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ORIGINAL ARTICLES

# Predictors of prognosis in type 1 myotonic dystrophy (DM1): longitudinal 18-years experience from a single center

Marco Mazzoli[1], Alessandra Ariatti[1], Gian Carlo Garuti[2], Virginia Agnoletto[3], Maurilio Genovese[4], Manuela Gozzi[5], Shaniko Kaleci[6], Alessandro Marchioni[7], Marcella Malagoli[4], Giuliana Galassi[1]

*[1] Department of Biomedical, Metabolic and Neural Sciences, University Hospitals of Modena, Italy; [2] Respiratory Unit, Mirandola Hospital, Italy; Cardiology Unit, University Hospitals of Modena, Italy; [4] Neuroradiology Unit, University Hospitals of Modena, Italy; [5] Radiology Unit, University Hospitals of Modena, Italy; [6] Department of Surgical, Medical, Dental and Morphological Science with Interest in Transplant, Oncological and Regenerative Medicine, University of Modena and Reggio Emilia, Italy; [7] Respiratory Diseases Unit, Department of Medical and Surgical Sciences, University Hospitals of Modena, Italy*

**The aim of the study was to identify possible predictors of neurological worsening and need of non-invasive ventilation (NIV) in individuals affected by myotonic dystrophy type 1 (DM1), the most common form of adult-onset muscular dystrophy.**

**Methods. A retrospective observational cohort study was undertaken. Thirty-three patients with genetic diagnosis of DM1 were followed at our Neuromuscular unit in Modena. Abnormal trinucleotide repeat (CTG) expansion of dystrophy protein kinase gene (MDPK) on chromosome 19q 13.3 was the prerequisite for inclusion. The number of CTG repeats was determined. All the participants were older than 14 at the time of enrolment, therefore they could be included into the juvenile or adult form of the disease. Participants were neurologically evaluated every 6-8 months up to 18 years. Neurological impairment was assessed by Muscular Impairment Rating (MIRS), Medical Research Council (MRC), and modified Rankin (mRS) scales. The independent variables considered for prognosis were age at first evaluation, duration of the disease, CTG repeat number, gender, and presence of cardiac and vascular morbidities.**

**Male patients were 51.5% and female patients 48.5%. Sixteen patients were younger than the mean age of 30.1 years, while the remaining 17 were up to 65. Twelve subjects (36.4%) underwent NIV before the end of follow-up. Muscle force and disability scores showed statistically significant deterioration ($p < 0.001$) during follow-up. The worsening was significantly higher among patients carrying higher number of CTG repeats and of younger age. The presence of cardio-vascular involvement has significant impact on neurological and respiratory progression.**

**Neurological worsening is predicted by CTG expansion size, young age and presence of cardio-vascular morbidities.**

**Key words:** myotonic dystrophy type I, muscular impairment rating scale, CTG trinucleotide repeat

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**Correspondence**
Giuliana Galassi
Department of Biomedical, Metabolic, Neural Sciences, University Hospitals of Modena, via P. Giardini 1355, Modena, Italy. E-mail: giulianagalassi@alice.it

**Conflict of interest**
The Authors declare no conflict of interest

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## Introduction

Myotonic dystrophy type 1 (DM1) is the most common form of adult-onset muscular dystrophy with a prevalence in Europe of 3-15/100.000 inhabitants [1-12]. DM1 is caused by an unstable expansion of the cytosine thymine-guanine (CTG) trinucleotide repeat located in the 3′UTR of *DMPK* gene, chromosome 19q13.3, encoding a serine/threonine protein kinase (DMPK) trinucleotide [8-19]. The disease is transmitted across generations in an autosomal dominant fashion with incomplete penetrance, variable phenotypic expression and somatic mosaicism [1,3,6,8-12,19-21]. Anticipation, i.e. increased number of CTG repeats in subsequent generations, is associated with increased severity of disease, more marked with maternal transmission [1,3,5,8,11,12,21-24]. The disorder is multisystemic and affects muscles and central nervous, ocular, respiratory, heart, digestive, endocrine systems in relation to the variable number of triple repeats in each organ [2-5,7-12,24-30]. Regular cardiological and ventilatory assessment is important in DM1, as respiratory failure accounts for almost 40% of mortality at an average age of 53 years [4,24,29-37]. The introduction of non-invasive ventilation (NIV) combined with treatments for cardiac manifestations have improved survival in DM1 [4,30-32]. Due to the broad clinical spectrum, DM1 subjects can be classified into five clinical forms according to age of onset of first symptoms: congenital, infantile, juvenile, adult, and late onset [24-26]. The progression rate of muscle strength loss in different phenotypes (juvenile, adult and late onset) needs to be documented and has an impact on patient prognosis. Our study was aimed at identifying predictors of neurologic and respiratory impairment in an Italian cohort of patients affected by DM1, followed longitudinally up to 18 years.

## Patients and methods

*Setting and study population*

We conducted a retrospective observational study in a single neuromuscular disease center (*Center for Neuromuscular Diseases, University Hospital of Modena, Italy*). Inclusion criteria were as follows: diagnosis of DM1 attested by clinical evaluation and molecular genetic testing. No patients at the time of enrolment had end-stage lung diseases (chronic obstructive pulmonary disease, interstitial lung disease, severe kyphoscoliosis and disorders of the chest walls). Between January 1st, 2000 and December 2018, 33 patients with clinical diagnosis of DM1 were included according to genetic diagnosis. All patients enrolled showed an abnormal expansion of CTG in the 3′ untranslated region of the Myotonic Dystrophy Protein Kinase (MDPK) assessed by long-PCR and Southern blot analysis gene (MIM #160900) The study design was approved by the local Ethical Committee (N°325/2019). A multidisciplinary team including neurologists, pulmonologists and cardiologists, evaluated the subjects during a follow-up which lasted from the first to the last visit or to death. Patients were followed for at least 24 months.

