

MYOTONIC DYSTROPHY TYPE 2 PREMUTATIONAL CONDITION: A PROXIMAL PROGRESSIVE MYOPATHY WITH PECULIAR PATHOMOLECULAR FINDINGS

L. SARACENO¹, R. CARDANI², R. VALAPERTA³, B. FOSSATI¹, G. CUOMO², E. COSTA³, G. MEOLA¹

¹Department of Neurology, IRCCS Policlinico San Donato, University of Milan, Italy; ²Laboratory of Muscle Histopathology and Molecular Biology, IRCCS Policlinico San Donato, University of Milan, Italy; ³Research Laboratories, IRCCS Policlinico San Donato, University of Milan, Italy;

Background

Myotonic dystrophy type 2 (DM2) is an autosomal dominant disorder primarily characterized by neuromuscular involvement with myotonia and progressive proximal myopathy, but is also variably associated with multisystemic manifestations such as early-onset cataract, cardiac conduction defects, glucose intolerance and hypogonadism. DM2 is caused by the expansion of tetranucleotide [CCTG] repeats in the intron 1 of *CNBP* gene on chromosome 3q1 that leads to nuclear accumulation of toxic mutant RNA. The *CNBP* gene contains the complex repeat motif (TG)_n(TCTG)_n(CCTG)_n. In the general population, the DM2 repeat tract is stable when the CCTG portion of the repeat tract is interrupted, most commonly by both GCTG and TCTG motifs. As in other disorders, the loss of sequence interruptions predispose normal alleles to expansion. Indeed uninterrupted CCTG sequences increase in length over time acquiring somatic, but also intergenerational, instability. The size of the CCTG repeat tract is below 30 copies in normal individuals [1], while in DM2 patients the range of repeat units is extremely wide, from about 75 to over 11000 units, with a mean of 5000 [2]. Minimum size of a pathogenic expansion is not well known and the smallest reported uninterrupted expansion in a DM2 patient was of 75 repeats [1]. Individuals, without signs and symptoms of DM2, but with unstable uninterrupted CCTG sequences, ranging from approximately 30 to 55 CCTG repeats, were considered "healthy premutated patients" [1]. However in the last years two DM2 patients respectively with [CCTG]₅₅ and [CCTG]₆₁ expansions have been reported in literature [3] without a description of their clinical and pathomolecular findings.

Aim

We report the first description of an Italian patient with Myotonic Dystrophy type 2 (DM2) clinical and histological muscular abnormalities, but associated with a short repeated -[CCTG]₃₆-uninterrupted allele (pre-mutational condition). To identify this DM2-linked myopathy the standard diagnostic approach routinely used to obtain DM2 diagnosis, which is Fluorescence in situ hybridization (FISH) in combination with MBNL1-immunofluorescence, has to be necessarily integrated with PCR and sequencing of *CNBP* gene.

Materials and Methods

We report the case of a 51 years old female patient with progressive proximal leg pain and weakness, leading to disabling walking impairment. She also complained of milder pain and hyposthenia in proximal upper limbs. Her father died for respiratory insufficiency at 69 years old and was affected by a not defined muscular dystrophy. After excluding others neuromuscular disorders (normal expression of dystrophin, caveolin and sarcoglycan proteins. Genetic test for FSH, DMPL, and laminin A/C was negative) genetic DM2 test was performed on DNA extracted from blood. Then a biceps brachii biopsy was carried out for histopathological evaluation. FISH in combination with MBNL1-immunofluorescence was performed to verify the presence of toxic RNA and MBNL1 nuclear foci. Alternative splicing of *CLCN1*, *MBNL1* and *INSR* was analyzed in muscle biopsy.

Results

High CpK enzymes (>220U/L), a mild increase of GGT and of arterial basal lactic acid (14,3 mg/dl) were evidenced on peripheral blood. Neurological examination confirmed muscular weakness of hip flexors (3+ MRC), knee extensors (4+) and shoulder abductors (4+). No clinical or EMG myotonia was evident. Muscle biopsies showed increase of central nuclei and of fiber size variability, type I and II fiber atrophy and nuclear clumps (FIG.1). DM2 test indicated a CCTG repetition number of 36 copies in *CNBP* gene. FISH associated with MBNL1-immunofluorescence performed on muscle sections did not show nuclear accumulation of mutant RNA or of MBNL1 (FIG.2) and alternative splicing of *CLCN1*, *MBNL1* and *INSR* was not altered (FIG.3).

