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(Myopathies, Cardiomyopathies and Neuromyopathies)

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*Founders: Giovanni Nigro and Lucia Ines Comi*

Three-monthly



G. CONTE ACADEMY - NAPOLI

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ORIGINAL ARTICLES

# AUTOMA: a wearable device to assess the upper limb muscular activity in patients with neuromuscular disorders

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**Inherited muscular dystrophies and congenital myopathies present in early childhood with progressive muscle weakness, determining severe motor limitations. Active surveillance and management of associated complications have improved ambulation, function, quality of life and life expectancy. The need for repeatable, objective and quantitative measures to monitor the clinical course of the disease is a current issue, particularly in the new era where new flows of therapies are proposed to the patients. In this scenario, we designed and tested a wearable device termed AUTOMA that is able to provide quantification of the muscular impairment in the upper limb upon isokinetic tests through the integration of a force sensor and an electric goniometer. This allows qualitatively estimating the muscular functions with a systematic procedure. We carried out a preliminary pilot study on 9 patients that revealed the suitability of AUTOMA as an objective measurement tool for diagnosing and monitoring neuromuscular disorders, and opens to a more extensive clinical study in which to test and validate our platform intensively.**

**Key words:** neuromuscular disorders, wearable devices, upper limb function, sensing, clinical monitoring, rehabilitation

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## Introduction

Neuromuscular disorders (NMDs) are a group of rare genetic diseases that induce a progressive disability in patients by affecting the muscular functionality. Duchenne Muscular Dystrophy (DMD) is one of such diseases that affects 1 in 3500 new-borns [1]. This idiopathic progressive pathology affects muscles from early life, threatening the ability to walk, to perform daily tasks with upper limbs, and inducing severe failures of the cardio-respiratory apparatus thus limiting life expectancy. Individuals with Becker muscular Dystrophy (BMD) have a more variable presentation and may continue to walk well into their fourth decade or later with a variable and partially unknown natural history.

Improved standards of care and the regular early-use of steroid treatments in DMD have changed the natural history of the disease, affecting both survival and time of loss of functional milestones [2,3]. More recently, there has been an increasing evidence of an additional benefit from new therapeutic approaches based on mechanisms targeting specific types of mutation [1,4]. To monitor the effect of such a therapy and to study the natural history of these young patients, over the last few years there has been an increasing attention to the identification of suitable outcome measures for clinical trials. At first, clinical research has focused on ambulant children only but, more recently, many studies have been carried out to reduce the existing gap in identifying suitable measurements for assessing upper limb functions across all stages of the disease.

In 2012, an international group of clinicians, physical therapists, patients, advocacy groups and industries developed the so-called Performance of the Upper Limb (PUL), a functional scale, based on scores awarded by operators, specifically designed for assessing upper limb function in DMD or BMD [5]. Meanwhile, a correlation was demonstrated between muscle strength and motor function among children and in the adult population [6]. An example of such score-based method is given by the Medical Research Council (MRC) index. This parameter ranges from 0 – no appreciable muscular contraction – to 5 – full muscular strength (normal power). The numerical value is subjectively assigned after detecting the muscular strength upon the application of a manual concentrated load on the limb [7]. However, is important to consider that the relationship between muscle strength and functionality is not linear. In general, upper limb functions become increasingly influenced by weakness in conjunction with contractures and/or growth, resulting in compensatory strategies and, ultimately, loss of function.

Finally, while several efforts have been made to identify outcome measures for DMD, other NMDs are still orphaned of functional measures and, there are still no measures capable of evaluating motor impairments of the upper limb in daily life. In fact, none of the routine rating scales captures the progressive muscle weakness representing the entire spectrum of the disease, in particular for patients at the weak end of the spectrum with low gross motor functions.

To overcome those limits, a large body of research has been carried out to develop equipment and devices able to perform motor tests, also in weak patients [8].

However, several limitations still exist in the use of such devices: i) a high level of subjectivity in the assessment of tests; ii) biased/incidental errors in positioning the devices on soft tissues of the patient (e.g., incorrect perpendicularity of the tools with respect to the directionality of the loads); iii) high footprint and invasiveness of isokinetic platforms for assessing force; iv) limited naturalness of the gesture [9,10].

In our work, we present the design and preliminary validation of a wearable mechatronic device named AUTOMA. Such a device is able to quantitatively assess upper-limb muscle strength upon quasi-static isokinetic loads and simultaneous determination of range of movements (ROMs) and motor functionality. This is a first step towards the definition of a set of wearables, non-invasive instruments to monitor DMD and, more broadly, NMDs.

## Materials and methods

*Hardware*

AUTOMA is conceived as a measurement tool to be worn by a patient and to be used by the therapist to evaluate in real time the impairment level of the upper limb muscles upon quasi-static loads.

Figure 1 reports the hardware parts that compose AUTOMA (Fig. 1A): i) two sensorized units (Fig. 1B1 and 1B2) to classify the typology of the limb movements and to measure the relative angle between the arm and the forearm; ii) a thermoformed polymeric bracelet to measure the external force applied by the therapist to the patient (Fig. 1C1 and 1C2) during the manual muscle test; iii) a wearable elbow sleeve or a shawl made of a washable material (e.g., lycra) on which the components are assembled to avoid a direct contact with the skin and to ensure a safe and comfortable wearability (Fig. 1D and 1E).

In detail, each sensorized unit is composed of a shell containing one endblock of a biaxial electro-goniometer SG150 (Biometrics Ltd, Newport, UK), and the predisposition for a 9-axis inertial platform ADATEC (Fig. 1B2) - for the classification of the upper limb movement but not implemented in the present study. The bracelet, fabricated in stereolithographic resin and thermoplastic polymer (Aquaplast®), shown in Figure 1C1-C2, serves as a holder for a uniaxial load cell FX1901-0001-0010 (Measurement Specialties Inc., Hampton, VA, USA). To improve comfort and to avoid a slippery direct contact between the polymeric structure and the elbow sleeve, we applied an internal coating made of polyurethane.

A first calibration of the sensors was performed on a bench by testing the loading cell and the electro-goniometer with calibrated weights applied by means of a dedicated indentation machine (Fig. 2A) and with a manual goniometer commonly used by therapists (Fig. 2B), respectively. In the first case, the calibration shows high linearity (error ~ 2%), while the angular resolution reaches values of 4°-5° due to the analogic/digital conversion (Fig. 2C). The correct donning of the device was checked

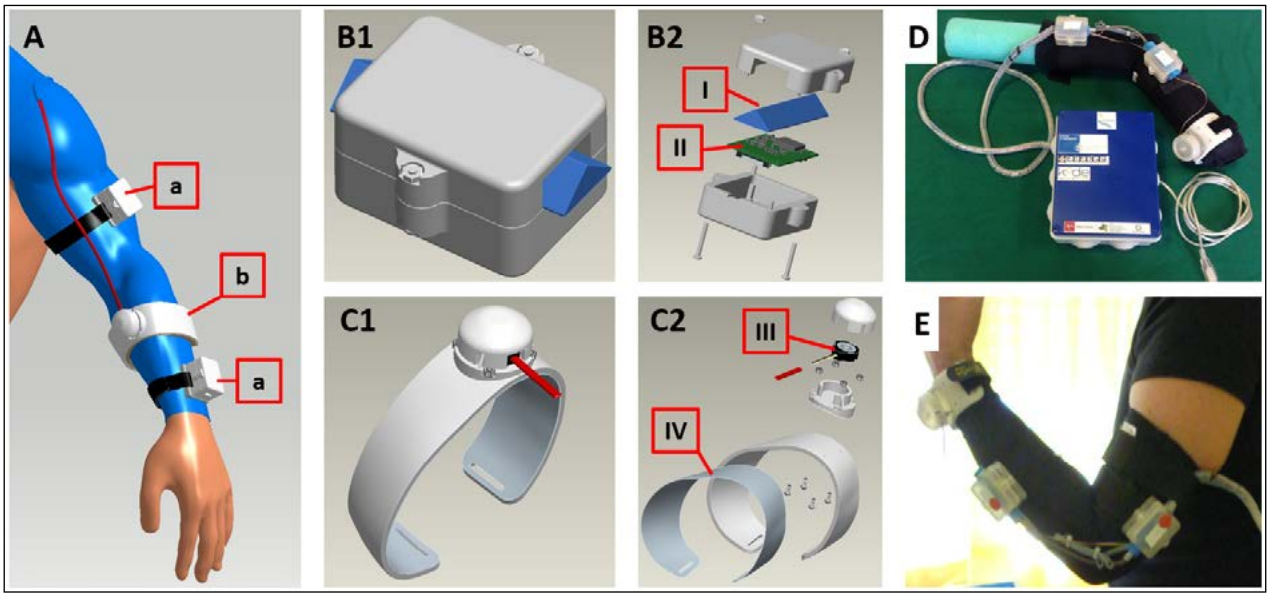


**Figure 1.** AUTOMA: hardware components. Panel A. 3D CAD model of AUTOMA composed of an elastic sleeve (in blue) and two types of sensors (a – electro-goniometer case; b – force sensor). Panels B1-B2: case for the electro-goniometer (I) and for the addition of a potential inertial unit - IMU (II) (not used for the validation in this study). Panels B3: Biometrics electro-goniometer SG150 model. Panels C1-C2: case (bracelet) for the force sensor (III) with an internal coating of polyurethane (IV) to assure comfort and to prevent slippage between the bracelet and the sleeve. Panel D. The complete system assembled on a dummy including the box containing the electronics. Panel E: AUTOMA worn by a subject: the bracelet was placed close to the wrist as specified by MMTs protocols implemented in the experiments.

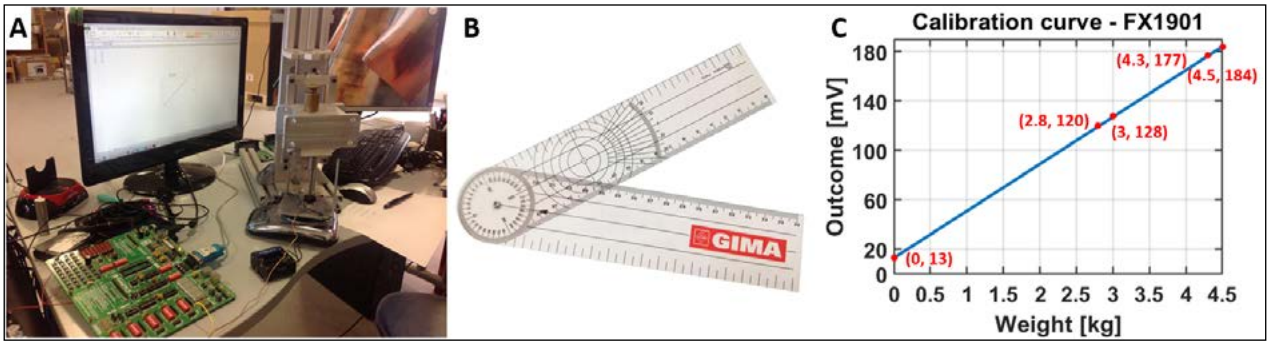


**Figure 2.** Calibrating AUTOMA. Panel A. Indentation machine. Panel B. Manual goniometer used by therapists. Panel C. Calibration curve. The red dots resemble the check-points for the calibration.

before the clinical trial. Within this phase, the electro-goniometer calibration was verified by means of a manual goniometer with AUTOMA already worn by the subject.

*Software*

Within the project developed, we implemented a platform named Health 360 to collect clinical data from AUTOMA and other sources. Health360 possesses a web-based modular architecture provided by a Software-as-a-Service (SaaS) model [11]. Data from AUTOMA can be embedded into Health360 in two manners, in both cases via communication through Application Programming Interface (API). The first approach, less computationally burdensome, relies on the communication of off-line previously analysed data; the second approach, computationally heavier, foresees the possibility of hosting data processing algorithms directly on the platform, therefore communicating raw data between AUTOMA and Health360. In both cases, Health360 will then merge data from AUTOMA with those from other sources, making them easy to be processed by Machine Learning algorithms and similar approaches. Data collected with

AUTOMA were processed using the R software (R Development Core Team) and later visualized using MATLAB (MathWorks, Inc., USA).

This will ultimately allow clinicians to collect, manage, and store data in the Cloud from tests that involve analogic and digital devices. Further details can be found in literature [12].

*Pilot study in patients*

We enrolled 9 subjects with neuromuscular issues (4 DMD patients, 3 BMD patients and 2 patients with a myopathic disorder, all males, age ranged 8-24 years) homogenously distributed across the MRC scale considering the upper limb performance. After being informed about AUTOMA and the procedures involved in the experimental design, patients provided a written consent to participate in the research program. With ethical approval (protocol 136/17, Tuscany Region Ethics Committee), the study took place at IRCCS Stella Maris Foundation (Pisa, Italy) and it was performed by professional therapists. Protocols included a first assignment of the PUL scores for the upper limb functionality and, using AUTOMA, the assessment through two specific items of the manual muscle test (MMT), namely MMT9 and MMT10, related to the extension and flexion of the elbow. Those exercises were independently evaluated by two operators and were repeated five times for each evaluator in a same session.

For each item, each trial was composed of three steps:

- first, the registration system is enabled with the operator supporting the subject's upper limb in the initial position without applying force on the load cell of the bracelet (time = 2 s);
- then, an isokinetic limb movement (to reduce force measurement artefacts due to inertial loads) was performed in elbow extension / flexion against operator resistance on the load cell, up to the patient's maximum range of motion for 2 s;
- after the completion of the test, the upper limb was brought to the initial position and the test repeated from point i.

Figure 3 depicts the phases of the tests on a subject. Compatibly with the patient's state of fatigue, both items were performed with 5 repetitions.

*Data analysis*

The experimentation and the analysis that follows focus on data recorded with the AUTOMA electro-goniometer and the load cell, while IMUs have not been preliminarily implemented since we decided to use these sensors in a future study, too, being AUTOMA designed to include them.

For each test, raw sensors signals have been initially processed using the R software off-line to identify the time interval in which the actual movement, and thus the force peak of interest, occurs. The data cleaning process consists of excluding force values below the threshold of 100 g (a threshold selected to exclude any accidental contact with the load cell), and then focusing on the angle signal *vs* time to select the interval in which test trend is similar to isokinetic trends as much as possible. Angle vs. time curves were firstly smoothed to estimate their first derivative. After that, we deemed the movements start when the angle first derivative respect to time falls over a selected threshold value of +25/-25 °/ms for MMT10 and MMT9, respectively. Finally, a linear regression model with time as the regressor was then elaborated in order to evaluate the angle *vs* time curve linearity, a benchmark meaning that the movement occurred uniformly and the test was isokinetic: when the R2 was greater than, or equal to, 0.8 the acquisition was considered adequately filtered, otherwise the acquisition was discarded (Fig. 4).

## Results

In this paper, we discuss the development of AUTO-

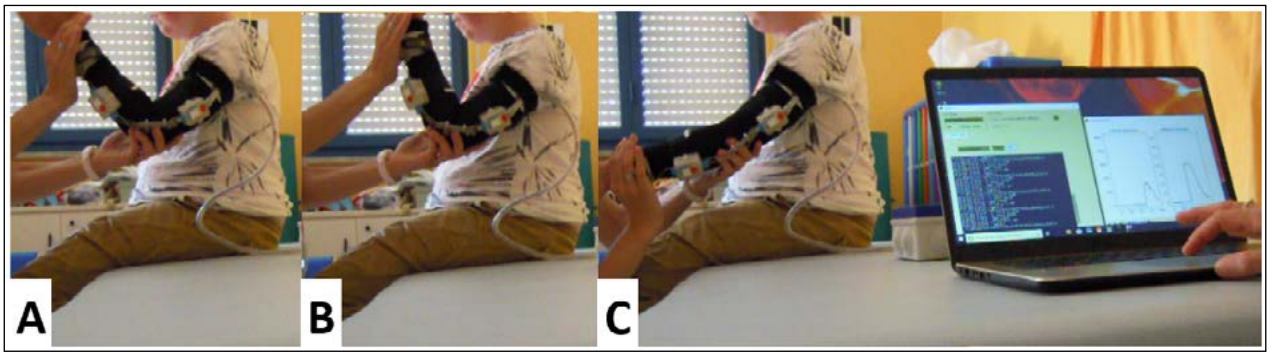


**Figure 3.** Isokinetic tests. Panels A to C show three different steps of the test with AUTOMA and the simultaneous data collection.