*Predictive variables and assessments*

Our genetic reference laboratory defined subjects as E1 with number of repeats less than 200, E2 from 201 to 699 repeats, and E3 from 700 and larger. All participants were older than 14 at the time of enrollment, therefore with juvenile or adult form of the disease [24-27]. Patients were further classified according to onset of symptoms at an age above or below 30.1 years, which was the mean in our cohort. Clinical onset in patients with very mild/asymptomatic disease was set at the date of their first clinical/genetic diagnosis or it was retrieved from previous medical history. For the specific purpose of the study, the following data were included: muscle impairment and disability scores, gender, serum creatine kinase (CK) titer assessed at least once per year, and electromyographical changes. In addition, the presence or absence of multisystemic involvement such as cardiac, vascular, endocrine, ophthalmological and hematological disorders was assessed at the time of enrollment and in subsequent follow-up. Each neurologist collected the informations at the time of enrollment on presence/absence of the above mentioned conditions.

Muscle strength was determined with manual muscle testing (MMT) by three neurologists (GG, AA, MM) using Medical Research Council Scale (MRC) in six muscles for each limb in upper (UE) and lower (LE) extremities: deltoid, biceps brachii, extensor digitorum communis, ileopsoas, quadriceps and anterior tibial. The maximal score for each muscle ranged between 0 and 5, and the total score obtained was 60. Disability was measured with the Rankin Scale (mRS) (range 0-5). Muscular Impairment Rating Scale (MIRS), a validated DM1-specific rating scale, was also used [9,25-27].

Respiratory assessments included arterial blood gas analysis and pulmonary function tests (PFTs) with evaluation of forced vital capacity (FVC), forced expiratory volume in 1 sec (FEV1) and FEV1/FVC (Tiffeneau index) performed at the time of enrollment and at every follow-up visit. Arterial blood gases were measured in samples drawn at rest from the radial artery. Arterial oxygen partial pressure (PaO2) and arterial carbon dioxide partial pressure (PaCO2) were determined within 10 minutes of sampling. Hypercapnia was defined as PaCO2 > 45 mmHg and hypoxemia as PaO2 < 80 mmHg 3,29-31. NIV (meaning the use for more than 4 hours/daily) included initiation of Nocturnal Positive Airway Pressure (NPAP), either continuous (CPAP) or bilevel (BPAP) 31.

NIV was planned by a pulmonologist in the presence of symptoms suggestive of chronic respiratory failure (dyspnea at rest, daytime hypersomnolence, orthopnea), plus at least one of the following criteria: FVC less than 50% predicted in seated position, apnea/hypopnea index greater than 15 events/hour, nocturnal arterial saturation less than 88% for more than 2 cumulative minutes, arterial pH < 7.35 and PaCO2 > 45 mmHg [2,4,31-33,35].

Cardiological evaluations included clinical assessment, basal blood pressure, ECG, 24h ECG Holter monitoring, and trans-thoracic echocardiography. Impulse conduction abnormalities and arrhythmias on a standard ECG, including sinus rate < 50 BPM, PR interval > 200 ms, QRS duration > 100 ms, left anterior or posterior fascicular block, abnormal Q waves, atrial tachycardia and fibrillation (AF) or flutter were considered indicative of cardiac involvement. We measured systolic left ventricular dysfunction as reduction of ejection fraction (EF) below 50% [4,22,29,30,38,39].

Only 4 cases could be studied with 1.5T Magnetic Resonance Imaging (MRI) scan of limb girdle, thigh and lower leg to assess muscular hypotrophy or atrophy, muscle edema and fat replacement on T1, T2 and on short time inversion recovery (STIR) sequences with fat suppression. After clinical indication, a brain neuroradiological study either with CT or MRI was obtained or reviewed in 17 subjects at the median age of 44.5 years (range 20-70, IQR 23.7). The retrospective quantitative evaluation of available images was carried out by two neuroradiologists looking for structural abnormalities, such as whole brain atrophy, and white matter subcortical and periventricular changes especially in the temporal pole [40]. White matter temporal changes were detected in 31.2% of the subjects. During follow-up, no patients exhibited detectable ischemic lesions.

*End points*

Primary end points of the study were analysis of clinical and genetic parameters associated with significant neurological impairment, defined as worsening in 2 out of the 3 neurological assessment scales applied. A change of ≥ 1 point in MIRS was considered significant, as well as a worsening of ≥ 7 points in total MRC score, of ≥ 4 points in UE MRC score, of ≥ 3 points in LE MRC score and a change of ≥ 1 point in mRS. Secondary outcome was the identification of clinical and genetic parameters associated with functional impairment and need for NIV.

*Statistical analysis*

Statistical analysis was performed using Stata 14.2 (Stata Corporation, College Station, TX, USA). Patient characteristics were analysed using descriptive statistics. Data were presented as median with minimum–maximum range or as mean with standard deviation (SD). For comparison of change between independent groups, Mann-Whitney U test was used for continuous variables and Mantel-Haenszel Chi Square Exact test for ordered categorical variables. The differences in MRC, mRS, and MIRS between baseline and times of follow-up were computed using t-test. Kaplan-Meier method was used to estimate progression with respect to outcomes; curves were compared with Log-rank test to determine any difference in terms of age at the time of onset of end points. Hazard ratio and 95% confidence interval (CI) from Cox proportional hazard regression model were used to estimate the risk of each end point, based on clinical attributes at baseline (i.e. gender, age, CTG expansion, presence of cardiovascular morbidities). The impact of clinical variables on MIRS and NIV requirement was evaluated using a logistic regression model. To improve statistical strength, genotypes based on number of CTG repeats were pooled into two groups that were E1 *vs* E2 plus E3. Clinical factors associated with *P* values below 0.05 in the univariable model were analyzed in a multivariable model with a stepwise forward selection. Missing data on MRC, MIRS, and mRS during follow-up were estimated by multiple imputation method (MI) using either linear or ordinal regression, if appropriate [41]. Variance analysis (ANOVA) and Tukey-Kramer test were used to compare clinical scores at baseline and at 24, 48, 72 and 96 months in patients having different genetic profiles.

