**SUPPLEMENTAL DATA: MATERIALS AND METHODS**

**Participants**

Six patients and six controls were recruited for this study. The sample size was limited due to both the very low prevalence of this disorder and the small number of patients who agreed to participate in this study. The patient inclusion criteria were to have confirmed DM1 diagnosis and a complete medical history and follow-up in the neurology department of the aforementioned hospital. Eligibility criteria for the six controls recruited for this study were to have no personal or family history of neuromuscular diseases and to be free of any other chronic disease. Patient muscle biopsies were obtained from the left *Biceps brachialis* (n = 5) and *Vastus lateralis* muscle (n = 1) in the neurology unit or in the ambulatory surgery rooms of our hospital during 2015 and 2016. Controls were healthy individuals – recruited from the Traumatology department, where they had previously undergone minor surgery. The control biopsies were obtained from the hand muscle *Abductor pollicis longus*.

**Clinical data**

We obtained – and updated – clinical information of DM1 patients from in the last visit’s medical records. We also reviewed the last ophthalmological, cardiological and respiratory examination by the corresponding specialists, including the electrocardiograms, echocardiograms and spirometry tests performed in the last year. We assessed the strength of the muscle – *Biceps brachialis* – used for the biopsy in the majority of patients (5 of 6) with the manual Medical Research Council (MRC) scale. We also assessed myotonia, by quantifying the relaxation time – defined as the time from the moment a muscle stops contracting until it relaxes completely, using a new method. In brief, we used an elastic goniometer to calculate the angle of separation between the middle phalanx of the index finger and the metacarpal of the same finger of the dominant hand. Once the goniometer was placed, the patient was asked to perform a maximal contraction in the handgrip (Jammar; Duluth, MN) for 2-4 seconds, and thereafter to relax back to the basal position (hand extension) as fast as possible. This procedure was repeated at least 5 times and an average value of the relaxation time (in seconds) was obtained. We assessed muscular impairment using the Muscular Impairment Rating scale (MIRS), and evaluated functional status and disability with the 6-minute walking distance (6MWD) test and the modified Rankin Scale (mRS), respectively.

**Myoblast cultures**

We isolated muscle cells were from muscle tissue by biopsy explants on culture plates treated with human plasma and gelatin 1.5% (1:2). We performed the cultures with DMEM supplemented with 15% of fetal bovine serum, 22% M-199, PSF 1x, insulin 1.74 μM, L-glutamine 2 mM, FGF 1.39 nM and EGF 0.135 mM. We purified myoblasts through CD56 magnetic beads according to manufacturer’s instructions (Miltenyi Biotec; Bergisch Gladbach, Germany), and they were further grown on pre-coated surfaces with 0.1% gelatin until 60-70% of confluence was achieved. Myoblasts derived from all DM1 patients and controls were grown simultaneously and plated on coverslips for immune studies. We collected muscle cells in pellets at the same passage of coverslips (from passages 4 to 6), which were frozen at -80°C for further DNA and RNA analysis.

**Expansion repeat sizing in myoblast**

To measure the expansion size in the myoblast cell lines, we isolated genomic DNA from myoblasts pellets. Briefly, we incubated cell pellets for 16 h at 37℃ with 20 mg/mL proteinase K, 1.2 mg/mL Tris-HCl pH 8.0, 0.6 mg/mL NaCl, 7.3 mg/mL EDTA and 10% SDS. The following day, we added 5.5M NaCl before ethanol precipitation. We quantified isolated DNA with Nanodrop ND-1000 (Themo Fisher Scientific; Waltham, MA).

We used long polymerase chain reaction (PCR)-southern blotting to determine the CTG repeat number in cell myoblasts. We amplified genomic DNA from myoblasts in three replicate reactions of 5 ng/μL, 30 ng/μL and 100 ng/μL. We used the primers MDY1D-F GCTCGAAGGGTCCTTGTAGCCG and DM1-rev GTTCCATCCTCCACGCAC. The set conditions of the long PCR were as follows: initial denaturation at 94°C for 4 min, followed by 35 cycles of denaturation at 94°C for 30 s and annealing-extension at 66°C for 8 min. We performed final extension at 65°C for 10 min. We electrophoresed PCR products in a 0.8% agarose gel at 80V for 900 min. We washed the agarose gel with acid solution (250 mM HCl) for 15 min, basic solution (0.5 M NaOH) for 30 min, and neuter solution (0.5 M Tris-HCl, pH=7.5, 1.5 M NaCl) for 30 min. Thereafter, we transferred the DNA in the gel through capillary electrophoresis to a nylon membrane (Roche, Basel, Switzerland). We fixed DNA to the membrane by heating the latter at 65ºC during 75 min. We used a concentration of 10 pmol/mL DIG-labeled LNA probe (5’-gcAgCagcAgCagCagcAgca-3’, where lower and upper-case letters represent unmodified and LNA nucleotides, respectively) to hybridize the membrane for 3 h at 70°C. We detected expansions through chemiluminescence of alkaline phosphatase-conjugated anti-DIG antibody and CDP-Star substrate, following manufacturer's instructions (Roche; Basel, Switzerland). To determine the CTG repeat number, we considered the most abundant band or the most intense part of the smear for diffuse bands, presumably corresponding to the most representative number of repeats present in the myoblasts.

