CLINICAL AND MOLECULAR INVESTIGATIONS IN ADULT AND JUVENILE DM1 PATIENTS

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Introduction

Myotonic Dystrophy type 1 **(DM1)** is a **multi-systemic autosomal dominant disorder** caused by a trinucleotice (CTG) expansion. The main **clinical feactures** are **myotonia, fatigue, skeletal muscle weakness and wasting**. At present, no effective pharmacological treatment and reliable biomarkers are available. Rehabilitative intervention might safely optimize muscle function and prevent additional disuse atrophy.

MicroRNAs (**miRNAs**) are small non-coding RNAs that regulate post-transcriptional mRNA expression and they are markedly stable in circulating body fluids. **miR-206, miR-133a, miR-133b, miR-1 are called "myo-miRNA" and are considered as markers of muscle regeneration, myogenesis and fiber type differentiation.**

Aim: We investigated the **use of microRNAs as circulating biomarkers** for DM1 in a clinical setting and used patients sera collected during a rehabilitative protocol

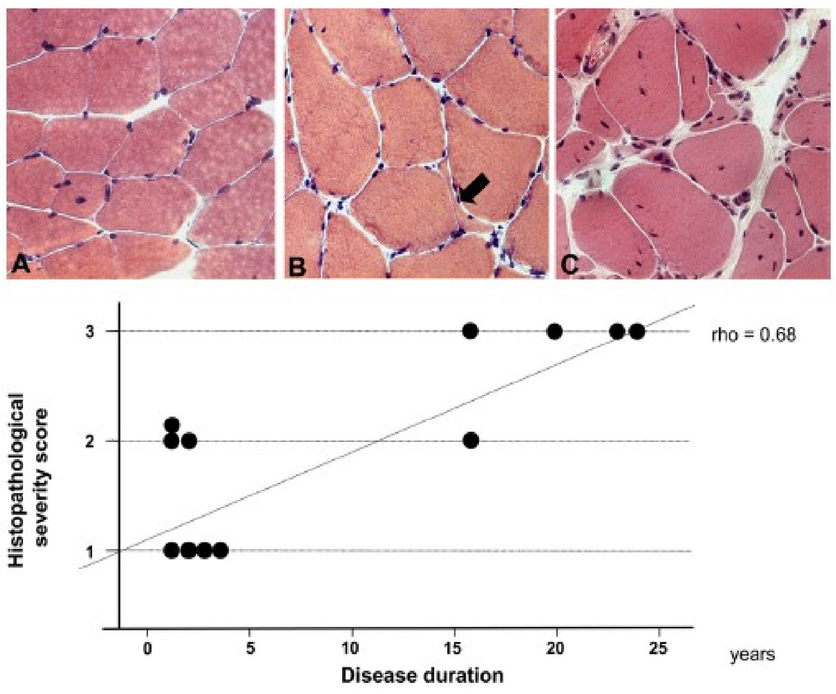
Methods

We investigated levels of muscle-specific myo-miRNAs (miR-1,miR-133a/b,miR-206) in muscle of 12 DM1 patients. Muscle fiber morphometry with a new grading of histopathological severity score were used to compare specific myo-miRNA level and fiber atrophy. Also we collected **serum of 10 DM1 patients** (9 male, 1 female) **before (T0) and after (T1) a period of physical rehabilitation and we measured circulating muscle-specific microRNAs**, miR-1, miR-206, miR-133 a and miR-133b by qRT-PCR.

The rehabilitation protocol has been recently published (Cudia et. Al 2016) and consists in Functional Electical Stimulation (FES)/ lower extremity training or erobic exercise for a period of 6-8 weeks. Functional electrical stimulation (FES) is a new rehebilitative approach that combines electrical stimulation with a functional task