## Results

*Patients baseline clinical data*

Demographic characteristics of patients are listed in Table I. Mean duration of follow up was 121.6 months ± 69.4 (median 100 months, range 24-220, IQR 125). Median duration of illness was 237 months (range 24-553, IQR 202). Demographic analysis of DM1 population showed a 1:1 male to female ratio with 17 males (51.5%) and 16 females (48.5%). The age of onset had similar gender distribution. Median age of diagnosis was 31 years (range 14-65). Ten patients (30.3%) had lower number of expansions (E1) whereas those classified as E2 were 39.4%, and E3 were 30.3%.

At baseline, the median total MRC muscle strength assessed with MMT was 48 (range 30-60, IQR 12; mean 48.8, ± 7.74), and 24 (range 18-30 IQR 8; mean 24.4 ± 3.5) in UE and 24 in LE (range 10-30, IQR 6, mean 24.3 ± 4.4). Regarding disability scores, mean MIRS was 3.66 ± 0.88 and mRS was 2.1 ± 1.30. At baseline, muscle strength, either computed as total or separately in UE and LE, and mRS were not significantly different among genders ($p = 0.66$); conversely, MIRS scores in females were

**Table I.** Summary of demographic and clinical characteristics of our patients.

| | | **Overall [33 (100%)]** |
|---|---|---|
| Gender [n (%)] | Male | 17 (51.5%) |
| | Female | 16 (48.5%) |
| Age of onset/diagnosis (y) [median (range)] | | 31 (14-65) |
| | Male | 32 (15-65) |
| | Female | 29.5 (14-53) |
| Duration of follow-up (mo) [median (range)] | | 100 (24-220, IQR 125) |
| Duration of disease (mo) [median (range)] | | 237 (24-553, IQR 202) |
| CTG repeat [n (%)] | E 1 | 10 (30.3%) |
| | E 2 | 13 (39.4%) |
| | E 3 | 10 (30.3%) |
| Death [n (%)] | | 4 (12.1%) |
| | Male [n (%)] | 2/4 (50.0%) |
| | Age (y) [median (range)] | 64 (54-67) |
| Pace maker or ICD [n (%)] | | 5 (15.2%) |
| | Male [n (%)] | 4/5 (80.0%) |
| | Age (y) [median (range)] | 51 (42-68) |
| Systemic involvement [n (%)] | | 30 (90.9%) |

significantly worse than in males (p = 0.03). Abnormal EMG findings were detected in 63.6% of cases, and CK level above 170 IU/L was found in 48.

*Multisystemic involvement*

Multisystemic involvement was detected in 90% of cases. Cataract occurred in 78.8%, while thyroid and endocrine dysfunction requiring treatments in 51.5%. The cardiac abnormalities were detected in 63,6% of cases and included conduction disturbances (66%) namely, prolonged PR interval (A-V block of I°), bradyarrhythmias, II° and III° atrioventricular blocks, AF, ventricular arrhythmias, left ventricular systolic dysfunction with depressed EF and clinical heart failure, and mitral valve prolapse. No ventricular tachycardia was detected. Vascu-

**Table II.** Outcome measures at baseline and at last follow-up in patients according to genotypes.

| | | **Overall** | | ***P overall*** | **Genotype E1** | | ***P*** | ***P basal*** | **Genotypes E2, E3** | | ***P*** | ***P final*** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **Base-line** | **Last f-up** | | **Base-line** | **Last f-up** | | | **Base-line** | **Last f-up** | | |
| EGAa | pCO2 (mmHg) | 41.8 (±6.4) | 43.0 (± 6.8) | 0.15 | 40.4 (± 4.04) | 40.3 (± 3.9) | 0.95 | 0.07 | 42.6 (± 7.1) | 44.2 (± 7.5) | 0.46 | **0.04** |
| | pO2 (mmHg) | 80.8 (±14.0) | 83.5 (± 13.1) | 0.33 | 86.9 (± 10.3) | 86 (± 8.1) | 0.83 | 0.07 | 78.2 (± 14.7) | 82.4 (± 14.8) | 0.33 | 0.24 |
| Spirometry | FVC (% predicted) | 75.4 (±18.5) | 67.2 (± 21.4) | **0.002** | 82.9 (± 7.4) | 78.9 (± 9.8) | 0.31 | **0.03** | 72.1 (± 21.04) | 62.1 (± .1) | 0.13 | **0.006** |
| MRC | Total | 48.8 (±7.74) | 37.8 (± 12.3) | **< 0.001** | 55.4 (± 6.1) | 50.4 (± 10.3) | 0.20 | **0.002** | 46 (± 6.6) | 32.3 (± 8.6) | **< 0.001** | **< 0.001** |
| | Upper limbs | 24.4 (±3.5) | 19.7 (± 5.81) | **< 0.001** | 27.4 (± 3.2) | 25.4 (± 5.0) | 0.30 | **0.003** | 23.2 (± 2.9) | 17.3 (± 4.2) | **< 0.001** | **< 0.001** |
| | Lower limbs | 24.3 (±4.4) | 18 (± 6.81) | **< 0.001** | 28 (± 2.9) | 25 (± 5.3) | 0.13 | **< 0.001** | 22.7 (± 3.9) | 15.1 (± 5.0) | **< 0.001** | **< 0.001** |
| MIRS | | 3.66 (± 0.88) | 4.24 (± 1.0) | **0.001** | 2.9 (± 0.99) | 3.2 (± 1.3) | 0.56 | **< 0.001** | 4 (± 0.6) | 4.6 (± 0.5) | **< 0.001** | **< 0.001** |
| mRS | | 2.1 (± 1.30) | 3.0 (± 1.4) | **0.001** | 1. 1 (± 1.2) | 1.6 (± 1.7) | 0.45 | **0.003** | 2.6 (± 1.0) | 3.6 (± 0.8) | **< 0.001** | **< 0.001** |