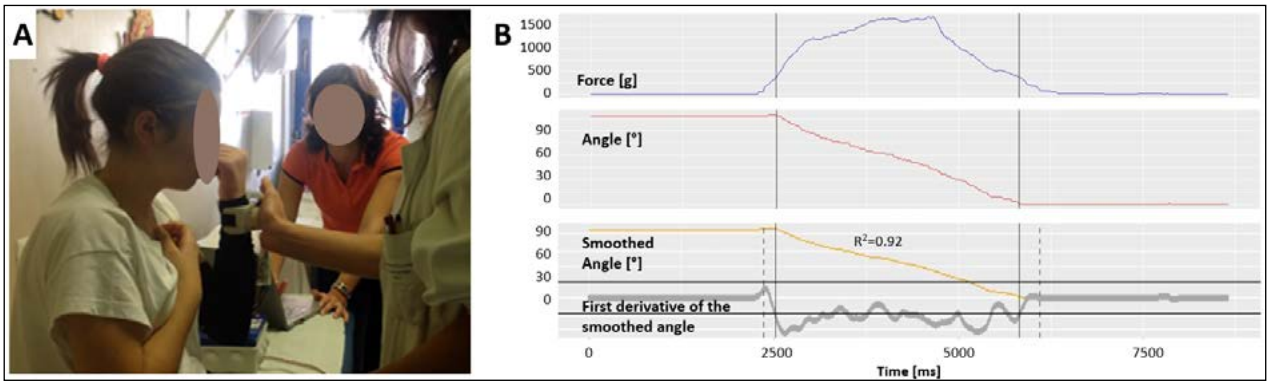


**Figure 4.** Gathering data with AUTOMA. Panel A. Using AUTOMA to perform MMT10. Panel B. Example of the acquisitions, showing the raw measured Force (g), raw angular displacement (degrees - °) and, in the bottom panel, the smoothed angle curve after filtering the signal (yellow line) and the first derivative of the smoothed signal. Note. Dashed lines are in correspondence of the first and the last Force numerical value above 100 g. Solid horizontal lines represent the bounds (+25/-25 °/ms) for the smoothed Angle derivative values. Solid vertical lines represent the first and the last value where the Angle derivative crosses the thresholds.

MA, a wearable device to assess the impairment level of the upper limb in patients with neuromuscular disorders.

We selected 9 patients with NMDs, previously classified with MRC scores of arms between 2 and 4, who agreed to participate to a pilot study designed to assess the capabilities of AUTOMA. We selected two specific tasks, namely the flexion and extension of the elbow (MMT9 and MMT10), to assess the muscular performance of the subjects, previously scored using PUL test. Following the criteria set by the experimental protocol, we had to discard part of the collected data (14% for MMT9 and 27% for MMT10), being not sufficiently linear, thus remodeling the structure of the dataset. Moreover, concerning MMT10, two subjects were not able to perform the MMT10 task due to their conditions and, therefore, were excluded from the statistical analysis. The final dataset is reported in Table I, where we also outline the average score assigned by the operators upon the items F to L (regarding elbow movements) of the PUL tests carried out before using AUTOMA. It is worth noting that the PUL score was not assigned to the 2 patients with myopathy since such a test is not standardized for this disorder.

Once the peak-force and mean angular velocity values were collected, they were compared across different levels of disease severity. Normality and homoscedasticity assumptions for both force and mean angular velocity were verified using Shapiro and Levene tests, respectively, separately for each disease severity and item (Tabs. II-III). Homoscedasticity assumption was accepted in all the cases at 5% significance level, but the Normality assumption was rejected in some disease severity groups for the mean angular velocity at 5% significance level. Thus, in order to perform comparisons across disease levels concerning the force, we used the ANOVA analysis that rejected the null hypothesis of no difference between different disease levels with an F value of 41.9 on 2 and 70 degrees of freedom (p-value 1.09e-12) and 61.59 on 1

**Table I.** Number of patients available for each disease severity level, ranging from 2 to 4 in the MRC scale and average PUL score for the upper limb tasks.

| MMT item | MRC score | | | Number of patients examined |
|---|---|---|---|---|
| | 2 | 3 | 4 | |
| MMT10 | 0 | 4 | 3 | 7 |
| MMT9 | 1 | 5 | 3 | 9 |
| Average PUL score | 17/34 | 32/34 | 34/34 | - |

Note. The average PUL score is not given for the patients with myopathy since the PUL test is not standardized test for such a disorder.

**Table II.** Shapiro-Wilk normality test p-values.

| MMT item | Index | MRC scale | | |
|---|---|---|---|---|
| | | 2 | 3 | 4 |
| MMT9 | Force | 0.583 | 0.400 | 0.241 |
| | Angular velocity | 0.0376 | 0.00572 | 0.446 |
| MMT10 | Force | - | 0.316 | 0.184 |
| | Angular velocity | - | 0.00215 | 0.362 |

**Table III.** Levene homoskedasticity test p-values.

| Index | MMT9 | MMT10 |
|---|---|---|
| Force | 0.583 | 0.400 |
| Angular velocity | 0.0376 | 0.00572 |

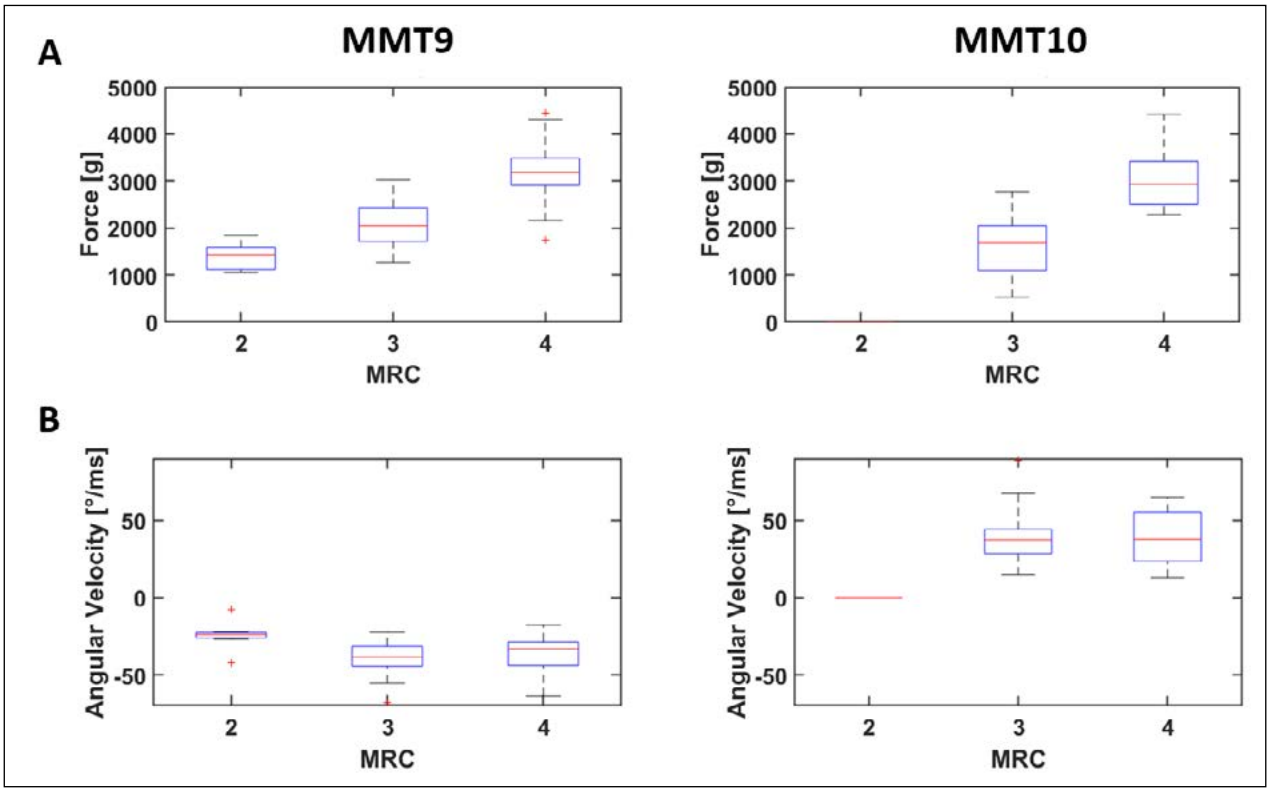


**Figure 5.** Whisker plots for the Force (Panel A) and the Mean Angular Velocity (Panel B) for each item in relation to the MRC.

**Table IV.** Results table of Tukey's method of honestly significant differences adjusted with the Bonferroni method for the force index in MMT9.

| Linear hypothesis | Estimate | Std. error | t-value | Pr(>\|t\|) |
|---|---|---|---|---|
| MRC 2 – MRC 4 = 0 | -1816.2 | 219.5 | -8.276 | 1.70e-11 |
| MRC 3 – MRC 4 = 0 | -1137.8 | 150.7 | -7.551 | 3.67e-10 |
| MRC 3 – MRC 2 = 0 | 678.5 | 193.7 | 3.503 | 2.42e-03 |

and 49 degrees of freedom (p-value 3.27e-10) for MMT9 and MMT10, respectively. Conversely, concerning the non-parametric Kruskal-Wallis rank sum test for the angular velocities, we accepted the null hypothesis of no difference (significance at 5%) between disease levels with a chi-squared value of 0.019, with 1 degree of freedom (p-value 0.89) for MMT10, and rejected it with a chi-squared value of 14.645 with 2 degrees of freedom (p-value 6.61e-04) for MMT9.

Since the MMT9 presents three distinct disease severity levels and both the ANOVA and the Kruskal-Wallis rank sum test highlighted significant (at 5% significance level) differences between groups, post-hoc tests were carried out using the Tukey's method with the Bonferroni correction and the Mann-Whitney test, respectively (Tabs. IV-V). All the post-hoc tests rejected the hypothesis (significance at 5%) of equality of mean/location shift of each disease severity group, except for the comparison between groups 3 and 4 for the mean angular velocity (see Figure 5).

**Table V.** Results table of Mann-Whitney test for angular velocity in MMT 9.

| Hypothesis tested (True location shift is not equal to 0) | W | p-value |
|---|---|---|
| MRC 2 *vs* MRC 3 | 383 | 2.5e-05 |
| MRC 2 *vs* MRC 4 | 118 | 2.5e-02 |
| MRC 3 *vs* MRC 4 | 312 | 0.19 |

The ANOVA/Kruskal-Wallis and post-hoc tests results, along with the plots in Figure 5, show a significant

stratification of the force levels reached by the patients with different disease severity, but not a significant difference between the angular velocities related to the movement.

## Discussion

AUTOMA is a device that belongs to the family of non-invasive platforms for collecting biophysical parameters. To date, a number of studies have been published demonstrating the capability of sensing systems of providing reliable datasets about signals like tongue muscle activity [13], blood pressure [14], presence of glucose in blood [15], heart rate [16], or sweat monitoring [17]. Novel hardware designs, along with the advent of artificial intelligence and cloud computing, have brought new opportunities for clinicians and operators to quantitatively diagnose and monitor diseases, giving new quantitative data cleaned from any subjective evaluation of biophysical parameters [18]. From a rehabilitation standpoint, such new approaches can lead to a paradigm change. In fact, by integrating these advanced and, often interactive, technologies, it is possible to create a renewed patient-specific awareness on the physiological conditions and, if applicable, to easy teach specific behavioral changes with low costs and intrusion [19].

Recently, Molina-Molina et al. published a work about a wearable system to perform surface electromyography to assess muscle activity. Their platform includes surface electrodes and a mobile computing to perform a cloud data analysis. Similar to AUTOMA, the authors carried out the experimental campaign by performing a set of isokinetic tests, thus minimizing the inertial artifacts. In contrast to AUTOMA that requires an elastic sleeve without a direct contact with the skin, their system requires a specific preparation of the muscle surface (e.g., shaved skin, cleaning with alcohol) that can affect the collection and reliability of the measurements. In addition, while AUTOMA has been preliminarily tested against traditional approaches for monitoring DMDs, Molina-Molina et al. did not report any comparison with other datasets [20]

The preliminary statistical analysis of the data collected with AUTOMA suggests that the device is capable of efficiently performing an objective quantification of muscle strength in the upper limb muscles. In particular, the plots reported in Figure 5 display a relationship between the peak force and the MRC scores, while they are not correlated with angular velocity. Therefore, we can conclude that the peak force can be considered an index for classification, independently of the numerical value of the angular velocity that is always kept constant (i.e., isokinetic test) by expert operators.

In view of this, AUTOMA is to be considered a sensorized tool that might assist clinicians to objectively detect the strength deficit and to monitor the evolution of all muscular pathologies including those, such as myopathies, for which PUL tests are not standardized. Thanks to its portability and to the integration of different sensors, AUTOMA could be considered easy to adapt in the clinical practice and can be customized directly onto the patient to optimize its ergonomic fit.

From the performance perspective, AUTOMA will enhance an unbiased evaluation of the health status of a patient, delivering a more precise classification of the disorder stages without discrepancies given by a subjective evaluation from different operators. Using such a device, clinicians will have the possibility to design customized therapies based on quantitative data that, as demonstrated, reflect and improve the outcomes from the subjective scores usually attributed by the operators to the patients.

## Conclusions

In the last decade, research studies have focused on quantitative measures that describe disease progression in the upper limbs, from early ambulant stages over transition stages to non-ambulant stages. Such approaches can be useful to trace the course of muscle weakness and to allow a better understanding of disease evolution and efficacy of interventions throughout the lifespan [21].

AUTOMA appears as a promising tool for monitoring NMDs from the early stages, since it is able to estimate, through a sensorized sleeve, variations of the muscle performance that could be difficult to discriminate manually based only on the experience of an operator. Moreover, being a simple and relatively cheap platform with low need for maintenance, AUTOMA could truly integrate the currently available bulky platforms, with the possibility to be easily transported and also used as a point-of-care solution for an in-home monitoring of NMDs.

From a technical standpoint, next steps include the addition of IMUs to better discriminate the angular displacements and to classify movement and fix the limitations observed in the present study. Such a methodology will include the implementation of wireless force/angular sensors to optimize portability and to increase the number of measured joints, together with the integration of other sensors (i.e., EMG, ECG, PZT Respiration) to monitor other key physiological parameters. Concerning the clinical side, we plan to start a new recruitment campaign with a larger number of patients in order to collect more data to stress the device and to perform a more robust statistical analysis, also including a correlation with the PUL scores.

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*Ethical consideration*

All tests were carried out following an explicit ethical approval (protocol 136/17, Tuscany Region Ethics Committee).

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*Conflict of interest*

All the authors declare no conflict of interest.

*Author contributions*

All Authors gave their approval.

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# Can symptomatic nmDuchenne carriers benefit from treatment with ataluren? Results of 193-month follow-up

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**Duchenne's muscular dystrophy (DMD) is an X-linked neuromuscular disorder caused by deletions (75%), duplications (15-20%) and point mutations (5-10%) in the dystrophin gene. Among the latter, stop-codon point mutations are rare. Female carriers of dystrophin gene mutations are usually asymptomatic as they are "protected" by the second X-chromosome, which produces a normal dystrophin protein. However, about 8-10% of them can present symptoms that set the clinical picture of the manifesting or symptomatic carrier. Although no causative cure there is for DMD, therapies are available to slow the decline of muscle weakness and delay the onset of heart and respiratory involvement. However, there is limited data in the literature documenting the treatment of symptomatic carriers, often entrusted to the sensitivity of individual doctors. In this paper, we report the follow-up outcomes of four European symptomatic nmDMD carriers treated with ataluren, overall followed for 193 months. Annual assessment of muscle strength, pulmonary lung function tests, and echocardiography, indicate a mild attenuation of disease progression under treatment.. There were no adverse clinical effects or relevant abnormalities in routine laboratory tests. We can conclude that ataluren appears to stabilize, if not slightly improve, the clinical course of patients with a good safety profile, especially if we consider that the treatment was late for 3/4 patients, at a mean age of 36.6 ± 10.6 years.**

**Key words:** Duchenne muscular dystrophy, nonsense mutations, symptomatic carriers, manifesting carriers; ataluren

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## Introduction

Duchenne's muscular dystrophy (DMD) is an X-linked neuromuscular disorder affecting muscles and heart in young boys [1,2], caused by deletions (75%), duplications (15-20%) and point mutations (5-10%) in the dystrophin gene. Among the latter, the stop codon point mutations are rare [3,4].

Females carrying a dystrophin gene mutation on one of the two X-chromosomes, are usually asymptomatic as they are "protected" by the

second X-chromosome which products a normal dystrophin protein. However, about 8-10% of these females can present symptoms, which causes the clinical picture of the *manifesting* or *symptomatic* carrier. Both terms have been widely used since the 1970s [5-12] to define *females with a history of Duchenne muscular dystrophy in their pedigree who have symptomatic weakness*. These females can also develop myalgia, cramps, fatigue, and show enlarged calf muscles (pseudo-hypertrophy). The severity of symptoms may range from a Duchenne-like progression to a very mild Becker-like phenotype. A considerable percentage of carriers may develop cardiomyopathy, at an advanced stage [13-15]. Cognitive impairment was also reported, mainly associated with mutations in the distal part of the *DMD* gene [16,17]. An increase in serum creatine kinase (CK) levels up to ten times the upper normal limit was reported in approximately 40-50% of carriers, especially in childhood [18,19]. Several mechanisms leading to reduced dystrophin production, were hypothesized to explain the onset of clinical manifestations and in particular the role played by the skewed X-chromosome inactivation (XCI). Though this role is still questioned, several papers [20-22] showed that DMD-manifesting carriers have a preferential inactivation of the X-chromosome carrying the normal allele, while non-manifesting carriers and healthy females showed a random (50:50) XCI pattern.

