First report of a family with a DMD out of frame exon 2 deletion associated with asymptomatic phenotypes.

G.L. Vita[2], M. La Rosa[1], M.G. Distefano[1], C. Barcellona[1],, M. Sframeli[1,2], C. Rodolico[1], F. Gualandi[3], C. Lunetta[2], G. Vita[1,2], A. Ferlini[3] and S. Messina[1,2]

[1]Unit of Neurology and Neuromuscular Disorders, Department of Clinical and Experimental Medicine, University of Messina, Messina
[2]Nemo Sud Clinical Centre for Neuromuscular Disorders, Messina
[3]Section of Microbiology and Medical Genetics, Department of Medical Sciences, University of Ferrara, Ferrara, Italy

Background

The severe Duchenne and milder Becker muscular dystrophy (DMD/BMD) are both caused by mutations in the DMD gene. Exon 2 is the most common site of duplication, but its deletion has never been described. The phenotypic difference between DMD and BMD patients is related to the reading-frame rule: in DMD mutations induce a shift in the reading frame leading to prematurely truncated, dysfunctional dystrophin; in BMD in-frame mutations allow the synthesis of deleted but largely functional dystrophins (*Aartsma-Rus et al. 2006*). Mutations in essential functional domains (NH3 and COOH terminal) are associated with severe phenotypes.

It has previously been reported a patient with a nonsense mutation (c.9G>Ap.Trp3X) within the first exons of the *DMD* gene, encoding the unique N-terminal domain of the dystrophin protein (ref) . Although this mutation would be expected to result in a DMD phenotype, the clinical phenotype was very mild. The authors identified the molecular mechanism responsible for the mild disease severity to be initiation of translation at two proximate AUG codons within exon 6.
It has been suggested that this may be a general mechanism of phenotypic rescue for point mutations within at least the first two exons of the *DMD* gene. *("DMD exon 1 truncating point mutations: amelioration of phenotype by alternative translation initiation in exon 6". Gurvich et al. 2009) .*

Case report

He first presented at age of 6 years for evaluation of an incidentally detected elevation of serum creatine kinase (550 iu/l; normal value < 200 iu/l).

MEDICAL HISTORY

No neuromuscolar disorder in his family
Normal early motor milestones

NEUROLOGICAL EXAMINATION

Normal

EMG

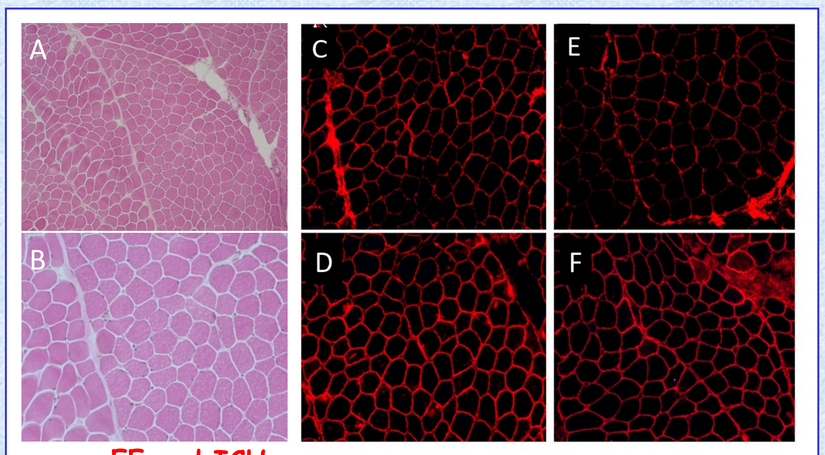
Mild myopathic pattern

MUSCLE BIOPSY

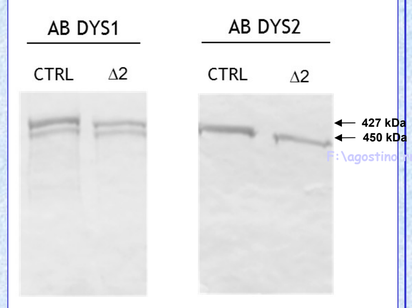
Slight fiber size variability and in some sections an increased number of central nuclei along with some densely stained hypercontracted fibers (Fig. A 10X E&E,B 20X E&E). Immunofluorescent analysis using NCL-DYS3 (NH2-terminal Fig. C) e NCL-DYS2 (COOH-terminal Fig. E) antibody showed the presence of fibers with low or irregular expression of dystrophin at the membrane with NCL-DYS1 antibody (Rod domain, Fig. D) it showed a mild reduction of expression. Caveolin showed a normal reactivity on all fibers.