EGA: arterial blood gas analysis. pCO2/O2: partial blood pressure of CO2/O2. FVC: forced vital capacity. MRC: Medical Research Council. MIRS: muscular impairment rating scale. mRS: modified Rankin Scale. The values are expressed as mean with standard deviation (SD) in brackets. P overall expresses the significance between baseline and last follow-up for each variable.
P *basal and p final* express respectively the significance of the comparisons between subjects carrying the different genotype at baseline and at the last follow-up for each studied variable. Significance: p < 0.05 two tailed. Significant results are in bold. See text for details.

lar comorbidities included severe hypertension (systolic blood pressure ≥ 180 mmHg and/or diastolic blood pressure ≥ 120 mmHg) and large vessel atherosclerosis; the latter, for statistical purposes, were pooled in the analysis with cardiac abnormalities. Regarding cardiac therapies, patients were treated according to published guidelines with angiotensin II receptor blockers, angiotensin converting enzyme inhibitors, beta-blockers, diuretics, oral anticoagulants, and flecainide, if appropriate [38,39]. Five patients had prophylactic pace-maker (PM) implantation; in 2 cases, an implantable cardioverter defibrillator (ICD) was subsequently applied.

## Predictors of neurological worsening

A statistically significant decline between baseline and last visit was seen in the whole sample for all the clinical outcome measures; 54.5% of subjects showed deterioration in all the scales. A significant decline in global muscle force (total MRC scores) was found in 27 cases (81.8%): loss of strength in LE was found in 78.6% of cases, and 66.6% worsened in UE with no significant difference between UE and LE (p = 0.87 and 0.10, respectively). The worsening occurred at mean age of 43.2 y (± SD 13.2) in LE and of 43.9 y (± SD 13.3) in UE. A worsening in mRS and in MIRS was found in 60.6 and in 63.6% of patients respectively.

*Expansion size*

Table II shows the measures of muscle strength, MIRS and disability scores obtained at baseline and at last follow-up in the whole cohort and in patient groups subdivided according to lower or higher than 200 CTG triplet repeats (i.e. E1 and E2/E3). The significance of clinical and functional scores in the whole cohort *(p overall)* and in those carrying genotype E1 were compared to pooled E2/E3 genotypes and estimated at the beginning and at the end of follow-up. The estimation of MRC, mRS and MIRS gave the following results: at baseline and at last assessment in group E1, neither MRC nor MIRS and disability scores worsened significantly (p > 0.05), whereas in E2/E3 groups the worsening in total MRC and MRC subdivided in UE and LE, in MIRS and mRS was statistically significant (p = 0.002, 0.003 and below 0.001, respectively). We further calculated clinical and functional scores at baseline and at end of follow-up between groups, and we found that groups E1 and E2/E3 were statistically different: basal p and final p expresses the significance of the comparison for each variable between the groups, E1 on one side and E2/E3 on the other.

We calculated with a Cox regression analysis the probability of surviving to worsening on MIRS in patients with different CTG expansion size: Kaplan-Meier curve is shown in Figure 1b (p = 0.016 at Log rank test). The latter result was confirmed by a logistic regression analysis, after adjustment with clinically relevant variables: higher than 200 CTG triplet repeat number had an independent prognostic effect on worsening in MIRS (HR 5.09, CI 1.34-19.36, p = 0.017) (Tab. III). Furthermore, we assessed the influence of genotypes on the probability of surviving to worsening in muscle strength assessed by total MRC, as shown in Fig 1d with Kaplan-Meier curve (p = 0.004 at Log rank). Figure 2a and 2c show the probability of being free from worsening in muscle strength for UE and LE in the three genotypes E1, E2, E3 (p < 0.001 at Log rank), with the number at risk and of survivors belonging to each genotype in relation to age. The box plot in Figure 2d shows MIRS measurements at baseline and at 24, 48, 72 and 96 months; a significant difference was found between genotypes E1 *vs* E2, and E1 *vs* E3 (p < 0.01).

*Baseline age*

Seventeen patients were older than 30.1 years; the oldest subjects carried either genotype E1 (7, 21.2%) or genotype E2 (9, 27.2%), and only 1 (3%) had genotype E3. At baseline, the youngest and the oldest subjects were

**Table III.** Results from univariate and multivariate Cox regression analysis of worsening on MIRS (≥ 1 point).

| **Worsening ≥ 1 point in MIRS** | | | |
|---|---|---|---|
| | **HR** | **95% CI** | *P* |
| Gender (female) | 1.13 | 0.46-2.77 | 0.78 |
| | (0.55) | (0.19-1.53) | (0.25) |
| Age at onset (> 30.1 y) | **0.26** | **0.10-0.68** | **0.006** |
| | **(0.26)** | **(0.09-0.78)** | **(0.01)** |
| CTG repeat number > 200 (i.e E2, E3) | **3.9** | **1.28-11.8** | **0.016** |
| | **(5.09)** | **(1.34-19.36)** | **(0.017)** |

HR: hazard ratio. 95% CI: 95% confidence interval. *p*: p-value. MIRS score. Age at onset > 30.1 years (y). CK: creatine kinase level ≥ upper limit of normal. Genotypes are classified according to CTG repeat number. Adjusted values are reported in brackets. Significance: p < 0.05 two tailed. Significant results are in bold.