From a clinical point of view, symptomatic carriers should be considered as affected as males with disease are, and be able to benefit from the same therapeutic opportunities.

There is no causative cure for DMD, but therapies are available to slow the decline of muscle weakness and delay the onset of heart and respiratory involvement. Among others, steroids, ACE-inhibitors and beta-blockers, are the gold standard of the treatment [23,24]. However, in the literature there is limited data documenting treatment of symptomatic carriers [25,26], often entrusted to the sensitivity of individual doctors.

In the last decade, different therapeutic approaches have been tested with encouraging results in patients with dystrophin gene deletions or duplications. Among them we mention gene therapy (which consists of introducing a transgene coding for full-length or a truncated version of dystrophin complementary DNA (cDNA) in muscles) [27], and exon-skipping techniques with antisense oligonucleotides which convert an out-of-frame mutation into an in-frame mutation [28]. For DMD patients having stop codon mutations in the *DMD* gene [4], potential drugs such as gentamicin[29] and ataluren (PTC124) [30] were explored as an alternative approach. These drugs allow ribosomal readthrough of premature stop codons, enabling the production of a functional dystrophin that might ameliorate the disease progression [30,31]. About 10-15% of DMD patients could potentially benefit from treatment with ataluren [31]. This drug has been available in Europe since 2014 [32] under the name (Translarna®).

In 2017, McDonald and al. [33] presented the results of a phase 3, multicentre, randomised, double-blind, placebo-controlled trial (ACT DMD) that assessed the ability of ataluren to stabilise ambulation, with a focus on a prespecified subgroup of patients with ambulatory decline. The primary endpoint of change in 6-min walk distance (6MWD) from baseline to week 48, with a hypothesis of a difference of at least 30m between ataluren-treated and placebo-treated patients, was not reached (difference 13.0 m [SE 10.4], 95% CI -7.4 to 33.4; p = 0.213). However, a benefit of ataluren was observed in the subgroup of patients with a baseline 6MWD between 300 and 400 m (difference vs placebo 42.9m [SE 15.9], 95% CI 11.8-74.0; p = 0.007) and confirmed in papers that appeared in subsequent years [34-36].

Articles recently published on the long-term ataluren treatment indicated a delay in loss of ambulation, as well effects on cardiac and respiratory parameters and upper limb motor function, even after loss of ambulation [37,38]. An early treatment with ataluren has also been suggested [39]. The response to the treatment with ataluren was investigated by D'Ambrosio et al. [40] in a 26-year-old symptomatic nmDMD female carrier who reported an improvement in motor skills after 9 months of treatment.

In this paper we report the follow-up outcomes of the patient described by D'Ambrosio et al., still on treatment with ataluren, together with those of three further European symptomatic nmDMD carriers overall followed for 193 months (average 48.25).

## Patients and methods

Clinical data of the four European DMD carriers so far treated with ataluren were retrospectively collected and included country's origin of female patients, age at first symptoms, age at muscle biopsy, time between first symptoms and muscle biopsy, age at genetic confirmation, time between first symptoms and laboratory abnormality or genetic confirmation. Age at first and last visit for ataluren, age at informed consent, prior and concomitant medications, age at start- and end-date of ataluren, duration of treatment, age at loss of ambulation were also collected. Motor function outcomes such as six minute walking test (6MWT), North Star Ambulatory Assessment (NSAA) total score, dynamic tests (Gowers time, time to climb 4 steps) were evaluated at the start and at the last visit; data on forced vital capacity (FVC) and left ventricular ejection fraction (LVEF) were also evaluated when available.

The drug was administered orally, at a dosage according to the weight of the patients.

Data are shown as mean, range and standard deviation when applicable.

## Results

*Baseline patient demographics & characteristics*

Demographics and characteristics of symptomatic nmDMD carriers treated with ataluren are shown in Table I. Two patients are from Italy, one from Israel and one from UK. The age of onset of the first symptoms was before 2 years in two, and at 17 and 30 years in the other 2 carriers. Muscle biopsy was performed in three carriers, immediately after the onset of symptoms in two, 4 years after the onset of symptoms in the third carrier. The mean time between the onset of symptoms and muscle biopsy was 1.3 years, ranging from 0 to 4 years. The mean age, at the molecular confirmation of the diagnosis, was 21, ranging from 3 to 38 years. The two carriers with onset of symptoms in childhood were on deflazacort, antioxidants, calcium and vitamin D3 treatment, which they continued to take concomitantly with ataluren.

The mean age at the first visit for ataluren was 26.9 (range 9.6-43 years); the start date of ataluren was between May 2015 and November 2017. All patients were under treatment at the time of last visit. The mean age at last visit was 30.7, ranging from 13 to 49 years. The average follow-up period was 48.25 months, ranging from 23 to 77, for an overall period of 193 months. During the follow-up, one patient stopped to walk after 6 years of starting treatment, at the age of 49. Another carrier is still able to walk with a waddling gait and lumbar hyperlordosis, but with a search for support. The other two carriers are still able to walk independently.

*Motor function tests*

6MWT was performed at the first visit in 3/4 patients, showing a mean value of 262 ± 10.47 m, but it was available for only one patient (217 m) at last visit. In the older carrier, an initial improvement under treatment was observed in 6MWT, passing from 270 up to 315m and followed by a gradual decline. NSAA total score was available at the first visit in two patients, showing a mean score of 22/34 which passed to 23.5/34 at the last visit. The time to get up from the floor, an ability present in 2/4 patients, changed from an average value of 8.95 sec at the first visit to 11.5sec at the last visit.

The percentage mean values of FVC passed from 89.7 ± 24.3 to 76.3 ± 20.4, with an annual average decline of 3.3%. The EF values, available in 2/4 carriers, varied on average from 65.5 to 61%, with an annual decline of 1.1% (Tab. II).



## Discussion

By definition, the term 'carrier' refers to someone who has a heterozygous mutation in his/her DNA, without presenting the symptoms related to the disease.

The prevalence of skeletal muscle damage among Duchenne female carriers, including asymptomatic carriers is estimated to be between 2.5-19%, and the incidence of dilated cardiomyopathy between 7.3-16.7% [13,14,16,41]. Viggiano et al. [21] observed that DMD carriers with moderate/severe muscle involvement exhibit a moderate or extremely skewed XCI, in particular if presenting with an early onset of symptoms, while carriers with mild muscle involvement present a random XCI. Moreover, when comparing muscle with heart manifesting carriers, the former group showed a higher degree of skewing [21,22].

The frequency of manifesting carriers complicated by cardiomyopathy increases with age [13,16,41] and studies begin to appear on how and when to best treat these patients [26,42]. However, there is limited high-quality evidence to guide the treatment of female carriers of Duchenne / Becker muscular dystrophy. The available evidence is

**Table I.** Demographics of symptomatic nmDMD carriers treated with ataluren.

| Reference Center | Current age (years) | Age at first symptoms (years) | Age at muscle biopsy (years) | Time between first symptoms and muscle biopsy (years) | Age at genetic confirmation of nmDMD (years) | Time between MB and genetic confirmation (years) | Time between first symptoms and genetic confirmation (years) |
|---|---|---|---|---|---|---|---|
| IT001 | 30 | 1.6 | 1.6 | 0 | 24 | 22 | 25 |
| UK001 | 13 | < 2 | 2.6 | 0.3 | 3 | 0.5 | 1 |
| IL001 | 49 | 30 | n.p. | n.ap | 38 | n.ap. | 8 |
| IT002 | 31 | 17 | 21 | 4 | 21 | 0.5 | 0,5 |
| Mn | 30.75 | 16.2 | 8.4 | 1.43 | 21.5 | 7.67 | 8.63 |
| Range | 13-49 | 1.6-30 | 1.6-21 | 0-4 | 3-38 | 0.5-22 | 0.5-25 |

Abbreviations: MB: Muscle Biopsy; n.p.: not performed; n.a.: not applicable

**Table II.** Clinical data of symptomatic nmDMD carriers treated with ataluren.

| Reference center | Age at first visit (years) forataluren | Ataluren start date | Duration of treatment (years) | Loss of ambulation (years) | 6MWD atataluren start date (meters) | 6MWD at ataluren end date (or last visit) | NSAA total score at ataluren start date | NSAA total score at ataluren end date (or last visit) | Gowers timeat ataluren start date | Gowers time at ataluren end date (or last visit) | Percentage FVC at ataluren start date | Percentage FVC at ataluren end date (or last visit) | Percentage ejection fraction at ataluren start Date | Percentage ejection fraction at ataluren end date (or last visit) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| IT001 | 26 | 01/10/2017 | 48 | 30 | 100,00 | u | n.p. | n.p. | u.t.p. | u.t.p. | 68 | 53 | 65 | 58 |
| UK001 | 9.6 | 01/12/2017 | 45 | | n.a. | n.p. | 31/34 | 32/34 | 2.5 | 2.7 | 116 | 91 | n.a. | n.a. |
| IL001 | 43 | 01/05/2015 | 21 | 49 | 270.00 | u.t.p. | p. | n.p. | u.t.p. | u.t.p. | n.p. | n.p. | n.p. | n.p. |
| IT002 | 29 | 23/11/2019 | 23 | | 255.20 | 217 | 3/ | 15/34 | 15.4 | 20.6 | 85 | 85 | 66 | 64 |
| | | | | | | | | | | | | | | |
| Mn | 26.90 | | 34.25 | 39.5 | 262.60 | | 2/34 | 3.5/34 | 8.95 | 11.65 | 89.67 | 76.33 | 65.50 | 61.00 |
| Range | 9.6-43 | | 21-48 | 30-49 | 100-270 | | 13-31/34 | 32/ | 2.5-15.0 | 2.7-20.6 | 68-116 | 53-91 | 65-66 | 58-64 |

Abbreviations: n.e. not evaluable; n. p.: not performed; n.a.: not available; u.t.p.: unable to perfor



mainly based on expert opinions and clinical experience.

Here, we report our experience in four symptomatic nmDMD female carriers treated with ataluren for 193 months overall. Routine investigations included muscle strength, dynamic tests, cardiac function and pulmonary function tests. We compared changes in 6MWT, Gower's time, FVC and LVEF at baseline and at the last visit from the start of ataluren. All patients were ambulant at the start of treatment, and two remained so at the last follow-up visit, after 48 and 45 months of treatment, respectively. Under ataluren, the annual assessment of muscle strength, pulmonary lung function tests, and echocardiography indicated a mild attenuation of the disease progression. No adverse clinical effects were reported by the patients nor relevant abnormalities observed in routine laboratory values.

We are aware that the study has the limitations of a retrospective study, which put together data collected spontaneously by researchers who wanted to test the efficacy of treatment with ataluren in nmDMD symptomatic carriers they had in care. The number of carriers treated may also seem too small, but we must remember that the estimated number of nmDMD patients is about 10-15% of the entire Duchenne population and that symptomatic carriers are an even smaller percentage.

Despite these limitations, we believe that ataluren has a good safety profile and stabilizes, if not slightly improves the clinical course of nmDMD female patients, in whom the treatment started much later than in affected males. However, larger clinical trials, and possibly on younger subjects are required to assess the role of ataluren and its long-term impact on disease progression in symptomatic nmDMD carriers.

*Ethical consideration*

The project was approved by the Ethical Committee of the University of Campania (Protocol number 769 of 23/11/2018).

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*Conflict of interest*

The Authors have no conflicts of interest to declare

that are relevant to the content of this article.

*Author contributions*

LP: conceptualization, methodology, writing original and draft preparation, writing review and editing, and supervision; AD, MG, MS, LPa, AT: investigation and data collection.

All authors have read and agreed to the published version of the manuscript.

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# Magnetic resonance imaging pattern variability in dysferlinopathy

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**The widespread use of magnetic resonance imaging (MRI) in the diagnosis of myopathies has made it possible to clarify the typical MRI pattern of dysferlinopathy. However, sufficient attention has not been given to the variability of MRI patterns in dysferlinopathy.**

**Materials and methods. Twenty-five patients with the clinical manifestations of dysferlinopathy were examined. For all patients, creatine phosphokinase levels were measured and molecular genetics were examined. In two patients, immunohistochemical examinations of muscle biopsies were performed. MRI scanning was included T2 multi-slice multi-echo, T1 weighted, T2 weighted and Short Tau Inversion Recovery T2 weighted sequences. Quantitative and semi-quantitative evaluations of fatty replacement and swelling of the muscles were undertaken.**

**Results. Variability in the MRI patterns was lowest in the pelvis and leg muscles and highest in the thigh muscles. Three main types of MRI patterns were distinguished: posterior-dominant (80%), anterior-dominant (16%), and diffuse (4%). Among patients with the anterior-dominant pattern, the collagen-like variant (4%), proximal variant (4%) and pseudo-myositis (8%) were separately distinguished.**

**Conclusions. Awareness of atypical MRI patterns in dysferlinopathy is important for increasing the efficiency of routine diagnostics and optimizing the search for causative gene mutations.**

**Key words:** dysferlinopathy, LGMDR2, LGMD2B, Miyoshi myopathy, MRI pattern, T2-MSME

**Abbreviations:** MRI: magnetic resonance imaging; T2-MSME: T2 multi-slice multi-echo; STIR, Short Tau Inversion Recovery; LGMDR2: limb-girdle muscle dystrophy R2; MRC: Medical Research Council; CK: serum creatine phosphokinase; DMAT: distal, with anterior tibial onset; (MDS: Muscular Dystrophy Score; SE: spin echo; Me: median; M: mean; CD: cluster of differentiation; TE: echo time; TR: repetition time; FOV: field-of-view; FF: fat fraction.

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## Introduction

Dysferlinopathy refers to a phenotypically heterogeneous group of hereditary muscular dystrophies caused by mutations in the *DYSF* gene (2p13), which encodes the transmembrane protein dysferlin (230 kDa) that is involved in the repair of muscle membranes [1]. Five main phenotypes are distinguishable: Miyoshi distal myopathy (OMIM# 254130), limb-girdle muscular dystrophy R2 (LGMD R2, OMIM# 253601); distal myopathy of the anterior lower leg (distal, with anterior tibial onset (DMAT, OMIM# 606768); proximal-distal form (a transitional form), asymptomatic hypercreatinephosphokinasemia [2,3] and congenital phenotype [4].

Increasing the diagnostic efficiency of dysferlinopathy can be achieved through a comprehensive analysis of clinical manifestations, as well as information concerning typical and rare magnetic resonance imaging (MRI) patterns for the distribution of fatty infiltration of muscles. The predominant involvement of the posterior and medial thigh muscle groups, the soleus muscle, and the medial and lateral head of the gastrocnemius has been described in previous publications [5-7]. One characteristic of dysferlinopathy is the development of edema prior to fatty replacement in the quadriceps femoris, the adductor magnus, and the posterior muscles of the thigh [8,9]. However, there are a limited number of reports suggesting high heterogeneity of MRI patterns in dysferlinopathy [10,11]. Hence, a more systematic description of the anatomical distribution of muscle wasting in dysferlinopathy, which has been analyzed in relation to clinical and molecular genetic investigations, would refine our understanding of disease pathology and improve diagnostic ability. Therefore, the purpose of the study was to determine the range of possible variants associated with the MRI patterns of the distribution of fatty infiltration of muscles in a cohort of Russian dysferlinopathy patients.

## Materials and methods

*Patients*

We examined twenty-five patients from a Russian cohort that consisted of twelve Avars, nine Russians, two Azerbaijanis, one Tatar, and one Kalmyk with dysferlinopathy. There were sixteen men 64% (95% Confidence interval (CI) 42-82) and nine women 36% (CI 18-57). Assessments of each patient's clinical status was carried out using the Vignos scale [12] and Muscular Dystrophy Score (MDS) [13]. Manual assessment of muscle strength was measured using the ordinal Medical Research Council (MRC) scale. A control group of twenty healthy volunteers (eleven men 55% (CI 31-76) and nine women 45% (CI 23-68) whose average age was (*Me*) 31 years (CI 21-40) was also included. All patients signed a voluntary, informed consent form in accordance with the requirements of the 2013 Helsinki Declaration and the local ethics committee of the Military Medical Academy S.M. Kirov (Russia) (protocol # 219, 12/02/2020).

