GENE THERAPY MEDIATED BY EXON-SKIPPING IN DUCHENNE MUSCULAR DYSTROPHY

Perelló, C. Grau en Genètica, Facultat de Biociències, Universitat Autònoma de Barcelona, Barcelona, Spain.

UAB Universitat Autònoma de Barcelona

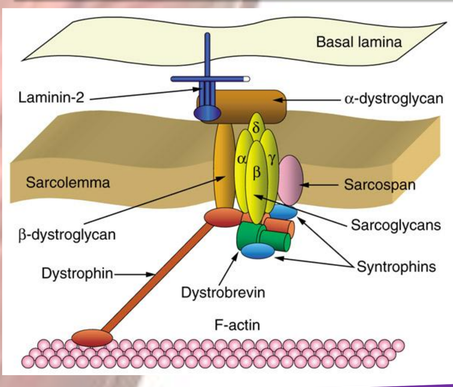
❖ ABSTRACT

Duchenne Muscular Dystrophy (DMD) is a neuromuscular degenerative disease linked to X chromosome caused by a mutation in the dystrophin gene. DMD affects 1/3500 males and causes premature death, before their 30s, due generally to cardiovascular dysfunctions. Patients have a early onset of the disease, between 2 and 5 years old, and at the age of 13 most of them have to use a wheelchair. There are three main treatments for the disease but only drugs are being used nowadays. Last investigations in the field propose cell therapy and gene therapy as alternatives.

❖ OBJECTIVES

The aim of the present review is to explain what are the causes of the disease and their consequences, specially in a molecular level. We focus the attention on the description of one of the most important treatments which is currently being tested, exon-skipping of the altered exons of the dystrohin gene.

❖ INTRODUCTION



What's the cause of DMD?

Mutations in the dystrophin gene (2.5 millions of bp with 79 exons) cause the truncation of the protein dystrophin which is mostly expressed in the muscular tissue and in the brain.

Dystrophin links the citoesqueleton and the extracellular matrix maintaining the stability during the contraction. When it is interrupted, in patients, there are problems during contraction.

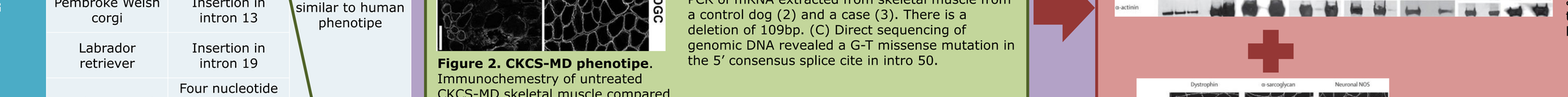
Patients have necrosis of the esqueletic muscular fibers and invasion of inflammatory cells. There is a degeneration of muscle which is substituted by fibroadipos tissue.

Which are the current treatments?

TREATMENT	EXPLANATION
Drugs	Glucocorticoids (prednisone and deflazacort) which increase strength and muscular function
Cell therapy	Introduction of precursor or stem cells into the affected tissue where are differentiated to normal muscular cells
Gene therapy	**Introduction of dystrophin gene or variants into the affected tissue. A type of gene therapy is Exon-Skipping**

❖ EXON-SKIPPING

The exon-skipping therapy consists on the introduction of antisense oligonucleotides (AOs) at local or systemic level to generate a shorter but functional dystrophin product. The AOs are short sequences (20-25bp) complementary to a pre-mRNA region near the acceptor o donor sites of splicing or between exons. As a result the splicing machinery is altered and the final mRNA, which is shorter, generates a truncated protein. This truncation doesn't include the internal region of the protein and the N terminal and C terminal regions, the most important ones, remain functional.



Animal models

ANIMAL	SUBTYPE/ BREED	MUTATION	OTHER CHARACTERISTICS
MICE	mdx	STOP in exon 23	Myofibers without dystrophin and some revertent miofibers
DOG	Golden retriever	Splice site point mutation, with skipping of exon 7 in transcript	Models more similar to human phenotipe
	German shorthaired pointer	Deletion encompassing the entire dystrophin gene	
	Cavalier king Charles spaniel	Splice site point mutation, with skipping of exon 50 in transcript	
	Pembroke Welsh corgi	Insertion in intron 13	
	Labrador retriever	Insertion in intron 19	
	Cocker spaniel	Four nucleotide deletion in exon 65	
	Tibetan terrier	Deletion of exons 8-29	
	Rottweiler	Nonsense point mutation in exon 58	

Types of AOs

There are two main types of chemicals used in clinical trials as AOs: 2'-O-methyl phosphorothiate antisense oligonucleotides (2'-O-MePS AO), and phosphorodiamidate morpholino oligomers (PMO). PMO has a stronger pairing to target RNA than RNA or DNA.