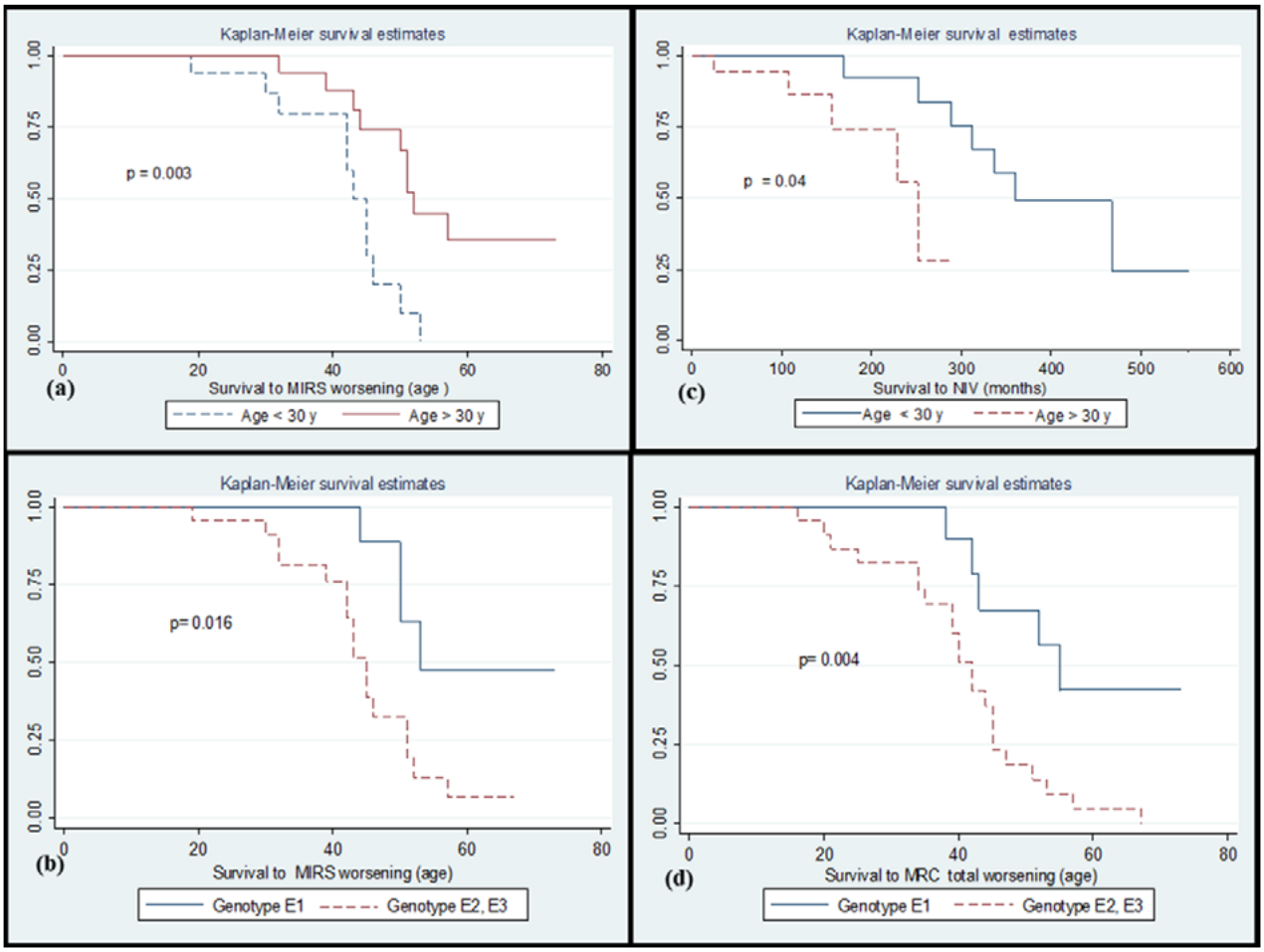


**Figure 1.** Cox analysis and Log rank test. **Fig 1a)** Kaplan-Meier survival curves expressing the probability of worsening in MIRS from age of onset in subjects with age either above or below the mean age $p = 0.003$, at Log rank test; **b)** Kaplan-Meier survival curves expressing the probability of worsening in MIRS from age of onset in genotypes E1 or E2 and E3, $p = 0.016$ at Log rank test; **c)** Kaplan-Meier survival curves expressing the risk of NIV from age of onset in subjects with age above or below the mean age, $p < 0.04$ at Log rank test; **d)** Kaplan-Meier survival curves expressing the probability of worsening in MRC total scores in genotypes E1 or E2 and E3, $p < 0.004$ at Log rank test.

not significantly different as measured by MIRS, mRS and muscle strength both in total ($p > 0.05$) and in UE ($p = 0.1$) and LE ($p = 0.09$).

Univariate Cox proportional hazard analysis showed that worsening in MIRS was significantly predicted by younger age at baseline. Kaplan Meier survival curves in Fig 1a show the probability of worsening on MIRS in subjects aged either above or below the mean of 30.1 years (Log rank test $p = 0.003$). Multivariate Cox regression analysis, as shown in Table III, confirmed lower risk of progression (HR 0.26, CI 0.09-0.78, $p = 0.01$) in the oldest patients. This result was further validated by a logistic model with multiple imputed data, where old age of onset (OR 0.30, CI 0.15-0.59, $p < 0.001$) and female gender (OR 0.15, CI 0.075-0.30, $p = 0.001$) showed a protective effect towards the risk of worsening on MIRS (Tab. IV).