*Laboratory studies*

The examination of patients included clinical and genealogical analyses, neurological examinations, electromyography, and laboratory diagnostic methods including measurements of serum creatine phosphokinase (CK). Molecular genetic studies of DNA samples were undertaken using next generation sequencing on an Illumina HiSeq 2000 platform (Illumina Inc., San Diego, CA). Confirmation of the results was performed via Sanger sequencing.

*Histological and pathomorphological analysis*

Open-incision muscle biopsies were performed in three patients for confirming pathogenic of mutations. A fragment (5x5x5 mm) of the lateral part of the quadriceps femoris was taken and prepared according to standard procedure. Longitudinal and transverse sections of the samples were stained with hematoxylin and eosin, and immunohistochemistry was performed using antibodies to dysferlin, dystrophin, smooth muscle alpha actin, Ki67, CD68, CD4, and CD8.

*Immunoelectrophoresis and western blotting*

Polyacrylamide gel electrophoresis and western blotting were performed as described previously [14]. All tissue samples were weighed, frozen and homogenized in 19 volume electrophoresis treatment buffer (e.g., 20 mg + 380 µl buffer) and given a loading concentration of approximately 2 mg in 30 µl volume [15].

*Magnetic resonance imaging of the pelvic girdle and lower extremities*

MRI scanning was performed from the anterior, superior, iliac spine to the lower third of the legs on an Ingenia 1.5T tomograph (Philips Healthcare, Eindhoven, Netherlands) using a body surface receiver coil. The protocol included T2-multi-slice multi-echo (T2 MSME), T1 weighted (T1w), T2 weighted (T2w) and Short Tau Inversion Recovery T2 weighted (STIR T2w) pulse sequences. The following are the acquisition parameters for:

- T1w spin echo (SE) in the axial and coronal planes: echo time (TE) = 10 ms, repetition time (TR) = 600 ms, number of repetitions = 1, tilt angle = 90°, refocus angle = 120°, field-of-view (FOV) = 450 × 450 mm$^2$, pixel size 0.6 × 0, 6 mm$^2$, number of slices = 30, slice gap = 10 mm, slice thickness = 10 mm;

- Axial T2w SE: TE = 80 ms, TR = 500 ms, number of repetitions = 1, tilt angle = 90°, refocus angle = 120°, FOV = 450 × 450 mm$^2$, pixel size 0.6 × 0.6 mm$^2$, number of slices = 30, distance between slices = 10 mm, thickness of slices = 10 mm;
- STIR T2w SE in the axial and sagittal planes: TE = 70 ms, TR = 4000 ms, number of repetitions = 1, tilt angle = 90°, refocus angle = 120°, FOV = 450 × 450 mm$^2$, pixel size 0.8 × 0.8 mm$^2$, number of slices = 30, distance between slices = 10 mm, thickness of slices = 10 mm;
- T2 MSME in the axial plane: TE in the range from 8 to 160 ms with a delta of 8 ms, TR = 2500 ms, number of repetitions = 1, tilt angle = 90°, refocus angle = 180°, FOV = 400 × 400 mm$^2$, size pixel 0.7 × 0.7 mm$^2$, number of slices = 10, distance between slices = 10 mm, thickness of the slices = 10 mm.

*Image analysis*

A quantitative assessment was carried out using a three-exponential calculation method with a division into water and fat signals from each muscle, according to the formula [16]:

$$S(TE) = A_f\left[c_l.exp\left(-\frac{TE}{T2_{fl}}\right) + c_s.\exp\left(-\frac{TE}{T2_{fs}}\right)\right] + A_m\left[exp\left(-\frac{TE}{T2_m}\right)\right],$$

where S (TE) is the signal for a given echo time; TE is the echo time; T2*fl* is the long relaxation time of the fatty component; T2*fs* is the short relaxation time of the fat component; T2m is the relaxation time of the water component in the muscle; *Af* is the coefficient reflecting the proportion of fat in the signal; *Am* is the coefficient reflecting the proportion of water in the signal; and *cl* and *cs* are the long and short coefficients of the bi-exponential fat model, respectively. To calculate the bi-exponential model of the fatty component in skeletal muscles, a separate segmentation of the subcutaneous fat was carried out.

The fat fraction (FF) was calculated as the ratio between the fat signal and the sum of the water and fat signals at TE = 0 ms. Based on the model presented in the above equation, this was determined by the formula:

$$FF = \frac{A_f(c_l + c_s)}{A_f(c_l + c_s) + A_m}$$

Mathematical analysis of the data was carried out using Python v.2.7.15 software. Segmentation of the muscles of the thighs and lower legs was performed manually using ITK-SNAP 3.8.0 software [17]. Fatty degenerative changes were scored semi-quantitatively according to the Lamminen-Mercuri scale [18].

*Statistical analysis*

The quantitative results of the study are presented as mean (*M*) or median (*Me*) values with 95% confidence intervals (calculated via the Klopper-Pearson method) depending on whether the data were normally distributed. Statistical significance was evaluated using the Mann-Whitney sign-rank test ($p < 0.05$).

## Results

*Clinical and genetic data*

The age of the patients at the time of the examination was (*Me*) 37 years (CI 31-43) and average disease duration was 10 years (CI 5-11). Miyoshi myopathy was diagnosed in 11/25 patients 44% (CI 24-65) and was characterized by the primary involvement of the calf muscles (atrophy, difficulty standing in socks, and contracture of the Achilles tendon). The LGMD phenotype was found in 14/25 - 56% (CI 34-76) of cases, with weakness of the proximal muscles of the lower extremities as the main clinical feature. The level of CK activity was 4547 U/L (CI 1204-8011). The functional status on the MDS was 32 points (CI 22-34), and the functional class according to Vignos was 2. Null homozygous or compound heterozygous mutations were detected in 9/25 – 36% (CI 18-57) of the cases, while in 16/25 - 64% (CI 42-82) of the cases at least one missense mutation was detected.

*Magnetic resonance imaging characteristics of muscle fatty replacement*

When assessing the frequency of involvement of the thigh and lower leg muscles in the myodystrophic process, fatty replacements of grade 1 or more was systematically observed in the semimembranosus, semitendinosus, gluteus minimus, tensor fasciae latae, and adductor magnus (100%) muscles in all patients. In these muscles, the highest degree of fatty replacement was most commonly found in the tensor fasciae latae (grade 4, 50%), gluteus minimus (grade 4, 26.4%), and semimembranosus (grade 4, 16.7%). Less often, fatty replacement was present in the gracilis (61.1%), obturator internus (64.7%), and the adductor brevis (66.7%).

In the calves, fatty replacement of grade 1 or higher was present in the soleus (100%), the medial head of the gastrocnemius (97.1%), the lateral head of the gastrocnemius (94.1%), and the peroneus longus (94.1%) muscles. Grade 4 fatty replacement was observed in the soleus (34.3%) and medial head of the gastrocnemius (32.4%) muscles. More rarely, the popliteus muscle was involved (17.6%). Muscles that were more severely affected by fatty replacement included the hamstring and adductor muscles of the thighs (Fig. 1).

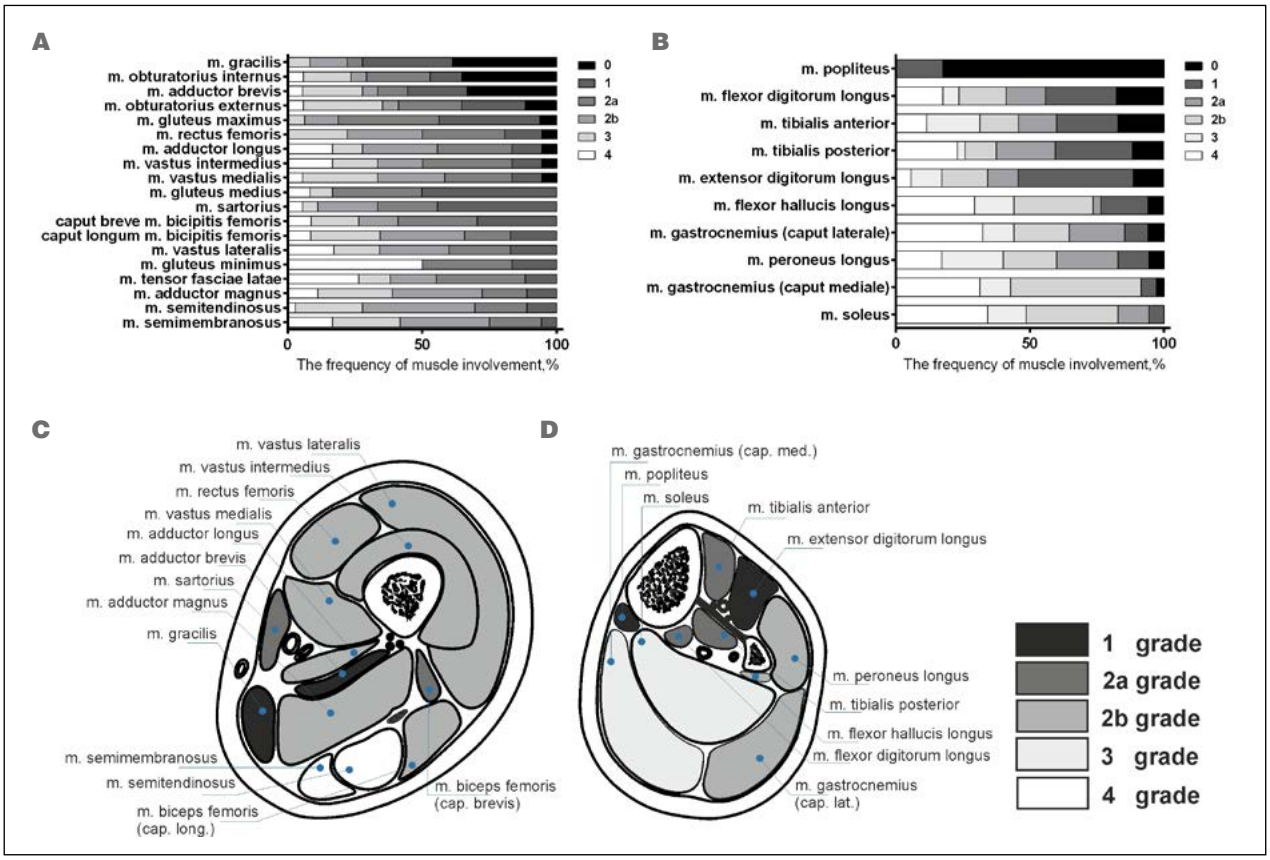


**Figure 1.** Fatty replacement in the pelvis, thigh, and lower leg muscles of patients with limb-girdle muscular dystrophy recessive type 2, *n* = 25 (a, thigh muscles; b, lower leg muscles). The fatty degenerative changes were scored according to the Lamminen-Mercuri scale. The colors indicate the grades of the Lamminen-Mercuri scale, presented as a stacked bar chart. Average magnetic resonance imaging distribution pattern of fatty replacement in limb-girdle muscular dystrophy recessive type 2 muscles (scoring according to Lamminen-Mercuri) in the mid thigh (C) and mid calf muscles (D).

In the pelvic muscles, edema was most often observed in the gluteus maximus (48%), obturator externus, gluteus medius, and obturator internus muscles. Maximum edema severity was characteristic in the gluteus maximus and medius muscles and was minimal (and less common) in the muscles that experienced early onset fatty infiltration (i.e., the tensor fasciae latae and gluteus minimus) (Fig. 2).

Patients with LGMD recessive type 2 (R2) presented with edema in most of the thigh muscles, with the exception of the tensor fasciae latae, semitendinosus, and semimembranosus due to earlier and more pronounced fatty infiltrations of these muscles. Edema was most pronounced in the muscles of the anterior thigh group (the rectus femoris and the vastus lateralis, intermedius and medialis), which was quantified by water T2 relaxation time (MSME). Through visual assessment (STIR), edema was also frequently observed in the anterior thigh muscle group including the vastus lateralis (81.5%), intermedius (70.4%), and medialis (59.3%); sartorius (51.9%); long head of the biceps femoris (51.9%); and the gluteus maximus (48.3%). Edema was less frequently observed in the gluteus minimus, tensor fasciae latae (7.4%), semimembranosus, and semitendinosus (11.1%) muscles.

In the lower legs, edema was detected in the popliteus, the flexor hallucis longus, and the extensor digitorum longus muscles. STIR-T2w visual assessment uncovered frequently occurring edema in the extensor digitorum longus (59.3%), tibialis anterior (48.1%), tibialis posterior (37%), and flexor digitorum longus (37%) muscles. Edema was rarely seen in the gastrocnemius (Fig. 2).

The severity and distribution of fatty replacement in the pelvic girdle showed little variability, with the gluteus minimus muscle being the most frequently affected. In the thigh muscles, it was possible to distinguish in this study three main MRI patterns of fatty replacement: posterior dominant; anterior-dominant and diffuse. However, other rarer anterior-dominant presentation, was also ob-

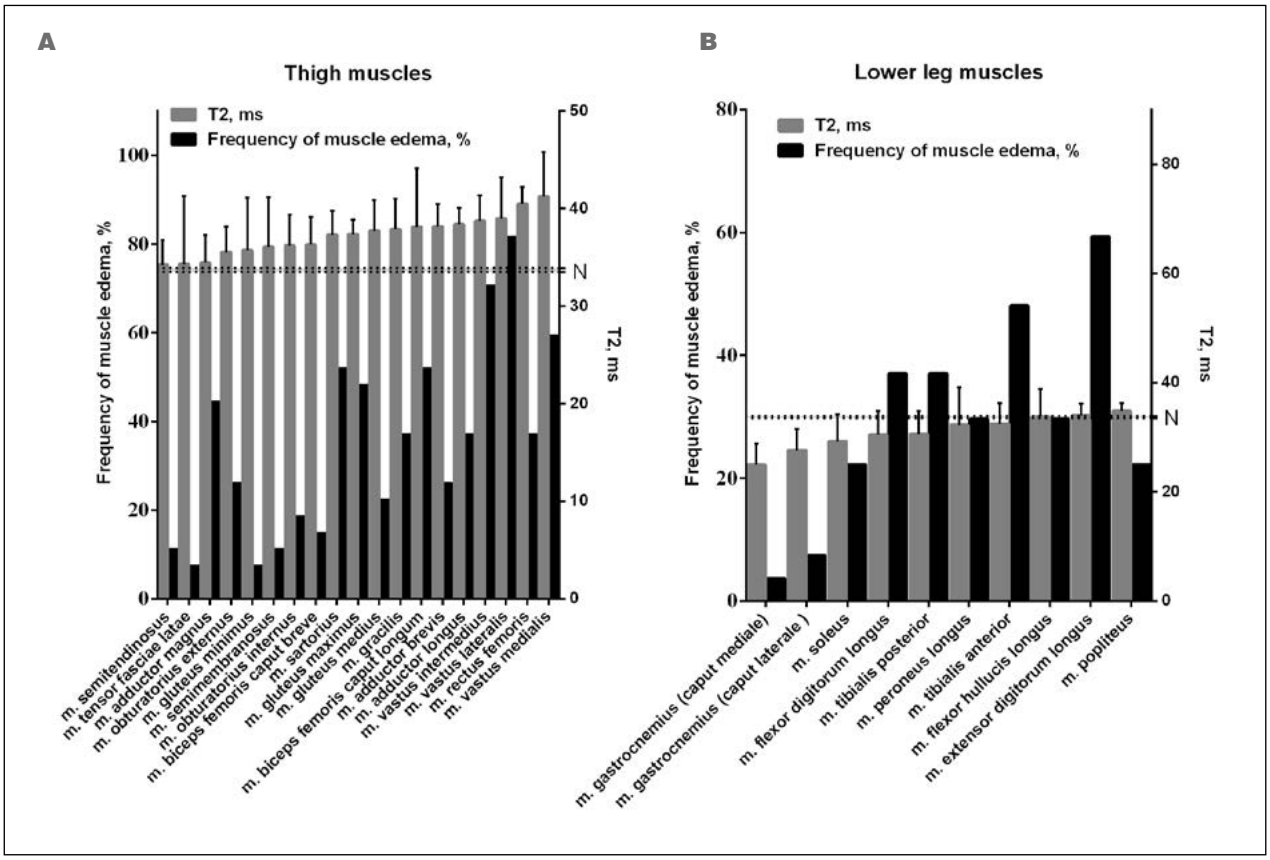


**Figure 2.** The frequency and severity of edema in the muscles of the thighs (A) and lower legs (B) in patients with limb-girdle muscular dystrophy recessive type 2, *n* = 25.

served such as the collagen-like anterior-dominant MRI pattern and the proximal variant. The pseudo-myositis pattern is a separate variant observed at the onset of dysferlinopathy (Tab. I).