Conclusions

We describe for the first time the clinical spectrum and pathomolecular findings of DM2 premutational condition. Patients with short CCTG uninterrupted repeats could present typical DM2 phenotype and histological features. FISH, the most simple tool routinely used to obtain a definite DM2 diagnosis, proved not to be a sensitive method to detect nuclear accumulation of premutated RNA, because the (CAGG)₅ probe doesn't detect very short sequences. Moreover MBNL1 immunofluorescence is negative and no splicing alterations could be identified because short CCTG expansions of premutated patients are not able to sequestrate MBNL1 in the cell nuclei. Consequently the only diagnostic approach adequate to detect this DM2-linked myopathy is to integrate FISH and MBNL1-immunofluorescence with PCR on peripheral blood and direct sequencing of the larger uninterrupted repeats. However haplotype analysis is currently in progress to assess whether this *CNBP* premutated allele derives from the same founder origin as the European DM2 mutation.

References

- [1]Radyanzky J, Surovy M, Polak et al. Uninterrupted CCTG tracts in the myotonic dystrophy type 2 associated locus. *Neuromuscul Disord*. 2013 Jul;23(7):591-8. doi: 10.1016/j.nmd.2013.02.013. Epub 2013 Apr 2.
[2]Liquori CL, Ricker K, Moseley et al; Myotonic dystrophy type 2 caused by a CCTG expansion in intron 1 of ZNF9. *Science*.2001 Aug 3;293(5531):864-7.
[3]Bachinski LL, Czernuszewicz T, Ramagli LS et al. Premutation allele pool in myotonic dystrophy type 2. *Neurology*.2009 Feb 10;72(6):490-7. doi: 10.1212/01.wnl.0000333665.01888.33. Epub 2008 Nov 19.