*Gender*

At the end of follow-up, muscle force in UE was diminished by 22% in male patients versus 17% in females. In LE, the decrease from baseline was 29% in males versus 23% in females. MIRS scores worsened by 17% in males versus 10% in females, and mRS decline reached 50% in males versus 32% in females. However, the proportion of worsening was never statistically significant between genders, and males did not differ from females in assessment scale scores at the end of follow-up (data not shown).

*Cardiac and vascular involvement*

In our cohort, during follow-up, cardiac involvement occurred in 75% of patients aged below 30.1 years *vs*

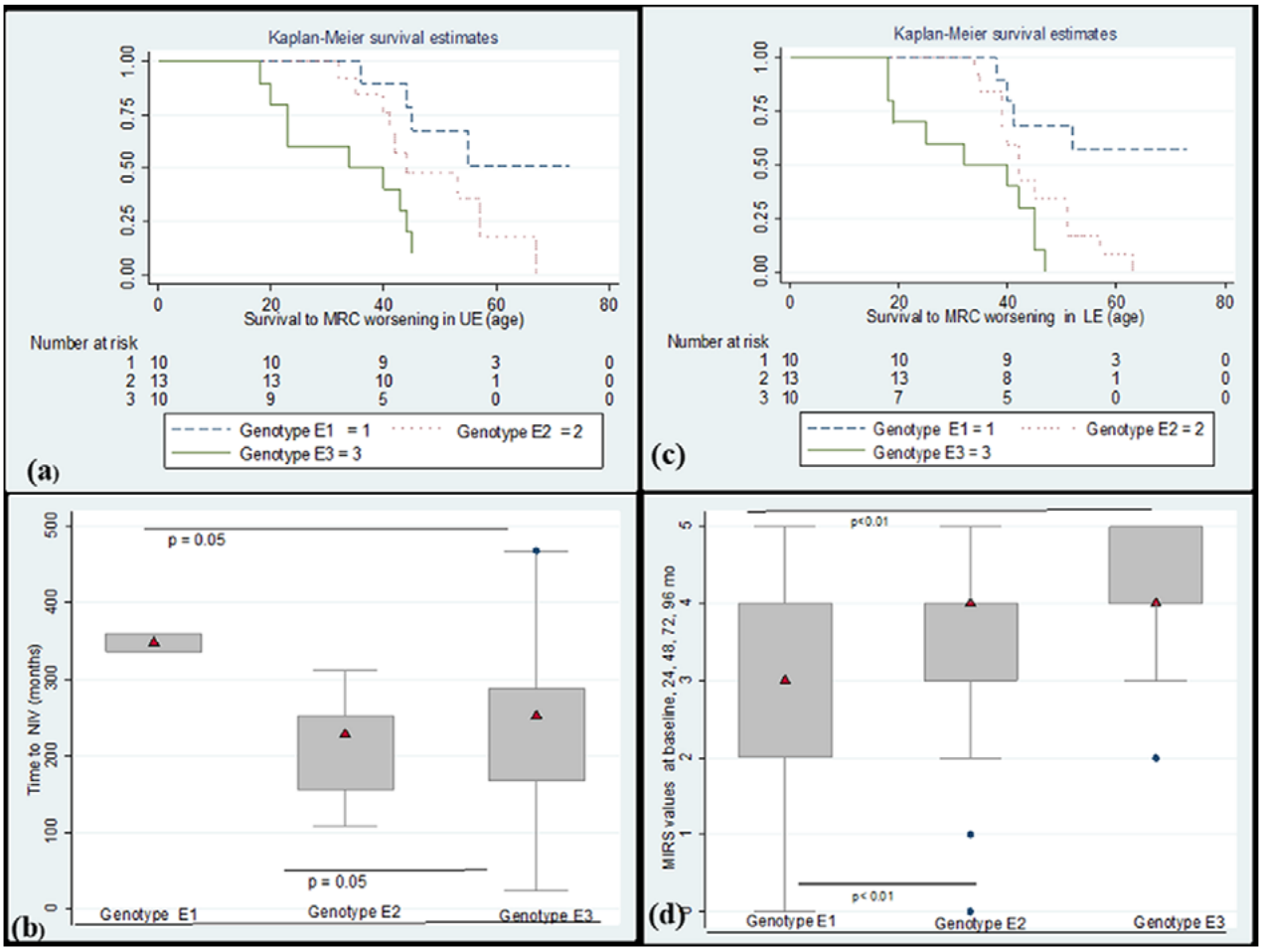


**Figure 2.** Cox analysis and Log rank test. **a) and c)** Kaplan-Meier survival curves expressing the probability of being free of worsening in strength, assessed by MRC in UE and in LE in the three genotypes. For both analysis $p < 0.001$, at Log rank test; **Anova model: b)** Box plot showing the time to NIV in months in the three genotypes. The boxes represent 25th, 50th and 75th percentiles. The diamond inside each box shows the median and the wriskers shows the range. Two comparaisons highlighted with lines are significant at TK comp test; **d)** Box plot showing the changes in MIRS at baseline and during follow-up at 24, 48, 72 and 96 months since onset in patients carrying different genotypes. The diamond inside each box shows the median and the wriskers show the range. Two comparisons highlighted with the lines are significant; p-value < 0.01.

52.9% in older patients, without statistically significant difference between groups.