*Cases illustrating atypical MRI patterns in dysferlinopathy*

**Patient 1**

A 47-year-old female first manifested the disease at 23 years of age with calf muscle atrophy. This was followed by difficulty in standing on the toes and an inability to run by the age of 25. At 30 years of age she began to notice weakness of the hip muscles when climbing stairs. Muscle strength was reduced in the anterior group of the thigh muscles. Extension of the lower leg was at 3/5 (MRC scale), flexion of the thigh was at 4/5, and in the calf muscles (flexion at the ankle joint) was 4/5. Achilles tendon contractures were observed up to 95°. The phenotype was Miyoshi myopathy at the grade 3 level on the Vignos scale, or 30 points according to the MDS. The CK level was 2573 U/L. A mutation was found in the *DYSF* c.5884C > T gene; p. (Gln1962*) in the homozygous state.

In the thigh, an atypically predominant involvement of the quadriceps (FF up to 85-90%) was noteworthy when compared with the muscles of the posterior and medial groups (FF of 10-55%) (Fig. 3). The typical preservation of the rectus femoris (60%) compared to the vastus lateralis, medialis and intermedius (FF up to 85-90%) was observed. In the posterior muscle group, the long head of the biceps femoris (FF up to 80%) was more affected than the short head (FF up to 43%), with significant asymmetry (FF up to 45% on the right and 80% on the left). The semimembranosus (FF of 35%) and semitendinosus (FF of 20%) muscles were less affected, and no edema was detected in these muscles. In the leg muscles, a typical pattern was observed with predominant involvement of the soleus and both heads of the gastrocnemius (FF up to 90%) that is characteristic of most dysferlinopathy patients. However, the tibialis anterior (FF up to 86%) was more affected than the peronei (FF up to 40%), which is in contrast to the typical pattern (Fig. 3).

**Patient 2**

The disease manifested in a 45-year-old female as

**Table I.** MRI patterns of dysferlinopathy.

| MRI pattern | Description | Number of cases |
|---|---|---|
| Posterior-dominant | Predominant fatty replacement of posterior thigh muscles | 20/25 - 80% (CI 59-93) |
| Anterior-dominant | Predominant fatty replacement of the quadriceps femoris muscle | 4/25 - 16% (CI 5-36) |
| Collagen-like anterior-dominant | Peripheral fatty replacement in the vastus lateralis, medialis and intermedius muscles and a central lesion in the rectus femoris | 1/25 - 4% (CI 0.1-20) |
| Proximal variant anterior-dominant | Severe damage to the thigh muscles, with minimal involvement of the lower leg muscles | 1/25 - 4% (CI 0.1-20) |
| Pseudo-myositis | Edema according to STIR without or minimal fat replacement on T1w | 2/25 - 8% (CI 1-26) |
| Diffuse | Mild / or moderate diffuse infiltration of the anterior and posterior thigh muscle groups equally | 1/25 - 4% (CI 0.1-20) |

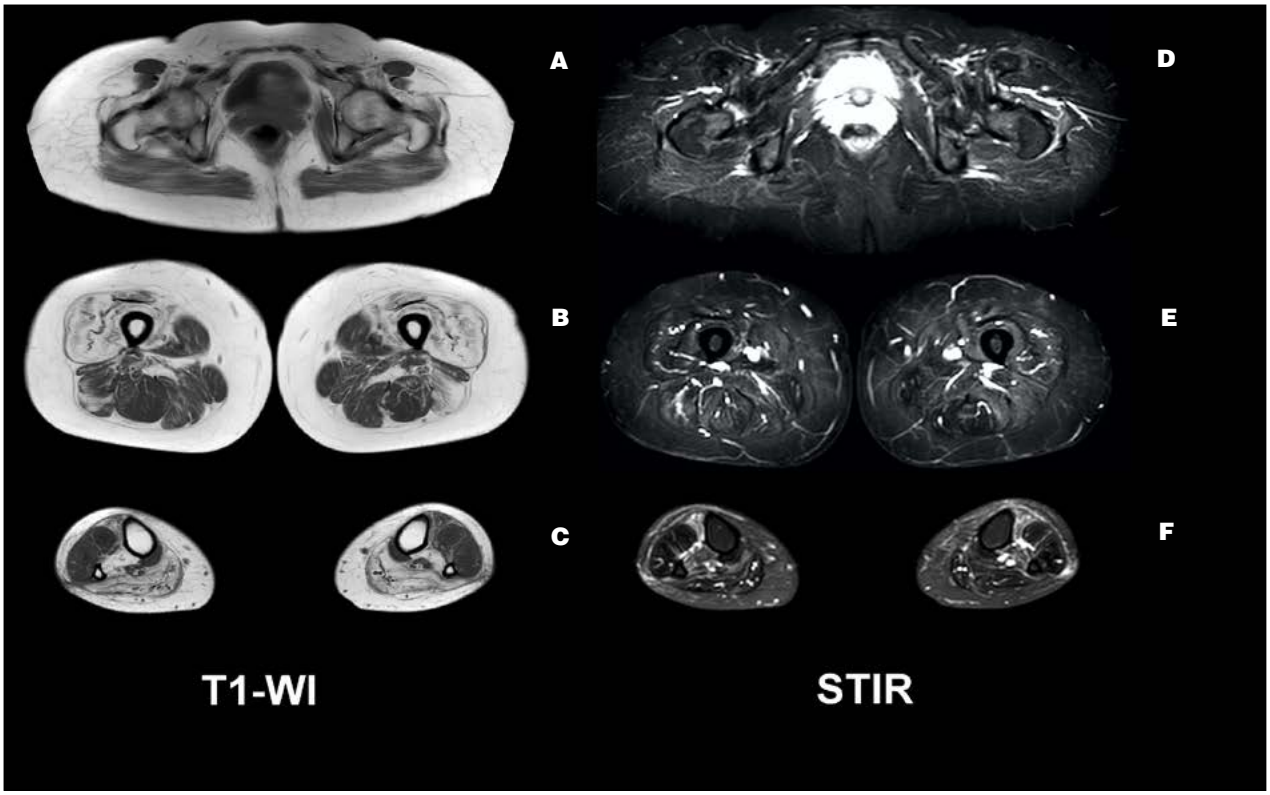


**Figure 3.** MRI (T1, Short Tau Inversion Recovery (STIR)) of the muscles of the pelvic girdle, thighs and legs of patient 1 with the Miyoshi phenotype (disease duration 24 years) (T1-weighted (WI) A, B, C; STIR D, E, F).

the pseudo-metabolic phenotype. Symptoms first began at the age of 22 with swelling of the left leg before each menstrual cycle in combination with weakness of the calf muscles. At 25, she began to notice weakness in the proximal lower extremities when climbing stairs. From the age of 31, her edema was bilateral and lasted 3-7 days per month, and her gait acquired the Trendelenburg sign, and from 43 years of age with moderate steppage gait. Muscle strength was reduced in the distal and proximal muscles of the upper limbs to 4/5 (MRC scale) (hand-grip dynamometry of 8/9 kgf). Strength in the hip flexors decreased to 4/5, while in the muscles of the extensor knee joints it was 2-3/5. In the distal areas of the lower limbs, strength was primarily reduced in the extensors of the feet to 3/5 when compared to the flexors at 4/5. Pronounced atrophy of the calf muscles was observed. The Achilles tendon flexion contracture was 102-114°. The phenotype was Miyoshi and grade 2 on the Vignos scale or 26 points on the MDS. The CK level was 1770 U/L. Mutations in the *DYSF* gene were detected c. 1116C > A. (p. Ser372Arg) with c.759G > C, (p. Gln253His) and was compound heterozygous.

Among the thigh muscles, there was predominant fatty replacement of the anterior thigh muscle group (up to

80-85%) with less involvement of the rectus femoris (FF up to 75%) compared to the posterior and medial muscle groups (FF of 40-70%), which was uncharacteristic for most patients with LGMDR2. The adductor group was characterized by lesser involvement (FF up to 40%) than the posterior group (FF of 50-70%) and exhibited a number of features, including the earlier involvement of the sartorius (FF of 35% - 2b st.) and an intact and hypertrophic gracilis muscle. One notable feature was the fibrotic changes in the distal semitendinosus muscle (Fig. 4B,E). In the lower leg, the triceps surae was preserved (FF of 85-95%), but the predominance of fatty infiltration in the tibialis anterior (FF of 85%) over the peronei (FF of 30%) was uncharacteristic (Fig. 4C,F).

**Patient 3**

This was a 15-year-old male who experienced disease onset at the age of 14 when he began to run more slowly, felt muscle weakness, and reported prolonged recovery after exercise. Muscle strength was slightly reduced in the flexors of the elbow joints 5/5 (MRC scale) and the flexors of the feet 5/5. The extension contractures of the Achilles tendons were 86-87°. The phenotype was Miyoshi and scored a grade 1 on the Vignos scale and 39 points on the MDS. The CK level was 8134 U/L. Mutations found in the *DYSF* gene were p.200_201delTGinsAT, (p.Val67Asp), in the homozygous state.

When investigating LGMDR2 at an early stage of the disease, it is possible to make a false diagnosis of inflammatory myopathy based on atypical MRI signs, including pronounced asymmetric edema on STIR-T2w images and minimal fatty infiltration in the adductor magnus and soleus muscles on T1-WI images. Such a pattern of early muscle changes in LGMDR2 is designated as pseudo-myositis or pseudo-metabolic [1,19] (Fig. 5).

**Patient 4**

A 35-year-old female first exhibited signs of the disease at the age of 30 with the development of pulling sensations in the calf muscles. This was followed by the acute development of weakness in the anterior thigh muscle groups and the extensors of the right foot after undergoing L5 radiculopathy. From the age of 31, the patient lost the ability to run and was unable to stand from a deep squat position on her own. Extension in the knee joint was at 3/5, flexion in the knee joint was 4/5 (MRC scale), and hip flexion was 4/5. The Achilles tendon contractures were up to 95°. The phenotype was LGMD, grade 3 on the Vignos scale, and 38 points on the MDS. The CK level was 2091 U/L. Mutations in the c.6313G > A gene

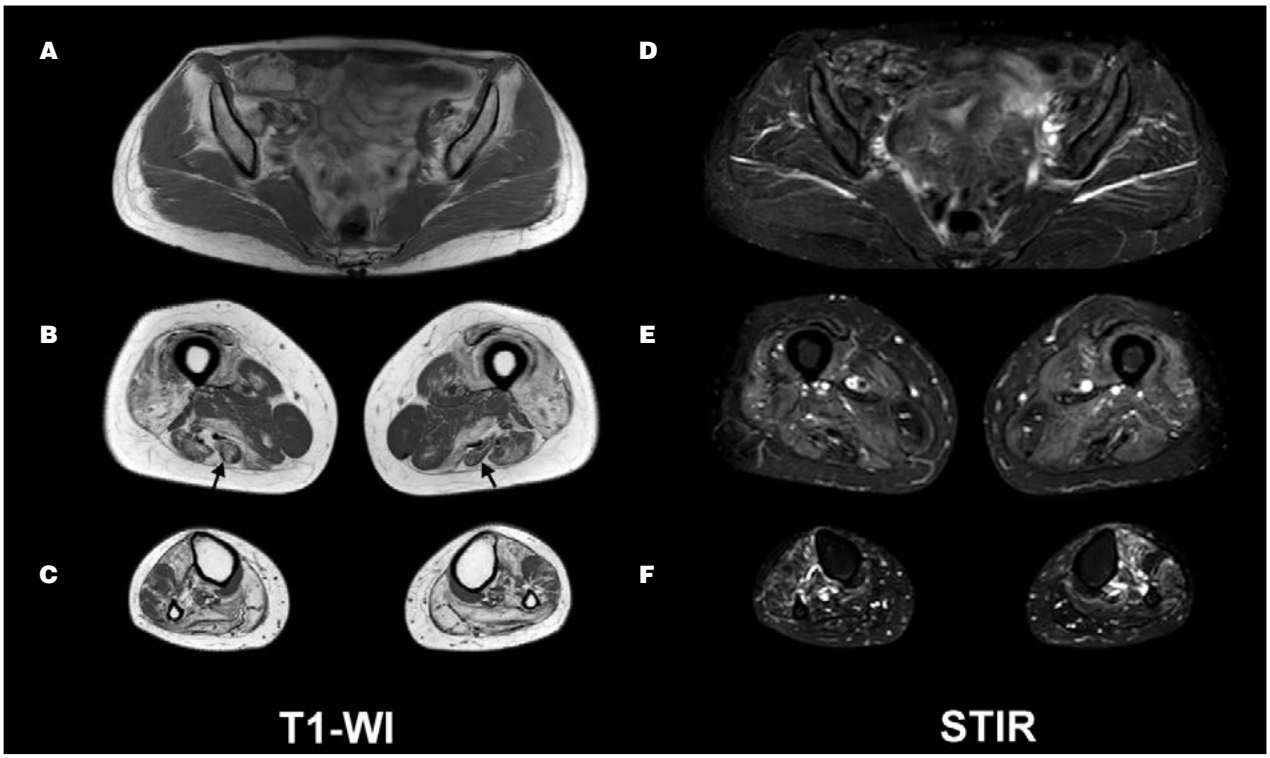


**Figure 4.** MRI pattern of fatty muscle infiltration of the pelvic girdle, thighs and legs of patient 2 (T1-WI A, B, C; Short Tau Inversion Recovery (STIR) D, E, F). Note fibrotic changes in the distal semitendinosus muscle (images B and E, marked with arrows).

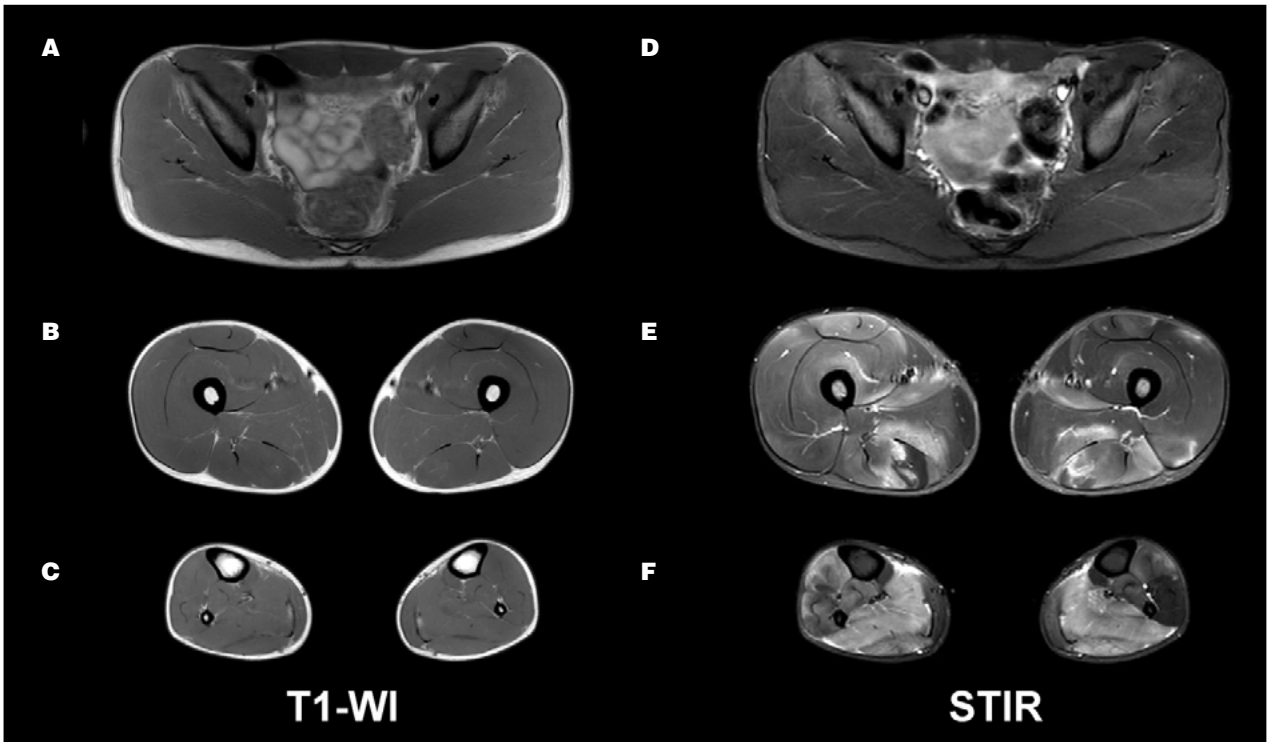


**Figure 5.** MRI pattern of the distribution of fatty infiltration in the muscles of the pelvic girdle, thighs and lower legs in patient 3 with a disease duration of 1 year. (T1-WI A, B, C; Short Tau Inversion Recovery (STIR) D, E, F). Arrows indicate fibrosed muscles.