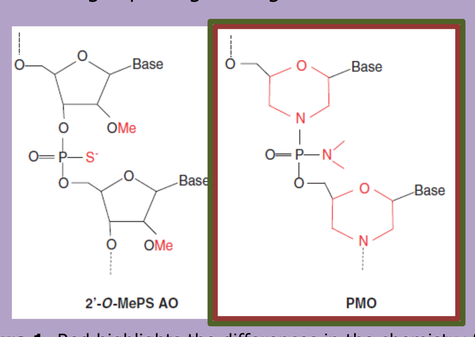


Figure 1. Red highlights the differences in the chemistry from RNA or DNA.

Preclinical assay

Genotipe and phenotipe are very similar to humans with DMD

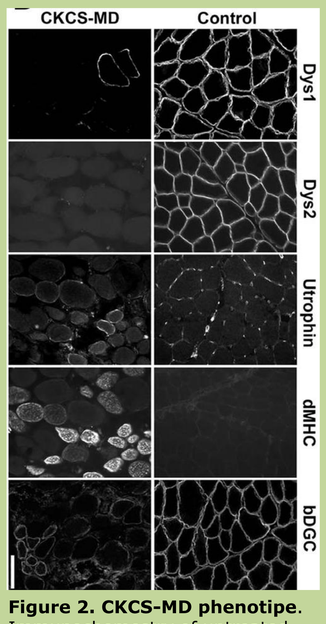


Figure 2. CKCS-MD phenotipe. Immunochemestry of untreated CKCS-MD skeletal muscle compared with control dog muscle

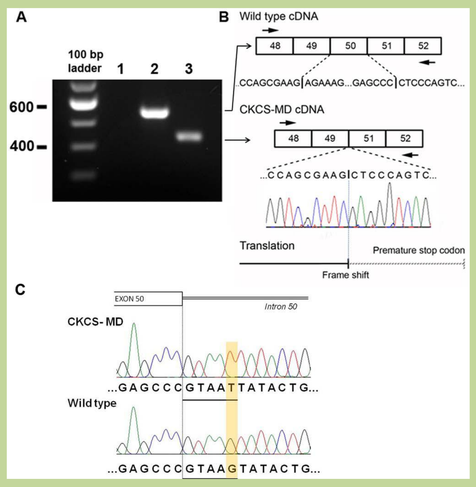


Figure 3. Genotype of CKCS-MD. (A and B) RT-PCR of mRNA extracted from skeletal muscle from a control dog (2) and a case (3). There is a deletion of 109bp. (C) Direct sequencing of genomic DNA revealed a G-T missense mutation in the 5' consensus splice cite in intro 50.

TREATMENT WITH AVI-4658 (PMO COMPLEMENTARY TO EXON 51)

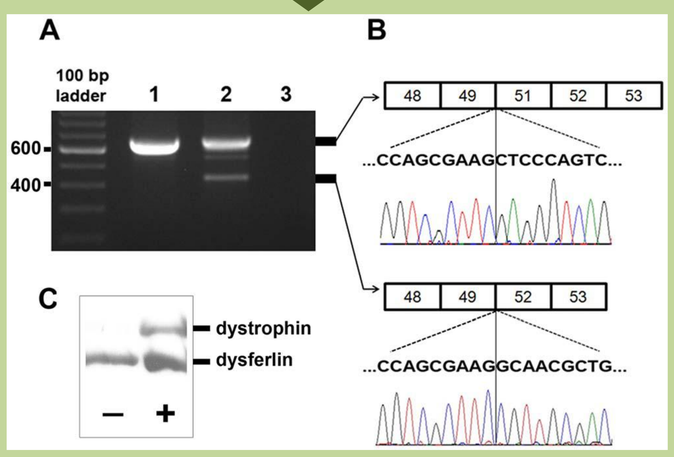


Figure 4.Antisense oligonucleotide mediated skipping of exon 51 restores protein expression. (A and B) RT-PCR of mRNA extracted from cultured canine myoblasts in absence (line 1) or presence (line 2) of AVI-4658. (C) Western immunoblot of protein extracts from treated (+) and untreated (-) CKCS-MD myoblasts demonstrate the re-expression of dystrophin following exon 51 skipping in treated cells.

