Myotonic Dystrophy type 2 in a cohort from southern Italy: a challenging diagnosis by biomolecular tests

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BACKGROUND

• Myotonic dystrophy type 2 (DM2) is an autosomal dominant multisystem disorder caused by the expansion of CCTG tetranucleotide repeats in the first intron of the *zinc finger nucleic acid-binding protein (CNBP)* gene on chromosome 3q21. Typically, disease onset is in the adulthood with proximal muscular weakness and myalgia as main muscle symptoms. However, clinical features are variable and non-specific, thus complicating the diagnosis.

MATERIALS AND METHODS

• We have evaluated a cohort of 25 patients with suspected DM2 at clinical and/ or histopathological data, followed in our Unit since 2005. Clinical features are summarized in Table 1.

•Molecular diagnosis was performed by genetic test on blood DNA (based on the combination of long-PCR and Southern blot analysis) and by fluorescence in situ hybridization (FISH) on muscle sections to verify the presence of nuclear toxic RNA accumulation. RealTime-PCR analysis was also performed on muscle biopsies to study the splicing pattern of several genes commonly involved in DM2 pathogenetic mechanisms.

RESULTS

•15 out of 25 patients were DM2 (10 F, 5 M); middle age 56,6 +/- 10 years

•Age at onset was 34.7 +/- 12,1 years and diagnostic delay 15,6 +-14,3 years

•The first symptoms more reported are pain and fatigue, followed by proximal muscle weakness , muscle stiffness and grip myotonia

• Grip myotonia was present in 10 pts

•CK level slightly increased in all patients except one (3000U/L)

•Among these 15 patients: 6 were also affected by thyroid diseases, 5 by cataracts and 2 by diabetes

•Clinical data are summarized in Table 2.

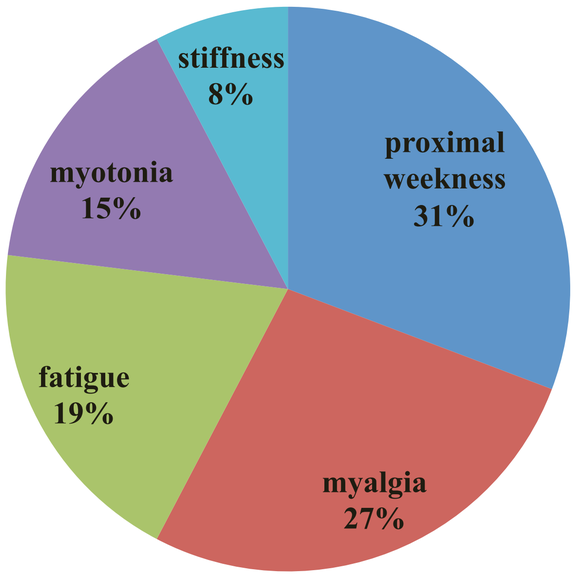
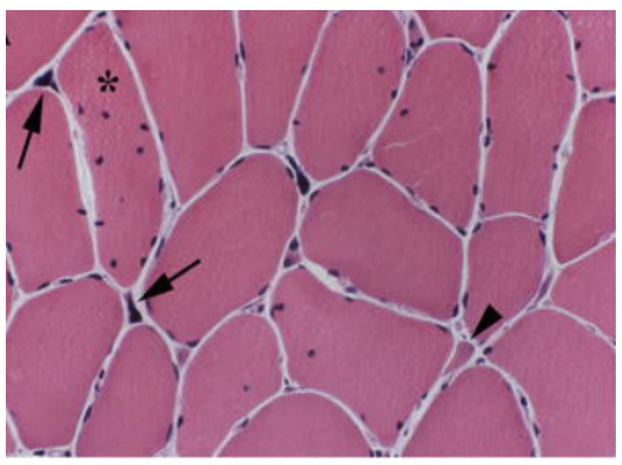