*DYSF* (p.Ala2105Thr) were detected; c.4282C > T (p.Gln1428Ter), and was compound heterozygous.

There was the predominant fatty infiltration of the anterior thigh muscle group relative to the posterior and medial groups. The distribution of fatty replacement in the anterior thigh muscle group was similar to the lesion pattern in congenital muscular dystrophies associated with type VI collagen mutations (target-like sign) (Fig. 6E). This collagen-like MRI pattern has also been described for LGMDR1 as an atypical variant associated with a severe disease course (Fig. 6D). The muscles of the medial and posterior groups exhibited a more pronounced fatty replacement in the right limbs (FF of 45-70% on the right side and 20-40% on the left). The asymmetric muscle involvement is a fairly common feature of LGMD recessive type 2 (R2) and, in this case, was possibly related to predominant loading on the right lower limb due to chronic left radicular pain syndrome (Fig. 6A-C). In the lower legs, the pattern of fatty replacement was classic dysferlinopathy, with asymmetric destruction of the soleus (FF up to 90% - 4 st. on the right and an FF of 15% - 1 st. on the left) and the peronei (FF up to 55% - 2b st. on the right and an FF of 10% - 1 st. on the left).

**Patient 5**

A 64-year-old female first experienced the disease at the age of 45 with gradually increasing weakness of the thigh muscles. By the age of 55, climbing stairs had become difficult. Muscle strength was reduced in the hip flexors (4 points), the leg flexors (4 points), and the deltoid (4 points). The knee and Achilles reflexes were reduced. The Achilles tendon extensor contracture was up to 98-99°. The phenotype was LGMD, grade 1 on the Vignos scale and 39 points according to the MDS. The CK level was 290 U/L. Compound heterozygote mutations were identified in *DYSF* c. 6116G > A (p. Arg2039Gln), c. 1692 + 8G > A. A muscle biopsy revealed a cytoplasmic pattern of muscle fiber staining and the absence of dysferlin in individual muscle fibers.

For this patient, who experienced a long course of the disease (9 years) against the background of age-related initial fatty infiltration of most muscles, minimal involvement of all gluteal muscles was observed (FF up to 25%). In the thigh muscles, predominant involvement of the vastus lateralis, medialis and intermedius (up to 35% - 2b) is atypical for most cases of LGMDR2. The rectus femoris was preserved (FF up to 5%) and relatively hypertrophied. The calf muscles presented with a minimal

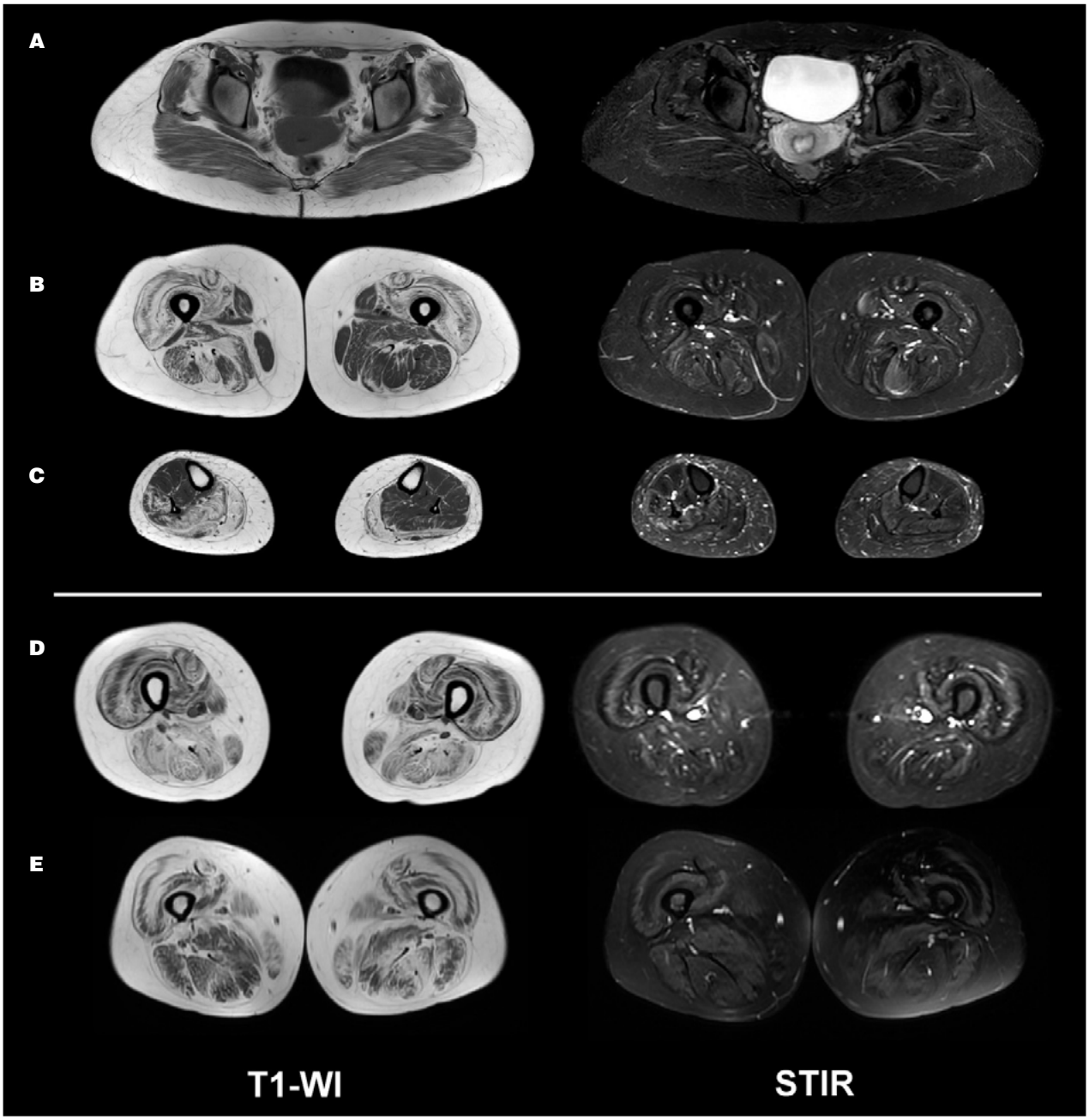


**Figure 6.** Collagen-like MRI pattern of muscle damage in the pelvic girdle, thighs and legs of patient 4, a 35 year old with limb-girdle muscular dystrophy recessive type 2 (LGMDR2) and a disease duration of 5 years (A, B, C). The MRI pattern of fatty replacement in the thigh muscles of a 27-year-old patient with LGMD recessive type 1 (D); and patient B, a 47 year old with congenital Bethlem myopathy (E). T1-WI, weighted; STIR, Short Tau Inversion Recovery.

involvement of the soleus (FF up to 30%) with signs of moderate hypertrophy of the medial head of the gastrocnemius (Fig. 7A-C). The diffuse and moderate muscle damage with a late disease onset was probably due to the retention of a certain amount of dysferlin protein expression, which was confirmed by immunohistochemistry and Western blot analysis of a biopsied muscle sample (Fig. 7G-I).

**Patient 6**

A 30-year-old male first experienced disease at the age of 19 when he developed weakness when rising from

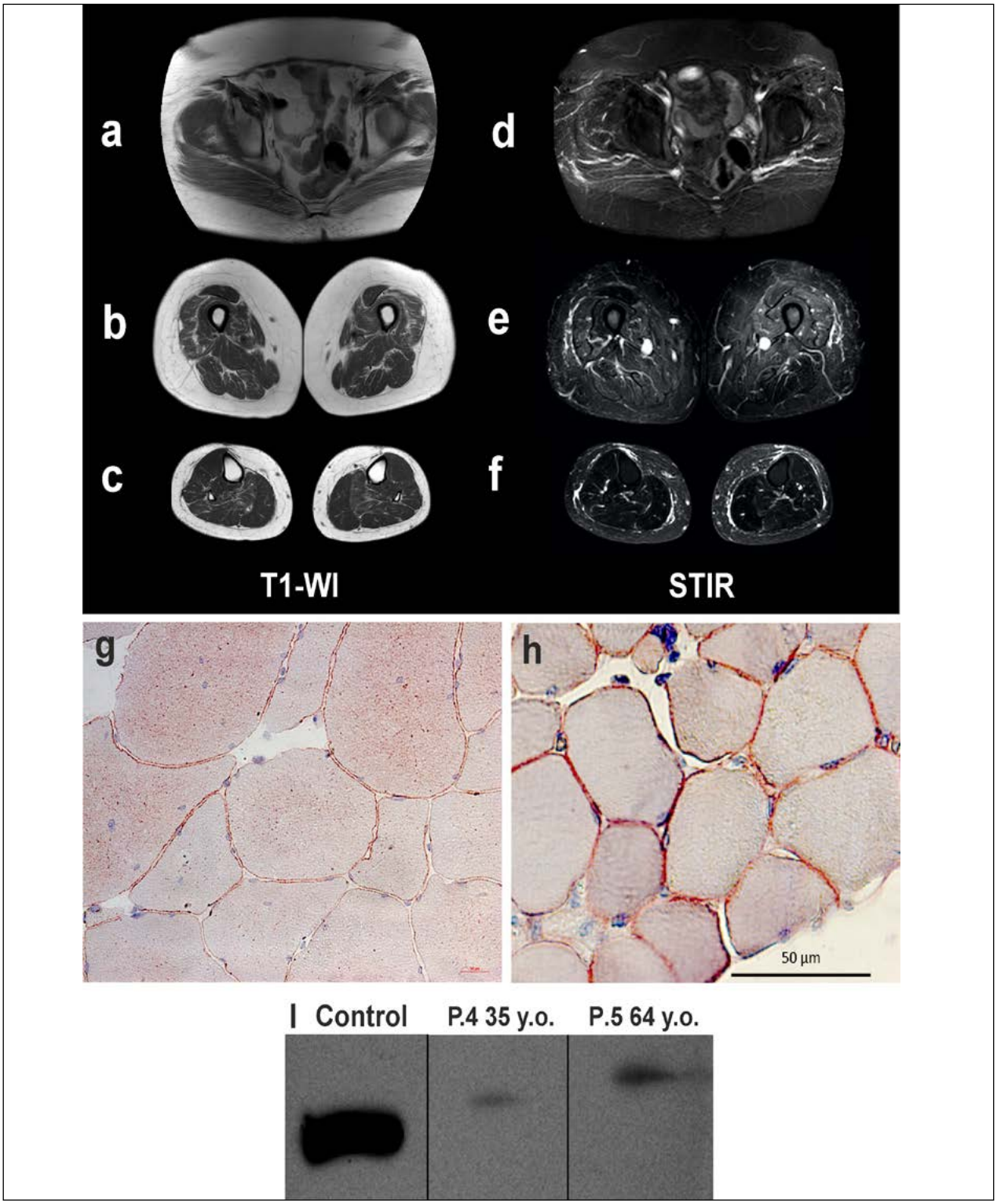


**Figure 7.** MRI pattern of fatty replacement distribution in the muscles of the pelvic girdle, thighs and legs of patient 5, a 64-year-old female with a disease duration of 9 years (T1-WI A, B, C; Short Tau Inversion Recovery (STIR)-T2w D, E, F). Immunohistochemical study of muscle biopsy samples from patients 5 (64 years old) and patient 4 (35 years old). Normal membrane dysferlin staining is observable in the vastus lateralis of patient 5 using antibodies to dysferlin (G). Control (H). Western blot analysis showed a low level of dysferlin protein expression in patient 4 and a decrease in expression of more than 60% in patient 5 (I).

a sitting position. From the age of 25, there was weakness in the calf muscles. At the age of 27, he began to notice weakness in the proximal and distal parts of the limbs. Muscle strength was reduced in the proximal (4/5 points) and distal segments (hand-grip dynamometry was 12/10 kgf) of the upper limbs. In the lower extremities, reductions were observed for hip flexion (4 points), knee flexion and extension (3 points), and foot extension (4 points). Tendon reflex was reduced in the knee; however, the Achilles reflexes ware normal. Tendon flexion contractures of the Achilles tendons it was 95/93°. The phenotype was LGMD, grade 3 on the Vignos scale and 32 points on the MDS. The CK level was 1930 U/L. Mutations in the *DYSF* gene were detected c. 1724T > S. (p. Leu757Pro), in the homozygous state.

Substitution of adipose tissue in the muscles of the thighs was typical, with predominant involvement of the posterior muscle group (FF up to 98%). In the quadriceps, the characteristic preservation of the rectus femoris (FF up to 30%) compared with the vastus lateralis, medialis and intermedius muscles (FF of 45-50%) was observed. Damage to the adductor group was atypical, with more pronounced fatty replacement in the adductor longus (FF of 95% - 4 st.) than the adductor magnus (FF of 84% - 2b - 3 st.). In this patient, despite the long course of the disease, minimal diffuse degenerative damage in the lower legs (FF of 10-30% - 1-2a st.) contrasted with the severe fatty replacement of the thigh muscles (Fig. 8).

## Discussion

Previous MRI imaging studies conducted with small patient samples using semi-quantitative T1w sequences have determined the main characteristics of muscle fatty

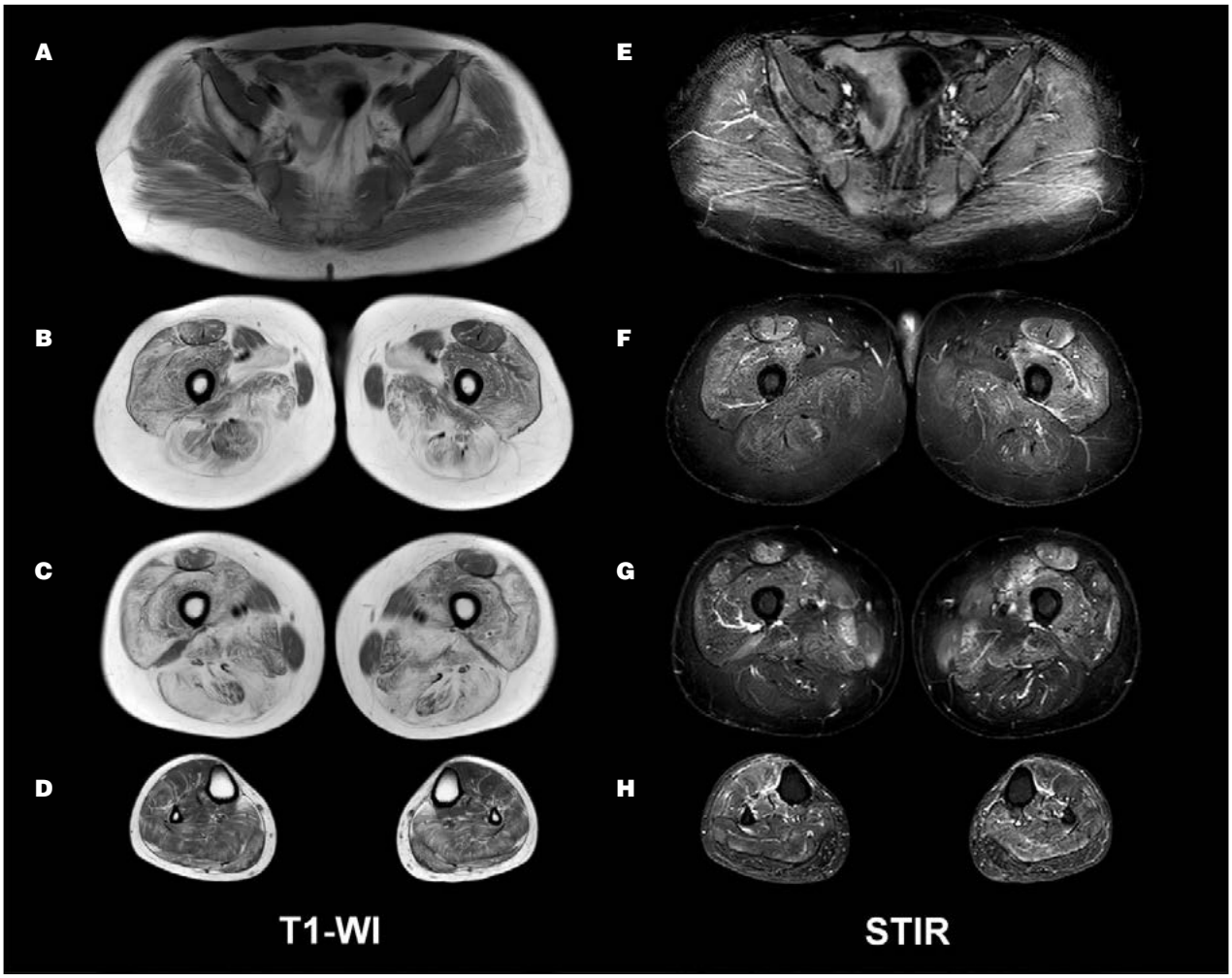


**Figure 8.** MRI pattern of the distribution of fatty infiltration in the muscles of the pelvic girdle, thighs and lower legs of a 30-year-old patient with limb-girdle muscular dystrophy recessive type 2 and a disease duration of 11 years (T1-WI A, B, C, D; Short Tau Inversion Recovery (STIR) E, F, G, H).

replacement patterns in dysferlinopathy using individual muscle Lamminen-Mercuri grades, grade frequency, and disease progression in the different muscle groups. In the largest study of 182 patients with dysferlinopathy, the idea of a typical MRI pattern and its evolution during disease progression was generalized [5]. Therefore, for everyday clinical practice, it is important to describe the entire spectrum of variants observable via MRI patterns of muscle damage in dysferlinopathy.