Clinical trial

This phase 2, dose-escalation study was performed with 19 patients between 5 and 15 years old with an out-of frame deletion eligible for correction by skipping of exon 51. These patients were treated with intravenous infusion of different doses of AVI-4658.

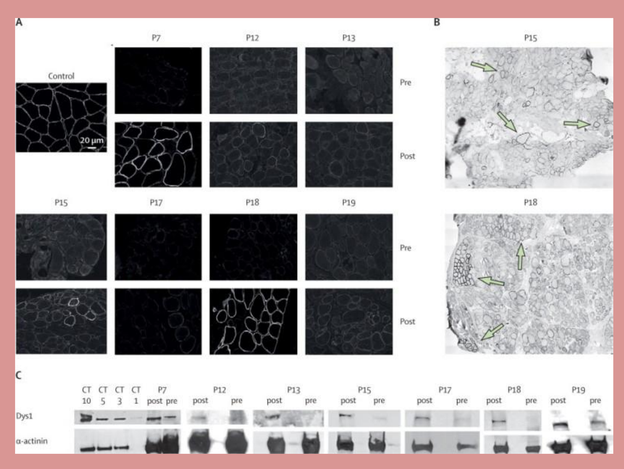


Figure 5. Dystrophin protein expression in the seven patients who responded to treatment. (A) Transverse sections of post and pre treated muscle specimens immunolabelled. (B) Post-treatment biopsy samples from patients P15 and P18. (C) Western blotting of pre and post-treatment muscle biopsy samples.

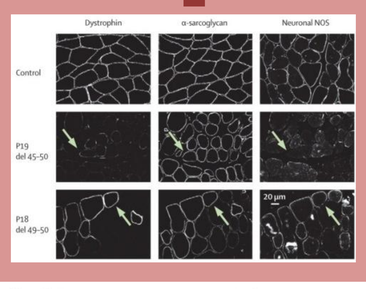


Figure 6. Post-treated biopsies. The expression of α-sarcoglican and neuronal nitric oxid synthase (NOS) in post-treated patients 18 and 19 is also restored in the sacolemma.

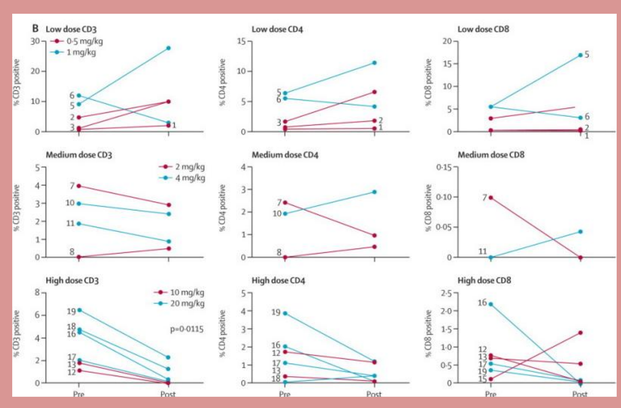


Figure 7. Inflammatory infiltrates quantification on pretreatment and post-treatment muscle samples. Muscle sections were incubated with antibodies againts CD3, CD4 and CD8

❖ CONCLUSION

The gene therapy of Duchenne Muscular Dystrophy has always had two main difficulties: the length of the gene and to target all the affected tissues by systemic administration. The exon-skipping seems to solve this two problems. On the one hand, the function of the protein is restored without needing to introduce the complete gene in a vector. On the other hand, some preclinical trials have restored dystrophin levels significantly in all the affected muscles. For these reasons, the therapy proposed in the present review supposes an encouraging approach to an effective treatment of the disease. However, there is still needed a more accurate technique with the final goal of offering to the patient a personal therapy in order to improve his life quality.

❖ REFERENCES

1. Sugita H, Takeda S. Progress in muscular dystrophy research with special emphasis on gene therapy. Proc Jpn Acad Ser B Phys Biol Sci. 2010;86(7):748-56.

2. Nakamura A, Takeda S. Mammalian models of Duchenne Muscular Dystrophy: pathological characteristics and therapeutic applications. J Biomed Biotechnol. 2011;2011:184393.

3. Walmsley GL,*et al*. A duchenne muscular dystrophy gene hot spot mutation in dystrophin-deficient cavalier king charles spaniels is amenable to exon 51 skipping. PLoS One. 2010 Jan 13;5(1):e8647.

4. Cirak S,*et al*. Exon skipping and dystrophin restoration in patients with Duchenne muscular dystrophy after systemic phosphorodiamidate morpholino oligomer treatment: an open-label, phase 2, dose-escalation study. Lancet. 2011 Aug 13;378(9791):595-605.