The presence of cardiac and vascular morbidities pooled together confirmed at 8-years' follow-up a significant impact on worsening in MIRS scores (OR 3.57, CI 2.00 -6.37, p < 0.002) (Tab. IV).

## Progression to NIV

NIV, either CPAP or BIPAP, was initiated during follow-up in 12 cases (36.4%) with equal occurrence between genders. The median age at NIV start was 50.5 years (Range 33-54, IQR12.5; mean 48.0 ± 9.41) and the median time from onset/diagnosis to NIV was 195 months (Range 24- 553, IQR 200; mean 214.9 ± 130.1).

*Expansion size and need for NIV*

Median time to NIV in genotype E1 was 348 months *versus* 240 months in genotype E3. The times to start NIV differed significantly among genotypes E1 *vs* E3, and E2 *vs* E3 (p < 0.05) as shown in Figure 2b. Regarding blood gas analysis, only pCO2 on last assessment differed in E2 and E3 group from basal pCO2 (p = 0.04), whereas among E1 patients the worsening was not significant (Tab. II). A significant difference between FVC value was detected between group E1, E2 and E3 already at baseline

(p basal = 0.03), and more obviously on last assessment (p = 0.006), confirming that FVC is a reliable indicator of progression [31].

*Influence of baseline age, gender and cardiac involvement on the need for NIV*

Among the 12 patients who received NIV, 41% (5) were older and 58% (7) younger than mean age. The median survival time to NIV was 324 months (Range 88-553) in the youngest, and 123 months (range 24-288) in the oldest subjects. Kaplan-Meier curve (Fig. 1c) indicated that the age of onset influenced survival to NIV: younger patients showed longer survival, but higher risk of NIV requirement, as demonstrated in univariate (HR 4.96, CI 1.07-22.8, p = 0.04) and multivariate Cox regression analysis (HR 7.54, CI 1.43-39.7, p = 0.01)(unshown results).

Regarding gender, the median time to NIV initiation did not show a statistically significant difference: 187 months in males (range 55-553), and 217 months in females (range 24-362).

The presence of cardiac involvement was independently associated with an increased risk of NIV requirement at 96 months, as resulted in MI data analysis (OR 11.5, CI 1.17-113.7, p = 0.03) (Tab. IV).

## Discussion

In the present study we describe the effect of clinical and genetic parameters on neurological and respiratory outcome in a group of DM1 patients followed-up to 18 years.

Knowledge about the progression of strength decline is crucial in DM1 [1-4,9,24-27]. Only a few longitudinal studies of DM1 have documented quantitatively the decline of muscle strength with repeated measurements [9,25-27,42]. We demonstrated a significant worsening at the end of follow-up in all selected clinical outcome measures. Regarding the progression in the distribution of strength loss, we could not find a statistically significant difference of decline in UE as compared to LE. This result differs from that obtained by other authors [1,9,25,27] and could be due to our sample size, to the key muscle group chosen, or to bias related to the measurement method. Interestingly, we recorded a difference in strength of UE and LE limbs among ventilated as compared to non-ventilated subjects (p = 0.01 and 0.02, respectively).

*Role of expansion size*

Although caution should be kept in using CTG repeat size to predict future symptoms, there is reliable consensus that patients with small CTG expansions generally have milder symptoms, whereas increased expansion size is broadly associated with earlier disease onset and increased clinical severity [1,4,8,9-12,24,27,30,31]. Vivekanand et al. [31] suggested a cumulative influence on disease severity of size of trinucleotide repeat and length of patient exposure to the expanded CTG, i.e. their age at presentation.

In our study, we confirm a significant association between worsening in all outcome measures and expansion size, as shown by survival graphs in Figure 1b and 1d. Furthermore, patients with high number of CTG repeats showed lower muscle strength as well as worse disability scores at baseline. Moreover, we found a statistically significant difference in extremity strength bewteen ventilated and non-ventilated subjects, meaning that respiratory impairment is linked to strength loss in the limbs [33]. Rossi et al. [30] concluded that CTG expansion is an independent predictor of respiratory restriction, and Boussaïd et al. [2,3] found that a higher CTG repeat number was associated with larger decreases over time in PFTs. Hawkins et al. [43] highlighted the issue of central versus peripheral respiratory control, where alveolar hypoventilation could be partially related to respiratory muscle weakness, and partially to involvement of the respiratory center in the brain stem, causing chronic hypercapnia. On the contrary, Thil

**Table IV.** Results from univariate and multivariate logistic regression analysis expressing the risk of NIV and of worsening on MIRS (≥ 1 point) as outcome.

| | NIV | | | Worsening on MIRS (≥ 1 point) | | |
|---|---|---|---|---|---|---|
| | **OR** | **95% CI** | ***P*** | **OR** | **95% CI** | ***P*** |
| | 1.1 | 0.26-4.54 | 0.89 | **0.35** | **0.20-0.59** | **< 0.001** |
| Gender (female) | (0.71) | (0.13-3.74) | 0.69 | **(0.15)** | **(0.075-0.30)** | **(< 0.001)** |
| | 0.53 | 0.12-2.2 | 0.39 | **0.34** | **0.10-0.60** | **< 0.001** |
| Age at onset (> 30.1 y) | (0.89) | (0.16-4.72) | 0.89 | **(0.30)** | **(0.15-0.59)** | **(0.001)** |
| | **12.1** | **1.31-111.2** | **0.028** | **3.73** | **2.15-6.48** | **< 0.001** |
| Cardiac /vascular comorbidities | **(11.5)** | **(1.17-113.7)** | **(0.03)** | **(3.57)** | **(2.00-6.37)** | **(< 0.002)** |

OR: odds ratio. 95% CI: 95% confidence interval. In brackets, adjusted results.
The last three columns display results obtained at 8-years'of follow-up with multiple imputation method (MI). (see methods for details) p significant < 0.05 two tailed. Significant results *are* in bold.

et al. [44] found no association between longitudinal lung function impairment and number of CTG repeats or blood gas analysis. Our results on respiratory impairment confirm that CTG expansion size showed a significant effect on respiratory impairment and NIV need.