One of the key MRI features of dysferlinopathy is muscle edema, which often leads to an erroneous diagnosis of inflammatory myopathy [20]. Edema of at least two or more muscles identified either qualitatively (STIR) or quantitatively (T2 MSME) was characteristic of all of our patients. Among the pelvic muscles, the most frequent and pronounced diffuse edema (according to STIR) was observed in the gluteus maximus and medius muscles, respectively. Edema was minimal and rare in the tensor fasciae latae and the gluteus minimus, which corresponds with previously reported data [7,11,17]. In our patients, most of the thigh muscles were characterized by the presence of edema, with the exception of the semitendinosus and semimembranosus muscles (11.1%). Edema occurred most frequently and was more pronounced in the anterior and medial groups of the thigh muscles including: the vastus lateralis (81.5%), intermedius (70.4%), medialis (59.3%); the sartorius and long head of the biceps femoris (51.9%); and the adductor magnus (46.6%), which is consistent with earlier studies [6,10]. Diaz-Manera et al. [17] has noted that edema in the sartorius, gracilis and rectus femoris mainly develop during the late stages of the disease.

Edema was most often observed in the extensor digitorum longus (59.3%), tibialis anterior (48.1%), tibialis posterior (37%), and flexor digitorum longus (37%), while the gastrocnemius was characterized by less swelling. In a relatively small number of cases, it has been shown that, in the early stages of the disease, edema is observable in the soleus and caput mediale m. gastrocnemii and, during the later stages, in the anterior and lateral muscle groups of the lower leg [6,21]. In a cohort of Chinese patients, Jin et al. [10] described the MRI pattern of thigh muscle edema in addition to the MRI pattern of fatty infiltration.

By comparing information concerning the MRI features of muscle damage during dysferlinopathy, we can confirm that severe edema occurs in muscles that are characterized by fatty infiltration and increased physical activity at this stage of the disease [17]. Therefore, for patients already exhibiting fatty infiltration in the posterior group of thigh muscles, edema in the anterior group will most often be observed and, subsequent to their fat replacement, edema will occur to a greater extent in the gracilis and sartorius muscles. In presymptomatic cases, edema is often detected only in the posterior and medial groups of the thigh muscles, as well as in the medial head of the gastrocnemius. Though muscle edema is usually observed in inflammatory myopathies [22], however it can be present in other genetic muscle disorders such as FacioScapuloHumeral muscular Dystrophy [23], LGMDR12 (already known as LGMD2L) [24] and Pompe disease [25].

With hundreds of dysferlinopathy cases now reported, the most common MRI patterns of muscle involvement have been unambiguously identified. However, insufficient attention has been paid to individual variability in the muscle damage of patients from various socio-ethnic groups. In our sample, no significant lesion variability was uncovered for the muscles of the pelvic girdle. In the calves, muscle damage variability was also minimal and exhibited earlier or simultaneous involvement of the tibialis anterior and posterior muscle groups. This was in contrast to the typical variant, which is predominant in the posterior and lateral groups. A similar type of variability has been described by Illa et al. [3] as a DMAT phenotype.

In our sample, the greatest variability of the lesions was characterized by the thigh muscles, for which three main types of MRI patterns were identified: proximal in 20/25 cases, 80% (range 59-93); anterior-dominant in 4/25 cases, 16% (range 5-36); and diffuse in 1/25 cases, 4% (range 0.1-20). The prevalence of the typical posterior dominant pattern was comparable to the results of Angelini et al. [17] but significantly higher than in a cohort of patients from China (56%) and Germany (40%) despite the absence of differences in gender or age in the compared groups [9,10]. Among patients with an anterior-dominant pattern, we distinguished the collagen-like variant, which was has been previously described in patients with congenital collagen VI associated myopathy and in severe LGMDR1 [26,27]. The diffuse MRI pattern that we identified in one case was much more common among patients in a Chinese cohort (12/57, 23%, range (13-37)) [10]. It should be noted that the diffuse nature of the lesions was observable from the onset of the disease, and was not a consequence of pronounced fat replacement during the late stages of dysferlinopathy, as presented by Arrigoni [6] in a quantitative analysis of thigh muscles with equal involvement of the anterior and posterior medial groups. In addition, the rare proximal variant was characterized by gross damage to the thigh muscles, with minimal involvement of the lower leg muscles, which often leads to an erroneous diagnosis of sarcoglycanopathy, Pompe disease, or LGMD type R9.

The pseudo-myositis MRI pattern occurred in 2/25, 8% (range 1-26) patients and was characterized only by the presence of edema according to STIR. There was an absence of muscle atrophy and fatty replacement, which may be a sign of an early stage of the disease. This MRI pattern may correspond to the previously described pseu-

do-metabolic phenotype [19]. The pseudo-metabolic variant was observed in 2% out of 193 patients from different European countries [28].

The relatively small patient sample size can be a limitation of our study. However, the diversity of ethnic groups included in the sample is a positive aspect.

## Conclusions

Increasing the efficiency of routine diagnoses of dysferlinopathy using MRI depends not only on knowledge of the typical distribution of fatty infiltration and muscle edema, but also on understanding the sequence of involvement of the muscle groups, as well as taking into account individual variants from MRI patterns.

*Ethical consideration*

None.

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*Conflict of interest*

The Authors declare no conflict of interest.

*Author contributions*

The Authors have contribuited equally to the work.

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# How to define and enhance diagnostic and assistance pathways in neuromuscular diseases during the COVID-19 pandemic: the concept of network

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**The main consequence of the COVID-19 pandemic has been to increase the distance between patients and their doctors and to limit the opportunities to compare experiences and clinical cases in the medical community. Based on this, we adopted a strategy to create networks with the ambition to break down these distances and to unify the process of care and management. Here we report the results and perspectives of our efforts and studies. A summary of the presentations on the topic, held during the webinars organized for macro-areas by the Italian Association of Myology with the aim of raising awareness among "non-expert doctors" who deal with neuromuscular disorders in the era of COVID-19 was collected and here reported. Although the macro-areas responded in different way to the problems of neuromuscular patients in the era of COVID-19, they all have tried to create a network between doctors and opportunity for education and information, with the secondary outcome to have shared process of care and management. Telemedicine, virtual meetings and the strengthening of national and international networks, through research projects, were the nodal and common points. Due to their complexity, neuromuscular diseases had already taught clinicians the importance of multidisciplinary confrontation. COVID-19 has further strengthened the need to create links between clinicians and experts, even of different nationalities, in order to guarantee to patients the best possible care, but above all, access and continuity of care even in critical periods. Adequate answers have been given to these problems, though there is still a lot to improve.**

**Key words:** neuromuscular disorders, COVID-19 pandemic, telemedicine, networks

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## Introduction

The impact of coronavirus disease 19 (COVID-19) pandemic in the care of many diseases, and in particular rare, chronic and disabling diseases such as Neuromuscular Disorders (NMDs) has been significant.

National health care services underwent a radical reorganization, with in-person consultations being postponed, and not considered urgent treatments delayed or canceled. In addition, there was a rapid implementation of remote approaches to patients [1].

Therefore, patients with NMDs not having guarantees of care and treatment as before the pandemic were at greater risk of developing severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) infection [2] and experienced deeper distress than to other patients, and prolonged home isolation [1].

A multicentric national survey [3] promoted by the Italian Association of Myology (AIM) showed since the first period of the pandemic, a significant malfunction of clinical and support services for patients affected by NMDs at national level, with the following outcomes. First, 40% of surveyed tertiary neuromuscular centers reported a reduction in outpatient visit and examinations, while 22% postponed in-hospital administration of therapies and the vast majority (93%) reduced or suspended rehabilitative services and on-site outpatient visits. Second, the possible worsening of all NMD conditions due to the indirect consequences of the pandemic SARS-CoV-2 infection, as well as the need to stop or significantly restrict the access to clinical trials, was also reported [3].

Because of these critical issues, the community of NMDs medical experts tried to reconsider the gold standard of excellence in disease management, evaluating the risk/benefit ratio of specific interventions (pharmacological and rehabilitative), with respect to the possibility of exposure to the disease. They also re-thought the clinical trial procedures to ensure remote participation in all phases of the study, from enrollment to drug administration and follow-up [4].

This effort was also made in Italy with the aim of creating an approach shared by all Italian third-level centers and facilitating an early diagnosis also at local level.

Thus, the AIM Board proposed a series of webinars aimed at raising awareness among "less experienced" doctors to create a network that could support patients, despite the lack of medical care and the isolation imposed by the COVID-19 pandemic.

Here we report the Italian experience derived from sharing a "new approach to care" during these webinars divided into two macro-areas of our Country (North-Center and Center-South Italy).

## Methods

The experience on the management of the neuromuscular patient during the COVID-19 pandemic, shared through lectures presented in the webinars and entitled "The management of the patient with neuromuscular disease in the pandemic period: from telemedicine to the vaccine experience" is proposed below by comparing, for simplicity, two national macro-areas.

## Results

*North-Central Italy*

In the North-Center, the first Italian macro-area that faced the spread of COVID-19 pandemic, the main strategy adopted was to create networks to reduce the distances imposed by the pandemic and to standardize the care and management process. This was possible by defining three types of networks, networks between stakeholders, regional networks and national/international networks. To improve the network between doctor and patient, face-to-face visits were replaced by telemedicine appointments. This modality cannot replace the direct neuromuscular examination but has the advantage of monitoring the patient's clinical status through anamnestic collection and the tests performed.

A rehabilitation experience using telemedicine was also reported [5]. Notably, a 28-year-old man with Charcot-Marie-Tooth disease who had received a probable SARS-CoV-2 infection, was able to benefit of continuing hand rehabilitation after tendon transfer surgery via telerehabilitation. It was also possible to advise the patient on ways to prevent the spread of infection and to cope with restrictions that limited outpatient visits [5].

In Padua, particular attention was paid to the reorganization of the paediatric palliative care (PPC) referral service. The improvement of their standard of care was achieved by collecting data on the consequences of COVID-19, by holding multidisciplinary meetings to share the approach with professionals involved in PPC in the Veneto region and by preparing educational material on COVID-19 for patients and their families. The data collected were used to assess real needs, and to develop a more appropriate reorganization model [6]. The following strategies were in particular put in place: (i) a 24-hour telephone service assisted by an experienced nurse; (ii) the reduction as much as possible of non-urgent home activities, to favor urgent follow up and critical interventions; (iii) the management of non-urgent needs through advice and 24-hour training for the COVID-19 emergency for healthcare professionals and families; (iv) monitoring and training of patients and families through the use of telemedicine through nursing telephone monitoring

every 15-30 days; (v) converting physiotherapy activity into therapist-led video call interventions; (vi) mandatory SARS-CoV-2 swab testing for patients and relatives prior their admission to the hospitals [6].

The PPC referral service in Padua collected also experiences in children with SMA who were obliged to postpone their hospital-based therapy (i.e., infusions of nusinersen®). Forty-eight % of parents of children with SMA perceived a worsening of muscle strength, although no correlation between delayed treatment and changes in functional scores in short and long term assessments was found [7]. This discordant perception is mainly due to a state of parental anxiety related to the suspension of therapy and physiotherapy, and to a perception of changes in their QoL.

In accordance with this statement, an important study was carried out in Lombardy with the aim to evaluate the consequences of COVID-19 pandemic measures and prolonged home isolation on quality of life (QoL) and perceived disease burden [8]. Between February and May 2020, 350 NMD patients underwent a telephone interview. The results showed that the virus outbreak impaired some aspects of QoL and affected access to outpatient care and ancillary services, with limited use of remote alternatives [8].

At the regional level, a study from the Liguria region analyzed the impact of SARS-CoV-2 infection on patients with NMD and in particular on those affected by Myasthenia Gravis and Guillain Barré syndrome (GBS), in consideration of their standard immunosuppressive therapy. The study concluded that SARS-CoV-2 infection could both cause GBS and affect the outcome of patients with non COVID-19 related GBS [9].

An empowerment of national and international networks was done to improve collaboration and encourage the birth of new research projects. Colleagues from the Liguria region participated in the national NeuroCovid project, a multicenter cohort study on neurological disorders associated with COVID-19, conducted in 51 centers in Italy, sponsored by the Italian Society of Neurology (SIN). It seems that a wide spectrum of treatable neurological manifestations may be associated with COVID-19 infection, including hypo-ageusia, hyposmia, acute ischemic stroke, delirium, headache, cognitive impairment, abnormal behavior or psychosis, seizures, GBS, severe encephalopathy with stupor or coma, dizziness, encephalitis and hemorrhagic stroke, and most cases occur in middle-aged adults with mild or severe respiratory syndrome.

On the other side, a national multicenter study under the leadership of tertiary NMD center in Milan documented the disease course and outcome of COVID-19 viral infection in NMD patients, and investigated the potential acute exacerbations of muscle symptoms in these patients. The study concluded that COVID-19 manifestations and morbidity in NMD patients were similar to those presented in the general population and there were no objective changes in disease course during COVID-19 infection.

*Central-Southern Italy*

The approach of the central-southern regions was different. As part of the reorganization of health- services, NMD experts valued the figure of the family pediatrician as key player in connecting patients and families to health professionals. In particular, the importance of the pediatricians in achieving an early diagnosis was underlined, and in line with a project shared by the Italian Society of pediatricians (SIMPe) and AIM, the training of these figures and their entrustment with the role of "case manager" of the clinical picture, were promoted.

A pediatric epidemiology and research network (RePER) was activated since the outbreak of the pandemic began [10,11], with web-based focus and training on rare conditions NMDs included. RePER created training web pages on various diseases with the aim of improving information, knowledge and care over time. These specific web pages are easy to consult and indicate the characteristics, the warning signs, the diagnostic possibilities, and the specialist reference centers for each disease. For some pathologies, experts have defined a summary of symptoms to consider when raising the suspicion of an infection, useful for implementing the likelihood of a timely diagnosis.

In order to strengthen the network between pediatricians and NMD expert reference centers and to increase knowledge on neuromuscular pathologies, RePER activated a series of web conferences held by experts for training purposes. Finally, the role of pediatricians as "case manager" has allowed a direct relationship with families and integrated commitment with other health professionals, school and specific needs.

Finally, the staff views on the changes in the care provided by a rehabilitation centre as part of a larger project investigating the impact of these changes on professionals, patients and their families were reported [12]. The survey was conducted using an open-ended questionnaire including six-items, on the practical and psychological aspects that emerged during the pandemic, in relation to the healthcare services provided by the centre and to the patients/caregivers conditions. The participants, most of them physiotherapists, highlighted 169 aspects emerging in the pandemic, 48.5% referring to the resources used to cope with critical issues and 51.5% concerning the difficulties encountered. Emotional aspects prevailed on practical aspects both in resources (52.4 *vs* 47.6%) and

in difficulties (57.5 *vs* 42.5%) categories. In particular, with regard to patients' resources, psychological benefits, despite the burden, were greater than practical ones (87 *vs* 13%), in the form of improved intra-family relationships, feeling more cared for, and satisfaction for the received care.

## Discussion

Although the experiences reported here, implemented to address the difficulties in maintaining adequate care of the NMD patients at the time of the COVID-19, are different and not integrated in a common national health plan, they summarize few salient and common points as the need for information, the need for training, the need for sharing. During the of COVID-19 outbreak, we saw that the best way to get assistance in NMDs was to strengthen or even create networking by considering all stakeholders.

In fact, only by creating shared paths and with well-organized flows at various levels, it is possible to overcome unexpected events, or insurmountable challenges such what we had to face with rare diseases in general and with NMDs in particular.

One tool that has proved useful in networking is telemedicine that has offered an immediate solution to break down both physical and psychological barriers. The use of telemedicine for visits, rehabilitation and training was the strategy shared by both macro-areas. The telemedicine model was developed not solely because of this need. Previous studies aimed to assess whether this method was adequate and comparable to face-to-face visits. Hosbon et al., in 2016 [13] found that Amyotrophic Lateral Sclerosis (ALS) patients treated with remote approaches had the same level of care and comparable survival as those with face-to-face visits. Furthermore, they reported that a virtual approach seems to reduce emergency room access and acute hospitalizations. Similar conclusions have been reached by Portaro et al. [14] for patients with Facio-Scapulo-Humeral Dystrophy (FSHD).

