2017.1271872
10. Aartsma-Rus A, Fokkema I, Verschuuren J, et al. Theoretic applicability of antisense-mediated exon skipping for Duchenne muscular dystrophy mutations. Human Mutation. 2009 Mar;30(3):293-299. DOI: 10.1002/humu.20918.
11. Bushby K, Finkel R, Wong B, et al. Ataluren treatment of patients with nonsense mutation dystrophinopathy. *Muscle Nerve*. 2014;50(4):477-487. doi:10.1002/mus.24332
12. Deconinck, N., & Dan, B. (2007). Pathophysiology of Duchenne Muscular Dystrophy: Current Hypotheses. *Pediatric Neurology, 36*(1), 1-7. doi:10.1016/j.pediatrneurol.2006.09.016