*Role of baseline age*

Different clinical phenotypes are recognized according to age of onset. De Antonio et al. [24] provided strong evidence for a disease classification model based on five clinical forms; their data demonstrated that all forms of DM1 differ in terms of CTG expansion size, frequency of symptoms and age of onset of disease manifestations. In our cohort, all the patients were older than 14 years at the time of enrollment, therefore they could be included into juvenile or adult form of the disease. We made the choice of splitting our sample into two groups, based on age of onset. Our patients, either younger or older than mean age, were not different at baseline in MIRS and muscle strength scores. However, as shown by the survival curve (Fig. 1a), we found an expected effect of younger age on progression: longer survival to MIRS worsening in terms of age for older patients as compared to younger ones. Regarding NIV requirement as end point, youngest subjects exhibited higher risk of occurrence, but longer survival to the event.

*Effect of gender*

At baseline neither muscle strength measures nor disability scores significantly differed between genders, except for MIRS where females were significantly worse than males at baseline. The latter finding is interesting and could be due to an uneven prevalence of some clinical signs in males and females. According to Dogan et al. [8], men more frequently exhibit myotonia, cardiac signs, restrictive syndrome and muscle weakness, whereas women more often show cataracts, dysphagia, digestive tract dysfunction, thyroid disorders and obesity.

At the end of follow-up the change in strength, MIRS and disability scores did not differ statistically between genders; however, at 8-years of follow-up, the risk of worsening in MIRS was significantly lower in females, suggesting a different rate of progression. Gagnon et al. [25] documented, in a 9-year study on 100 DM1 subjects, a higher loss in distal muscle groups with a decrease over 50%. This significant loss of strength was found for both men and women separately; however, men had greater strength decline over time than women for all muscle groups, suggesting that the stronger the participants were at baseline, the more important was the strength loss at 9 years. Hammaren et al. [26] pooled results from different clinical phenotypes in a 5-years' study on 43 patients with assessment only of lower limb strength: their female patients showed a statistically significant change at five years in hip flexors (-1.3%), whereas in males there was a significant decrease in all examined muscle groups. Mathieu et al. [42], in 50 DM1 subjects studied cross-sectionally showed a decrease of 1.2-1.6% per year for proximal muscle groups and of 2.0-3.0% per year for distal muscle groups with no significant difference between genders (0.99% in females and 1.54% in males per year).

*Cardiac and vascular involvement*

Cardiac complications are the second leading cause of death in DM1 [4,5,8,22,28, 30,31,37,45-47]. Overall, the cardiac phenotype of DM1 is complex and includes an approximately three-fold higher risk of sudden cardiac death compared to age-matched healthy controls.[29,30] Metabolic abnormalities, including diabetes mellitus, hypertension, atherosclerosis, hyperlipidemia are known risk factors of anticipated mortality in DM1 [4]. Moreover, an autonomic nervous system dysfunction, diagnosed on the basis of heart rate variability and increased temporal dispersion of myocardial repolarization argues in favor of a heart involvement in DM1 that might go beyond the known conduction system involvement [4]. Cardiac arrhythmias are common in DM1 [4,29,46] and have been shown by Kaminsky et al. [29] to have a broad correlation in severity with age, muscular impairment, male gender, lung involvement and extent of the molecular defect.

Cardiac involvement in DM1patients occurs as a degenerative process, with progressive fibrosis and fatty replacement of the myocardium, which involves not only the specialized conduction system, but also other areas, initially unaffected, of the working atrial and ventricular myocardium [46,47]. As pointed out by Russo et al. [47] this pathologic substrate facilitates conduction disorders and systolic dysfunction in populations with DM1. Several clinical, metabolic and endocrine features of DM1 phenotype usually affect the elderly, and signs of accelerated aging in DM1 are cardiac conduction disturbance and endocrine abnormalities, as glucose intolerance and dyslipidemia [29]. Cardiac symptoms and signs and other vascular morbidities in our patients had an incidence similar to previous reports [4,7,29,30]. Another interesting finding to be further investigated was the increased risk for NIV requirement in patients with cardiac involvement, possibly reflecting the interrelated multisystem nature of the disease [29,30].

## Limitations of the study

Inherent limitations of this study lie in the design based on a retrospective analysis of clinical medical records although most of the patients were followed pro-

spectively at our Center since the time of the diagnosis for a very long longitudinal care. A further limitation might the small sample size which is due the fact that we included only subjects with a diagnosis confirmed by genetic molecular testing in the subjects. However, our data are comprehensive and repeatidly validated by three neurologists

## Conclusions

By concluding, large CTG expansions and age of disease onset were significantly associated with neurologic and respiratory impairment during a follow-up period of more than 24 months. Moreover, our study suggests that females showed lower relative risk of neurological worsening at 8 years. Finally, multisystem involvement, especially through cardiac diseases, exerts a significant negative effect on neurological progression and respiratory impairment. These results should be confirmed in prospective trials on a larger population of DM1 patients**.**

In a clinical setting, the management of DM1 patients should be carried out with a multidisciplinary approach which includes a periodic cardiac and respiratory assessment [4,29-31,46,47,51-54]. Particular attention should be paid especially to those patients who carry large CTG expansions.

*Disclosure statements*

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The study was approved by the Ethic Committee AVEN of Modena.

Informed Consent was obtained from the patients involved in the study.

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