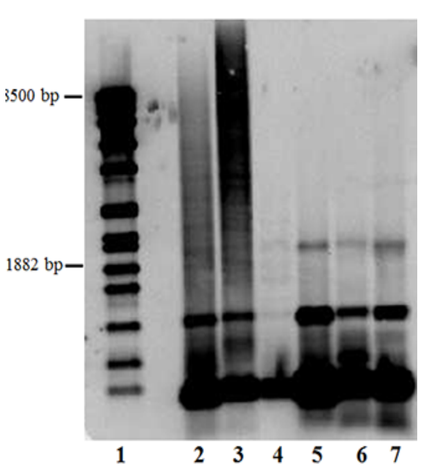
Table 1. Presenting symptoms



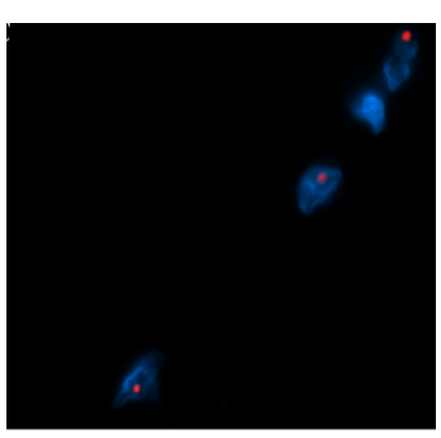
Muscle biopsy: hematoxylin and eosin (H&E) staining showed high fiber size variability with several atrophic fibers and nuclear clumps

Pts	Age of onset (years)	Clinical presentation	CK U/L	Grip myotonia	Proximal weakness	Myalgia	Systemic features	Genetic test	FISH analysis
1	24	fatigue	550	no	no	yes	Hashimoto thyroiditis	+	+
2	46	proximal muscle weakness	225	no	yes	yes	thyroid nodular goitre	+	+
3	17	myotonia	600	yes	no	yes	no	+	+
4	38	myalgia	391	yes	yes	yes	thyroid nodular goitre	+	Not done
5	41	myotonia	500	yes	yes	yes	cataracts	+	Not done
6	37	myalgia	500	yes	yes	yes	thyroid nodular goitre/ cataracts	+	+
7	16	fatigue	321	no	yes	yes	thyroid nodular goitre	-	+
8	49	myalgia	250	no	yes	yes	no	+	+
9	35	fatigue	250	yes	yes	yes	no	+	+
10	44	stiffness	3000	yes	no	yes	Dermato-myositis/ cataracts	+	+
11	34	proximal muscle weakness	357	yes	no	yes	diabetes	+	Not done
12	18	fatigue	258	yes	yes	no	no	+	Not done
13	54	myalgia	300	no	yes	yes	diabetes	-	+
14	43	proximal muscle weakness	190	yes	yes	no	Hashimoto thyroiditis	+	Not done
15	71	myalgia	268	yes	yes	yes	cataracts	+	Not done

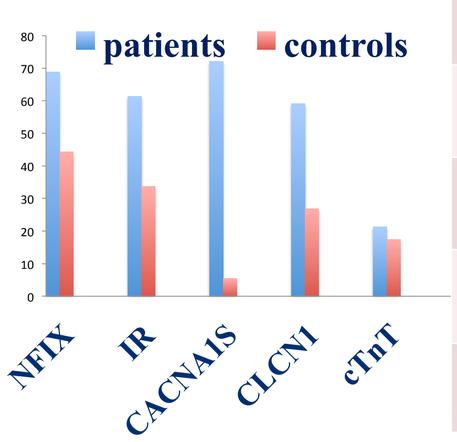
Table 2. DM2 patients data



Results of diagnostic test: Line 1 is the Marker VII; Line 2 and 3 are two positive pts; Lines 4, 5, 6, and 7 are healthy subjects



FISH on muscle section from a DM2 patient: direct visualization of mutant messenger RNA containing CCUG repeat (red spots)



Splicing alteration in pts compared to controls : NFIX (nuclear factor IX),IR (insulin receptor), CACNA1S (CA voltage-gated channel sub a1A), CLCN1(voltage-gated chloride channel),cTnT (cardiac muscle troponin T)

DISCUSSION

• The wide clinical spectrum makes the DM2 diagnosis quite challenging. In fact, long-PCR and Southern blot analysis do not allow always DM2 diagnosis because of the extremely large size and somatic instability of the DNA expansion. Our study underlines the limits and pitfalls of DM2 current diagnostic methods. These results strongly suggest to perform a combination of FISH and genetic molecular test (long-PCR and Southern blot) to increase the DM2 diagnostic sensitivity.

REFERENCES

Hilbert JE, Ashizawa T, Day JW, Luebbe EA, Martens WB, McDermott MP, Tawil R, Thornton CA, Moxley RT 3rd (2013) Diagnostic odyssey of patients with myotonic dystrophy. J Neurol 260:2497–2504.