WESTERN BLOT ANALYSIS

Reduction of the expression and molecular weight with both antibodies (Dys1 and Dys2) in the patient (Δ2) compared with control(CTRL). The protein had a smaller molecular weight (~410kDa)



EE and ICH



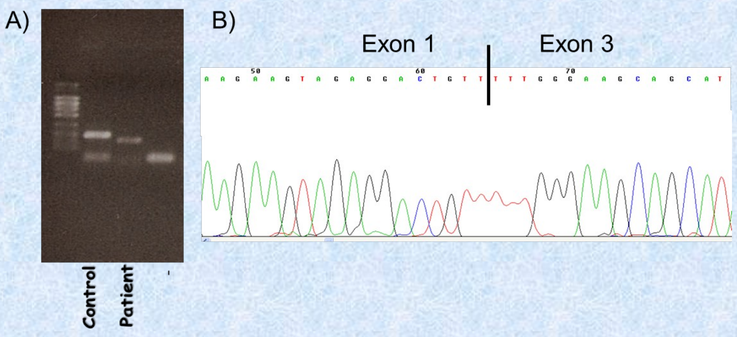
Western-blot analisys

Out-of-frame deletion of exon 2 was identified by MLPA and better defined by CGH-DMD array

CGH-Array: CGH profile of the entire X chromosome detecting a 12.983 bp deletion including exon 2 and part of introns 1 and 2. A microhomology of 5 bp (CTGTG) is found at the junction between the distal and proximal sequences.

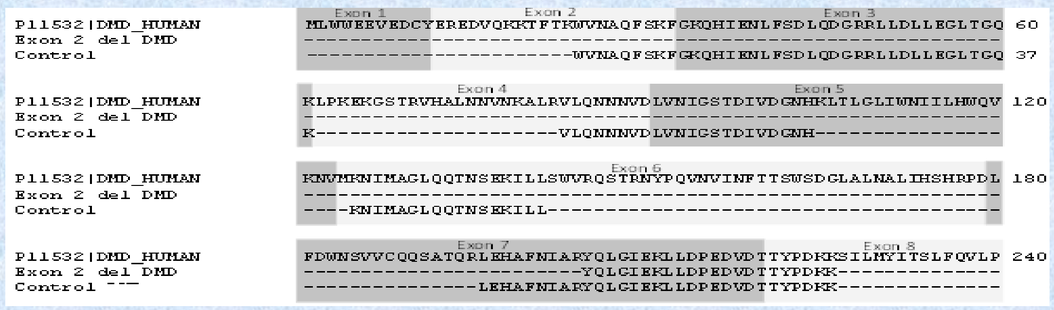
RNA ANALYSIS

RNA was analyzed by RT-PCR amplifying the region surrounding the mutation (M1 promoter -exon3). We detected a reduction of molecular weight in the amplification product from patient with respect to control (A). Sequencing revealed a junctions between exons 1 and 3 (B), confirming exon 2 deletion.



MASS SPECTROMETRY

The expression of a shorter isoform with no peptide sequence encoded by exon 1-6 was confirmed by mass spectrometry (MS).



FOLLOW-UP VISIT AT AGE 18 YEARS:

MEDICAL HISTORY

Subclinical hypothyroidism . (FT3, FT4, TSH normal).
Mild muscle pain associated with muscular efforts
Occasional muscle cramps
Easy tiredness in climb paths
No myoglobinuria

PHYSICAL EXAMINATION

Overweight (Weight 105 Kg height 181 cm).
Valgus foot

NEUROLOGICAL EXAMINATION

Normal

Gowers Manovre: negative.
North Scale Ambulatory Assessment: 34/34.
10 metres running: 2,8 sec
6 Minute Walking Test: 602 metres
AFO: absent

SPIROMETRY

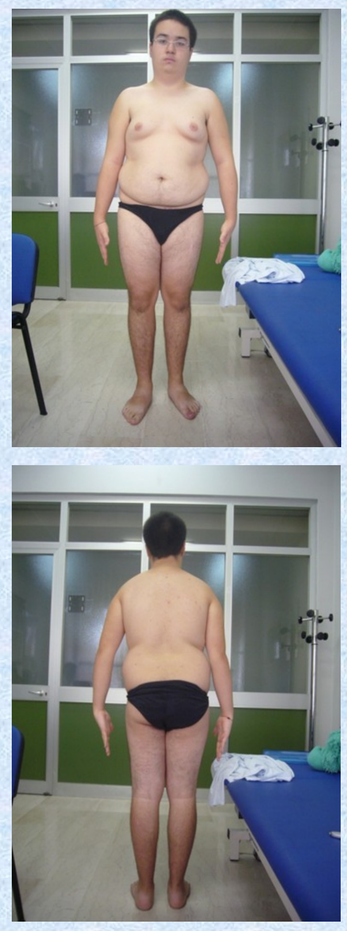
Normal (FVC) 4,79 L (88% of expected value)