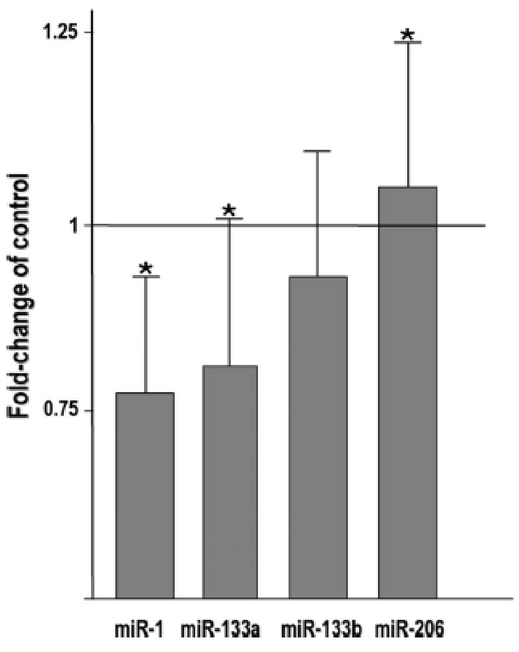
Results

HISTOPATHOLOGICAL CHANGES OBSERVED IN MUSCLE FIBERS



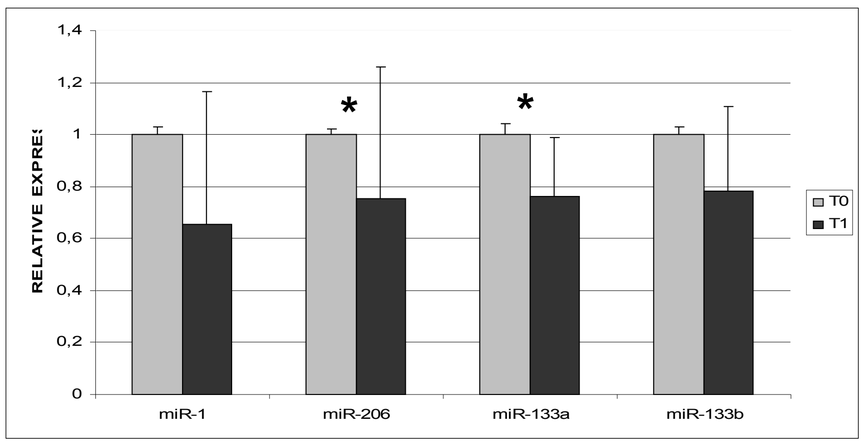
Different extent of muscle histopathological changes observed in DM1 patients. Histopathological score 1: ***A*** increased fiber size variability, occasional internal nuclei. Score 2: **B** atrophic fibers) *(arrow).* Score 3: ***C*** interstitial connective tissue increase, nuclear clumps, atrophic fibers. The ***lower panel*** Disease duration shows a correlation between the histopathological severity score and years of disease duration (Rank Spearman test: p= 0.68, p<0.02)

MYOMIRNAS IN MUSCLE BIOPSIES



Histograms showing myo-miRNA expression levels in muscle of DM1 patients: as compared to controls, **miRNA-1 and miRNA-133a were significantly decreased. MiRNA-206was significantly elevated.**

SERUM MYOMIRNAS IN DM1 PATIENTS BEFORE (T0) AND AFTER (T1) PHYSICAL REHABILITATION



Histograms showing the levels myomiRNAs in serum of DM1 patients before (T0) and after (T1) physical rehabilitation.

VALUE OF THE OUTCOME MEASURES RECORDED AT BASELINE AND FOLLOW-UP

Table 1

		Participants							
		Participant 1		Participant 2		Participant 3		Participant 4	
	Outcome Measures	Baseline	Follow-up	Baseline	Follow-up	Baseline	Follow-up	Baseline	Follow-up
FES group	MRC quadriceps (R/L)	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
	MRC hamstrings (R/L)	5/5	5/5	4/4	4+/4+	5/5	5/5	4−/4−	4/4
	MRC tibialis anterior (R/L)	3−/3−	3/3	3+/3+	4/4	3−/3−	3+/3+	2/2	3−/3−
	MRC gastrocnemius (R/L)	5/5	5/5	3+/4	4+/4+	5/5	5/5	4/4	4/4
	6MWT (meters)	487	535	190	290	425	490	290	312
	10MWT (seconds)	7	6	14	11	6	7	11	10
		Participant 5		Participant 6		Participant 7		Participant 8	
		Baseline	Follow-up	Baseline	Follow-up	Baseline	Follow-up	Baseline	Follow-up
Resistance aerobic group	MRC quadriceps (R/L)	5/5	5/5	5/5	5/5	4+/4+	4+/4+	5/5	5/5
	MRC hamstrings (R/L)	3/3	3/3	4+/4	5/4	4/4	4/4	4−/4−	4−/4−
	MRC tibialis anterior (R/L)	3/3	3/3	3+/4	3+/4	2−/2−	2−/2−	3+/3+	4/4
	MRC gastrocnemius (R/L)	4/4	4/4	5/5	5/5	4/4	4/4	5/5	5/5
	6MWT (meters)	275	285	500	575	92	125	273	315
	10MWT (seconds)	14	9	8	6	32	26	6,5	6,46

R, right; L, left; 6MWT, 6-minute walking test; 10MWT, timed 10-m walk test.

FES Group showed an improvement of muscle strength at the end of training (increment of the score in the MRC Scale, 6 MWT, reduction time to walk 10 m. Resistance-Aerobic Group shoewed increase in MRC score, improvement of 6 MWT. A larger effect of resistance-aerobic training in reducing the time to cover 10 m was founf as compared to FES treatment.

We observed **down-regulation** of all myomiRNAs studied after 6/8 weeks of rehabilitation treatment, **significant for miR-206 and miR-133a.**

We also recorded outcome measures at baseline and follow-up, we showed an improvement of muscle strength at the end of training: increment of the score in MRC scale, 6 MWT, reduction time to walk 10 meters.

Conclusion

This study validates the clinical use of microRNAs after the first discovery in DM1 (3). In our investigations in muscle and serum, some microRNA (miR-1, miR-133a, miR-133b, miR-206) appear promising in detecting changes in DM1 in natural history and during rehabilitation. They correlate with functional outcomes, we found that reversal of muscle atrophy in DM1 might be revealed by decreased microRNA levels.

Bibliography:

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