Muscle histopathology

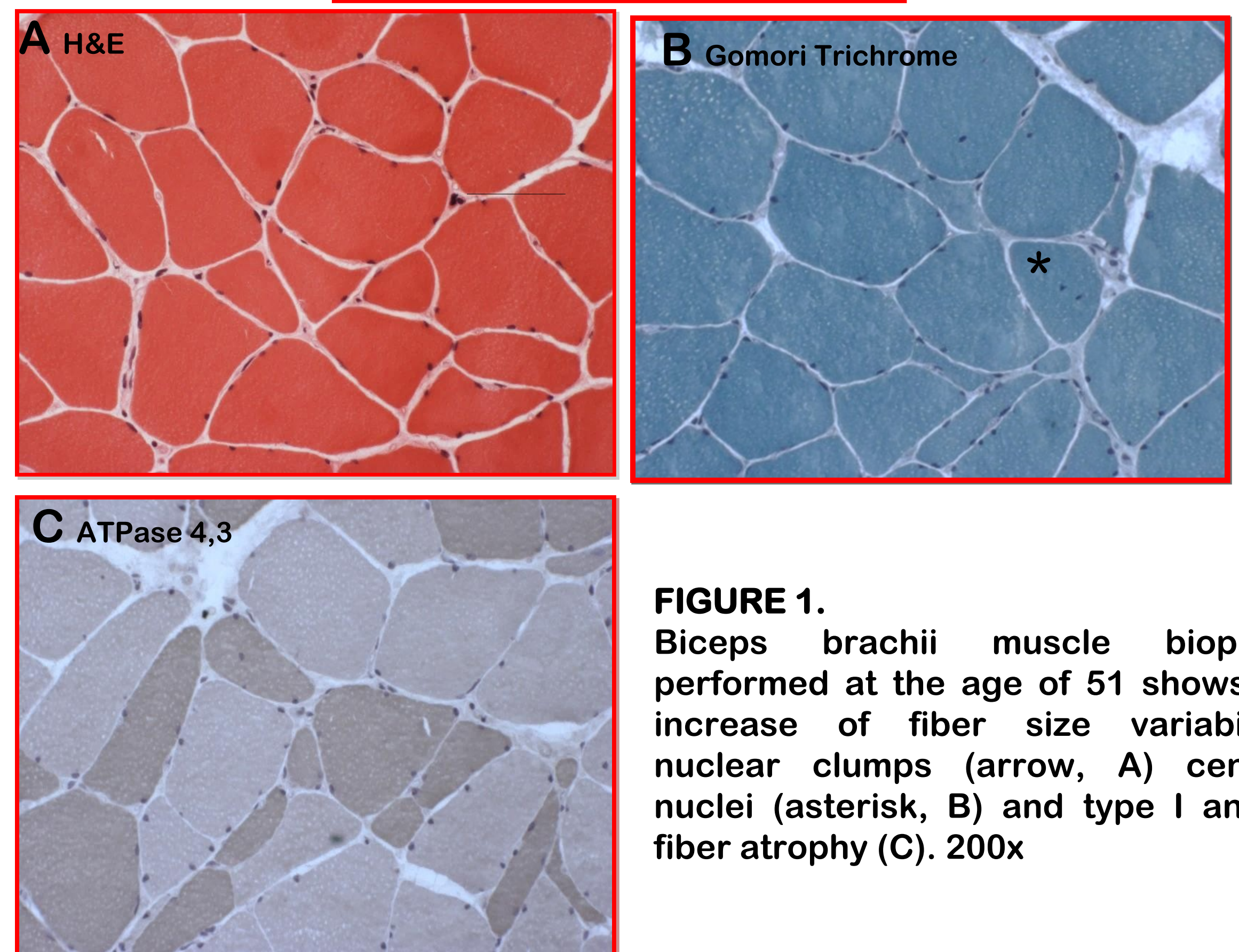


FIGURE 1. Biceps brachii muscle biopsies performed at the age of 51 shows an increase of fiber size variability, nuclear clumps (arrow, A) central nuclei (asterisk, B) and type I and II fiber atrophy (C). 200x

Fluorescence in situ hybridization +MBNL1 immunofluorescence

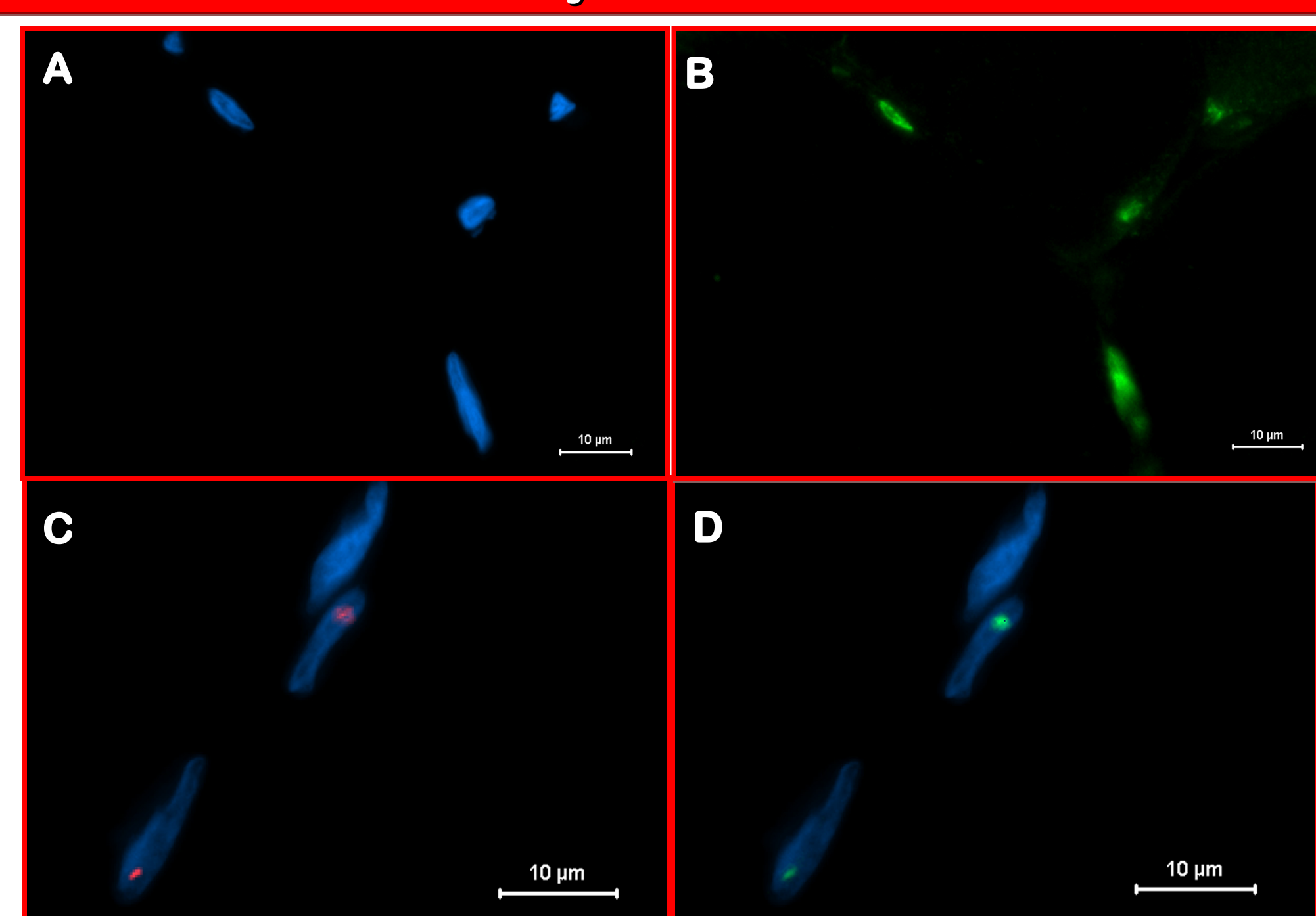


FIGURE 2: FISH + MBNL1 immunofluorescence shows no nuclear accumulation of mutant RNA (A) or of MBNL1 (B) as observable in a DM2 patient with 2000 CCTG expansion (C, D). MBNL1 shows a diffuse nuclear staining (B).