**3D imaging of RNA foci and MBNL1**

To detect RNA foci and MBNL1, we performed fluorescence in situ hybridization (FISH) and immunostaining. In brief, coverslips containing 60-70% of confluent myoblasts were fixed with 4% PFA and permeabilized with 0.3% Triton X/PBS for 10 min at room temperature (RT). Thereafter we placed them into a humidified chamber and incubated them overnight at 37°C in hybridization buffer: Cy3-(CAG)10 probe 0.01 µM, 30% formamide, 10% dextran sulfate, 2mM of vanadyl, 0.02% BSA and 2X SSC. The following day, we washed coverslips three times with 30% formamide/2X SSC for 3 min at 45°C and two times with 1X SSC for three min at 37°C, and did a final wash with 1x PBS for 3 min at RT. Thereafter, we blocked coverslips with 1% goat serum for 1 h in a humidified chamber at RT and thereafter incubated them with the primary anti-MBNL1 antibody mouse 3A4 (sc47740, Santa Cruz Biotechnology; Dallas, TX) 1:100 dilution at 4°C overnight in a humidified chamber. After three washes with PBS, we incubated the cells with secondary antibody anti-mouse Alexa 488 (A11001, Invitrogen by Thermo Fisher Scientific) and diluted then in 1% goat serum/PBS (1/500) for 45 min at RT. After incubation, we washed the cells three times with PBS and mounted them using ProLong Gold antifade reagent with DAPI (Invitrogen; Carlsbad, CA).

We studied colocalization of RNA foci and MBNL1 using a Zeiss LSM 710 confocal microscope (Jena, Germany) equipped with a 63×/1.4 NA oil immersion objective. We acquired image Z-stacks for each channel sequentially with the following parameters: *i)* pinhole size: 1 Airy; *ii)* XY pixel size: 130 nm; and *iii)* Z pixel size: 300 nm. We analyzed a minimum of 10 myoblasts per cell line and took Z-stack images with an interval of 0.3 µm, covering the total thickness of myoblasts within a 6.3–8 µm range. After their acquisition, we processed the images (cropping and thresholding) using Fiji (ImageJ distribution) software. We generated isosurfaces and 3D video animation using Imaris and Zen Black program, respectively.

**Alternative splicing analysis and DMPK expression**

We extracted RNA from myoblast pellets using TRIzol™ Reagent (Thermofisher), following the manufacturer’s instructions. We treated RNA with *DNAse I* and converted it to cDNA using High-Capacity cDNA Reverse Transcription Kit (ThermoFisher). We analyzed DMPK expression with quantitative PCR (qPCR) using Taqman Fast Advanced Mastermix and the following custom Taqman assay (ThermoFisher): forward AGCCTGAGCCGGGAGATG, reverse GCGTAGTTGACTGGCGAAGTT and probe AGGCCATCCGCACGGACAACC (2). We analyzed the results with the 2-ΔΔCT method using human large ribosomal protein (RPLPO) as endogenous control. We analyzed alternative splicing of MBNL1, INSR and ATP2A1 with reverse transcript (RT)-PCR. PCR For RT-PCR analysis, we kept annealing temperature at 58ºC for 50 cycles and used the following primers: MBNL1 forward GCCCAATACCAGGTCAACCA (3), MBNL1 reverse GGCCTCTTTGGTAATGGGGG (3), INSR forward GAGCTGGAGGAGTCCTCGTTTAG, INRS reverse TCGATGCGATAGCCCGTGAAG, ATP2A1 forward CTCATGGTCCTCAAGATCTCAC (4) and ATP2A1 reverse AGCTCTGCCTGAAGATGTGTCAC (4). We tested the amplified products in a 3% high-resolution agarose gel and quantified bands with ImageJ. We calculated the percentage of exon inclusion and exclusion isoforms as follows:

$$\frac{exon eclusion OR inclusion band}{(exon exclusion + exon inclusion band)}×100$$

**Statistical analysis**

We analyzed the relationship between molecular (3D) and clinical data using Pearson’s correlations after checking normality with the Kolmogorov-Smirnov test. We applied the Mann Whitney’s *U* test to compare expression and alternative splicing profiles in DM1 vs control myoblasts. We performed all statistical analyses with the Graphpad Prism 5 software, with the level of significance set at 0.05.

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