CARDIOLOGICAL EVALUATION

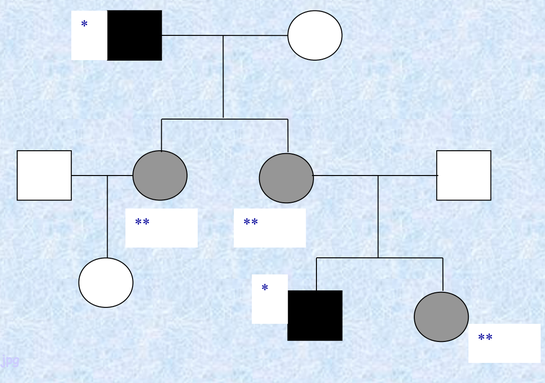
Normal. FE: 61%

MUSCLE MRI

Mild fatty infiltration of the lower limb muscles (Fig. 1)

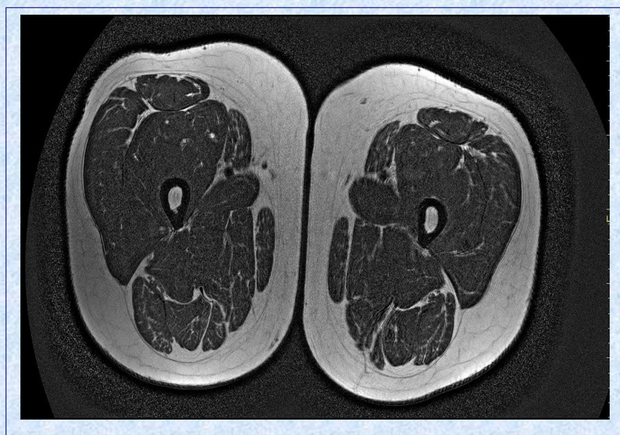


Molecular study was expanded to the patient's family with the results shown in the pedigree.

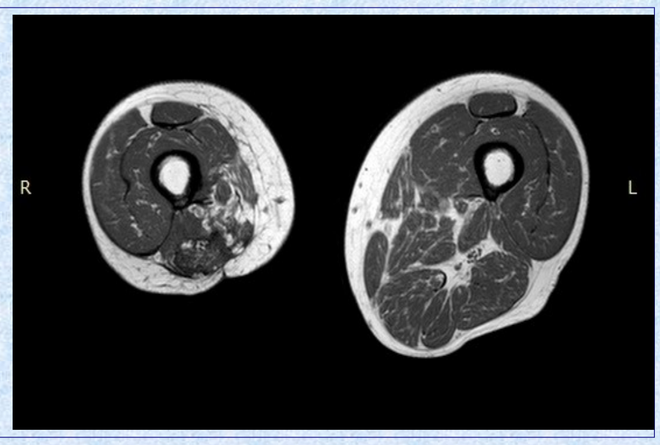


Mother and sister were asyntonmatc carrier **. The grandfather, who showed the same genetic pattern, had a normal neurological examination and muscle MRI (Fig. 2) at the age of 80 yrs.

MUSCLE MRI



Mild muscle gfat infliltration in thigh posterior compartment (Fig.1)



Mild fatty infiltration of the lower limb muscles compatible with age (Fig. 2)

CONCLUSIONS

- Our previous work demonstrated the presence of a IRES (internal ribosome entry site) within exon 5, activated from disruption of the reading frame caused by deletion of exon 2, which allows an alternate translation initiation beginning in *DMD* exon 6 that leads to expression of a highly functional N-truncated dystrophin, supporting a novel therapeutic approach for patients with mutations within the 5' exons of DMD (Wein et al. Nature 2014).

-This report shows for the fist time that deletion in exon 2 with the expression of N-truncated but functional isoform of dystrophin can lead to a phenotype which remains asymptomatic throughout the advanced age.

REFERENCES

- **Duplications in the DMD gene.** Aartsma-Rus et al. . Human Mutat. 2006
- **Dystrophin: more than just the sum of its parts.**Le Rumeur et al. Biochim Biophys Acta. 2010
- Gurvich et al. Hum Mutat. 2009
- **A novel DMD IRES results in a functional N-truncated dystrophin, providing a potential route to therapy for patients with 5' mutations.** Nicolas Wein et al. Nat. Med. 2014