Alternative splicing

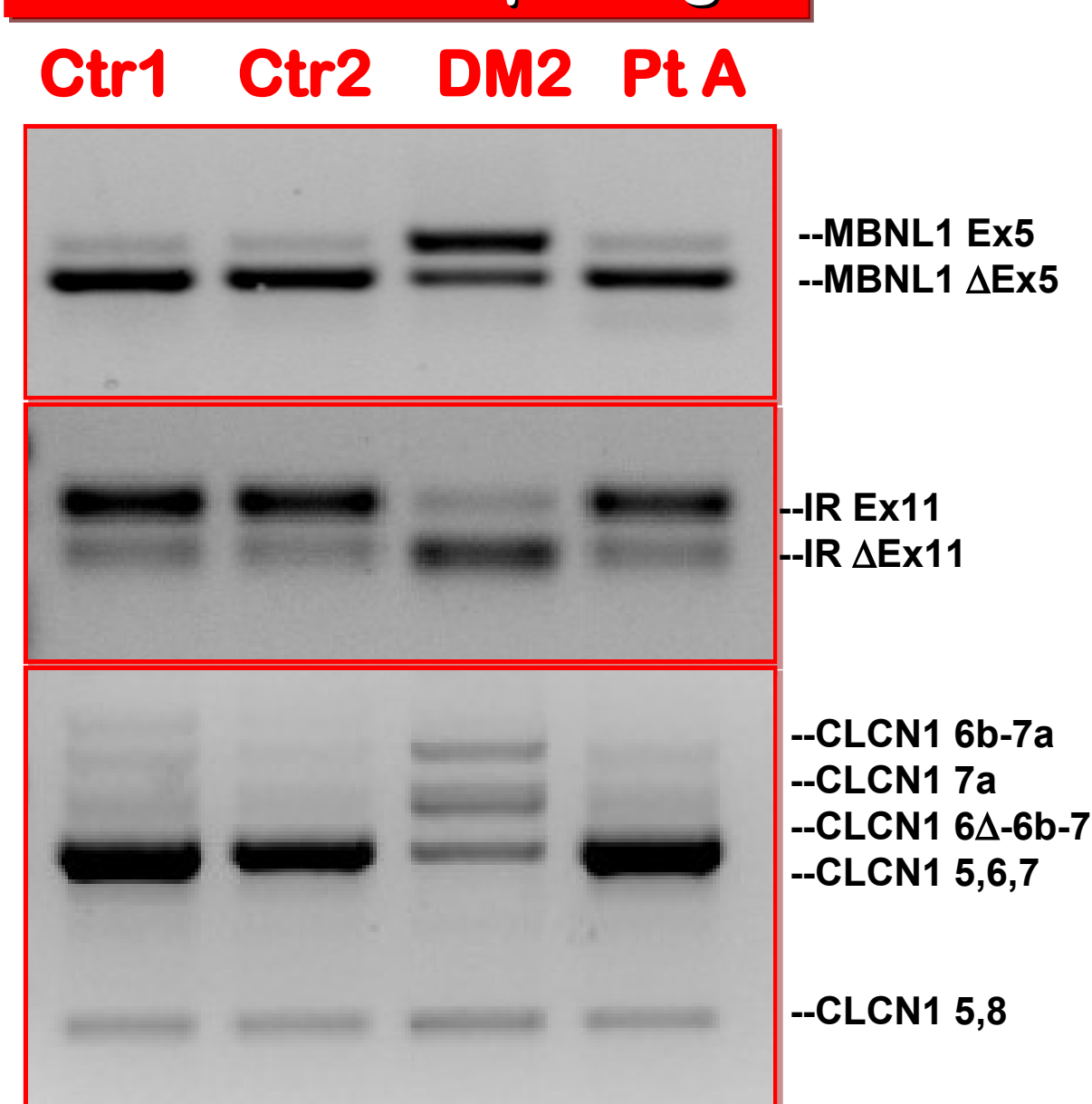


FIGURE 3: Similarly to healthy patients (Ctr), no alterations of alternative splicing of MBNL1, Insulin Receptor (IR) and CLCN1 genes have been evidenced by RT-PCR analysis performed on biceps brachii muscle biopsies from Patient A (Pt A). DM2 patient, used as positive control, shows splicing alterations of all the genes examined.