Another useful tool was the implementation of online sharing of critical care and second opinion through videoconferencing consultation. This tool in the age of information technology should be encouraged, by allowing for more frequent meetings, the emergence of new research opportunities and in general, more shared decision-making.

Furthermore, the empowerment of national and international networks has allowed the definition of common guidelines for the management of neurological complications from COVID-19 infection and for vaccinations.

In conclusion, the COVID-19 pandemic has confronted the healthcare system with an unexpected difficulty in managing patients with rare, debilitating and complex diseases, such as neuromuscular diseases. However, the resilience of the clinicians who deal with these pathologies has made it possible to find and put in action strategies that reduce the distances by favoring connections at multiple levels of intervention (Fig. 1). Of course, the path is still too long, web connections are not always optimal and education to use new tools is not widely spread. However, we are aware that, as a popular saying goes "the road is harder when you're headed for the sky".

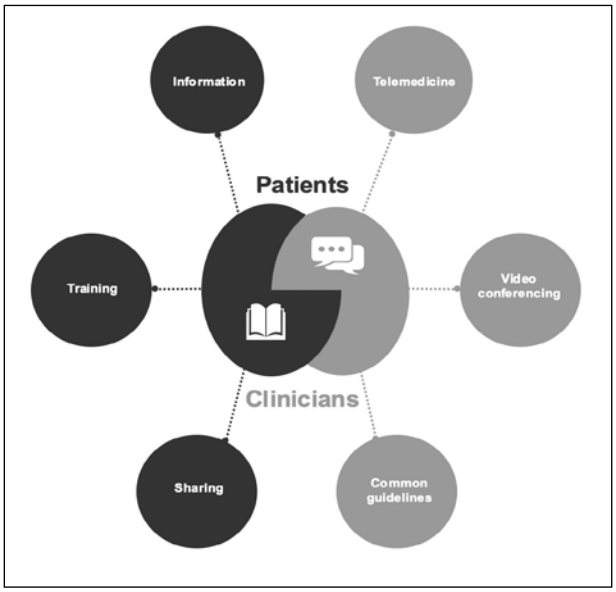


**Figure 1.** Graphic layout of action strategies implemented to overcome the difficulties imposed by the COVID-19 pandemic.

*Ethical consideration*

No mention is made of sensitive data referable to patients.

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*Conflict of interest*

All the authors declare no conflict of interest.

*Author contributions*

GA and LP conceptualized this study after having investigating the interest in sharing the information of the

various co-authors. CA, DG, MG and MGi acquired and described the data concerning their territorial reality.

GM and GA analyzed the available data in the light of the literature and wrote the draft, under the guidance, supervision and methodology of LP. GA and LP reviewed the final version of the paper

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CASE REPORTS

# BAG3-related myofibrillar myopathy: a further observation with cardiomyopathy at onset in pediatric age

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**Myofibrillar myopathies are a heterogeneous group of neuromuscular disorders characterized by degeneration of Z-disk, causing the disintegration of myofibrils. They may be caused by mutations in different genes, among these, the BAG3 gene (Bcl-2 associed-athanogene-3) encodes a multidomain protein that plays an important role in many cellular processes. We report the case of a 16-year-old male who at 4 years of age presented with a hypertrophic obstructive cardiomyopathy, then developed axonal sensory motor polyneuropathy, muscle weakness, rigid spine, severe kyphoscoliosis and respiratory failure. Muscle biopsy showed the typical hallmark of myofibrillar myopathy with abnormal cytoplasmic expression of multiple proteins. Ade novo heterozygous common mutation in the BAG3 gene with a c.626C > T (p.Pro209Leu) was discovered on NGS genetic analysis. Mutations in the BAG3 gene are causes of a severe and progressive condition and natural history data are important to be collected. An early diagnosis is critical for prognostic implications in cardiomyopathy and respiratory failure treatment.**

**Key words:** BAG3, myofibrillar myopathy, cardiomyopathy, pediatric neuromuscular disorder

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## Introduction

Myofibrillar myopathies (MFM) are a heterogeneous group of neuromuscular disorders caused by mutations of different genes that share myopathological features, first of all the disintegration of Z-disks followed by myofibrillar disruption and ectopic accumulation of multiple proteins [1]. Each gene produces a mutated protein which is an integral part of the Z-disk or is closely associated with it. The clinical manifestations of various subtypes can change and may include different age of onset (from childhood to late adulthood) and distal more than proximal weakness, cardiopathy, respiratory failure, cataracts or peripheral neuropathy in various

combinations. The diagnosis of MFM is based on clinical findings, electromyography, nerve conduction studies and muscle histology. The most frequent inheritance pattern of the MFM-causing genes is autosomal dominant, and in a significant number of patients the mutation occurs *de novo* [2-3].

An early-onset subtype of MFM is caused by a mutation in *BAG3* gene on chromosome 10, encoding for the antiapoptotic Bag3 (Bcl-2 associed-athanogene-3) protein. Bag3 is a multi-domain protein that regulates the Hsp70 family of molecular chaperones and that interacts with many other polypeptides, strongly expressed in skeletal and cardiac muscle and at lower level in other tissues. Bag3 is involved in a panoply of cellular processes such as development, apoptosis, autophagy, cytoskeleton organization, cell adhesion and motility [4].

The clinical presentation of BAG3-related MFM is usually characterized by limb and axial muscle weakness, peripheral neuropathy, cardiomyopathy and respiratory failure. In these patients the mutation P209L re-occur at a high frequency. In the typical presentation of MFM due to BAG3 mutation, childhood cases can be particularly severe with rapid progression of the clinical picture and death in early adolescence [5-7].

With the aim of contributing to better defining the natural history of BAG3-related MFM, we report here a Caucasian 16-year-old male patient with the p.Pro209Leu (c.626C > T) in exon 3 mutation and cardiomyopathy as first clinical sign associated with peripheral neuropathy and MFM due to BAG3 mutation.

## Case report

The 16-year-old Caucasian male had a negative family history for neurological disease and normal physiological history. Developmental milestones were normal. At 4 years a heart murmur was discovered and cardiological examinations led to a diagnosis of hypertrophic obstructive cardiomyopathy (HOC). He started therapy with metoprolol. At 8 years, he was hospitalized for fatigability and gait abnormalities. Increased creatine kinase (583 U/L) was detected, and HOC was stable. A diagnosis of myositis was made. The symptomatology then improved, but he was referred to the Pediatric Neuromuscular Clinic for associated mildly clumsy gait. Genetic analysis for Friedreich's ataxia was negative. Neurological and neuromuscular examination at the age of 10 showed mild girdle and distal lower limb weakness (deltoids, biceps and triceps brachialis 4/5, tibialis anterior e peroneal muscles 4/5), pes cavus, rigid spine and ankle contractures with toe walking, positive Gower's sign and absence of deep tendon reflexes in upper and lower limbs. Romberg maneuver was positive. Creatine kinase was increased (843 U/L). Nerve conduction study showed a severe sensory-motor axonal polyneuropathy with upper limbs motor conduction median nerve 37.5 m/s and ulnar nerve 40 m/s and slowed F wave. In the lower limbs, the compound motor action potential and the sensory action potential were not evocable.

Muscle biopsy revealed, on the hematoxylin and eosin staining, myopathic changes with marked fiber size variability, atrophic fibers occasionally angulated, abundant centrally placed nuclei, necrosis and mild increase in connective tissue (Fig. 1A). Type I fibers were predominant. Multiple small vacuoles were present in numerous fibers (asterisks, Fig. 1A) and eosinophilic areas were observed in the cytoplasm of many fibers (arrows, Fig. 1A), the same strongly reactive with the Gomori trichrome staining (arrows, Fig.1B). Immunofluorescence analyses revealed ectopic expression of different sarcomeric protein like such as alphaB-crystallin (Fig. 1C) and myotilin (Fig. 1D).

Ultrastructural analysis confirmed the presence of severe signs of myofibrillar disruption with accumulation of electron dense granulo-filamentous materials in the intermyofibrillar space (Fig. 1E, Z-disk streaming, Fig. 1F) and abnormal extension of electron dense Z-bands (Fig. 1G).

*Genetic analysis*

LMNA, Desmin, alfaB-crystallin, myotilin e LDB3 genes didn't show mutations. We performed the analysis of an NGS panel that include 169 genes associated with neuromuscular conditions (MotorPlex) 8. Coverage was at least 50x for > 98% of target. We also included parents in the NGS study, and we discovered a single heterozygous mutation p.Pro209Leu ( c.626C > T NM_004281.4 ) in exon 3 of BAG 3 gene. This variant is currently not listed in gnomAD, and it is predicted to be pathogenic according to the ACMG/AMP criteria confirming the diagnosis of BAG3-myopathy. It was a de novo mutation, absent in the parents.

At 4 years the electrocardiogram showed left atrial enlargement and left ventricle hypertrophy, while the echocardiogram showed hypertrophic obstructive cardiomyopathy with a peak left ventricular outflow tract (LVOT) gradient of 50 mmHg; the interventricular septum thickness was 16 mm, the middle septum 17 mm, the posterior basal septum and the anterior basal septum18 mm. The systolic function was normal (left ventricle ejection fraction, EF: 50%), while in diastole the left ventricle had high filling pressure. Subsequently, during the follow-up period, the features of the cardiomyopathy changed, the LVOT obstruction disappeared, but the restrictive pattern worsened with increase in the filling pressure of the left ventricle. The cardiac catheterization showed slight post-

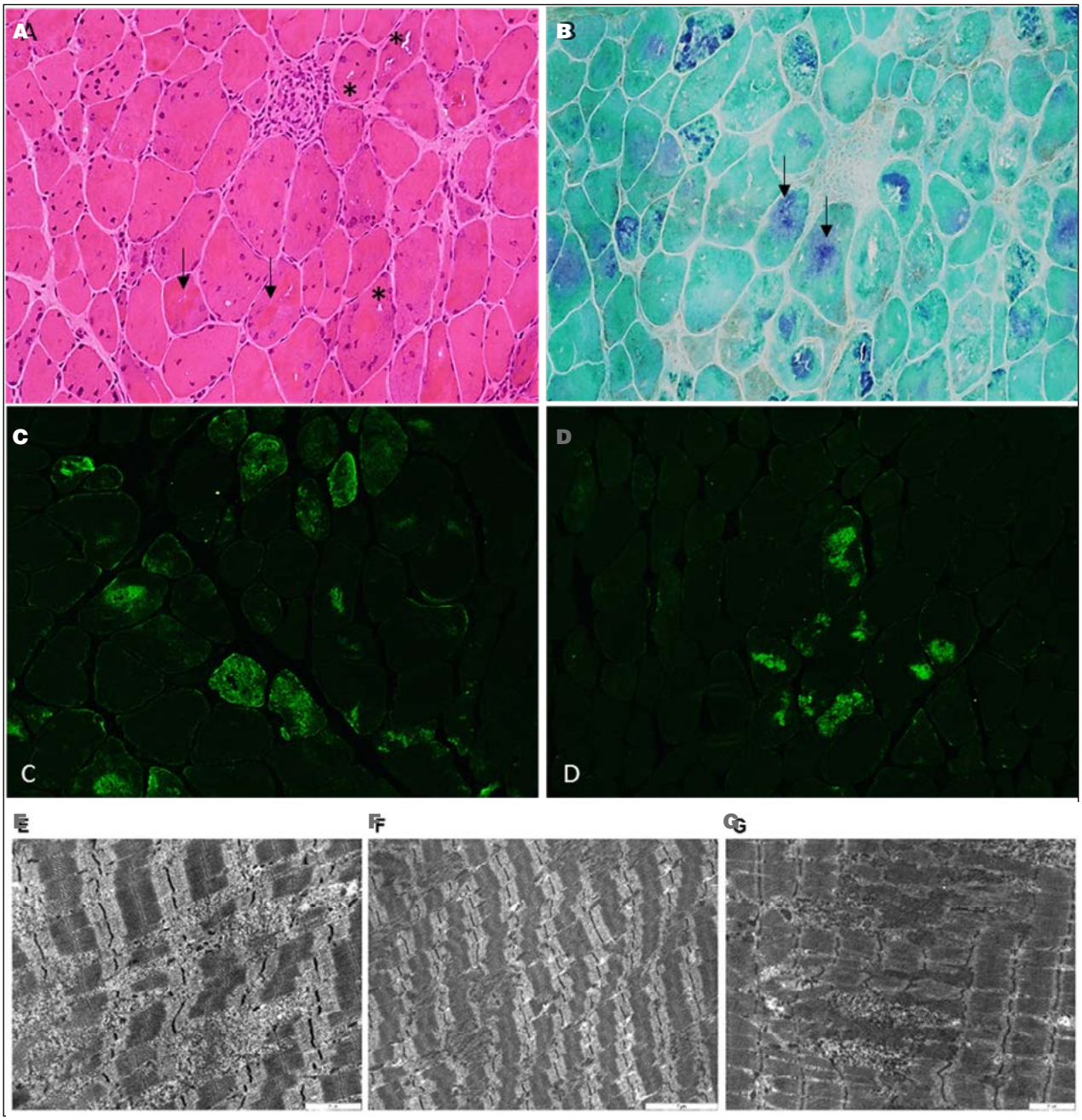


**Figure 1.** Morphological analyses of patient skeletal muscle tissue. **A)** hematoxylin and eosin (H&E) histological staining: protein inclusions and vacuoles indicated by arrows and asterisks, respectively (magnification 20x); **B)** trichrome Gomori staining: protein inclusions indicated by arrows (magnification 20x); **C,D)** immunofluorescence analyses with anti-alpha B crystallin(1C) and anti-myotilin (1D) antibodies showed protein aggregates inside the fibers (magnification 10x); **E-G)** ultrastructural analyses with Transmission Electron Microscopy (TEM) showed abnormal myofibrillar structures and Z-disk streaming.

capillary pulmonary hypertension with mild increase in vascular pulmonary resistance.

At 11 years hypercapnic restrictive respiratory failure (paCO2 84 mmHg) occurred. Hospitalization in the Cardiological Intensive Care Unit and pulmonary assessment were necessary. Since then he needed non-invasive ventilation (NIV), first only at night, then also all day. Idebenone 600 mg/day was added to the therapy.

At 16 years, standing is possible with support and he is able to walk with support for a few minutes. Kyphoscoliosis is very severe. He becomes tired very easily. The weight is 33 kilos. The EF of the left ventricle is 55%. Dysphagia is not present and speech is fluent. He presented an episode of acute congestive heart failure which was treated till the remission of the acute phase. His cognitive performances are very good.

## Discussion

BAG3-related MFM is a rare condition, in most cases due to a *de novo* mutation, and causes severe symptoms with rapidly progressive muscle, nerve, respiratory and heart involvement. The heterozygous mutation p.Pro209Leu (c.626C > T) has previously been identified in several patients and seems to be particularly associated with a severe neuromuscular phenotype. Some variability in the onset and severity of the different symptoms seems to be, even if almost all pediatric cases reported in the literature have a negative prognosis, with quickly progressive worsening of cardiac and respiratory features until exitus, usually occurring within the second to third decade of life (Tab. I). Muscle pathology shows disrupted Z-disks, disorganization of sarcomeric structures, cytosolic aggregated proteins and ectopic accumulation of various myofibrillar proteins and organelles, sign of protein quality control (PQC) and proteolytic systems dysfunction [9]. Studies on a zebrafish model of BAG3 P209L [10] demonstrated a relation between mutated BAG3, protein aggregates and autophagy (a degradation mechanism for damaged proteins in older cells) impairment. However, these studies revealed that mutated Bag3 maintained its function and did not cause protein aggregation but only myofibrillar disruption. Moreover, they demonstrated that aggregate formation was due to the gradual reduction of BAG3 availability caused by itself trapping inside the aggregate. Dysfunctional autophagy was reported in dilated cardiomyopathy due to BAG3 p.Pro209Leu mutation in patient cardiac tissue which demonstrated an increase in autophagy and mitophagy markers [11].

The clinical phenotype in our patient is characterized by early onset HOC, early multiple contractures with rigid spine, non-severe proximal and distal weakness, severe axonal motor sensory neuropathy and severe progressive respiratory failure. Symptoms started with HOC at the age of 4 and the evolution of the clinical picture was initially relatively slow. A similar early onset of HOC is reported in other cases, while sometimes heart symptoms appear later, in the adolescence. In BAG3 mutated cases cardiomyopathy can be isolated and progressive and often leads to heart transplantation before the onset of other symptoms. Currently, at the age of 16, in our patient the cardiological picture shows stability of concentric ventricular hypertrophy with preserved global function. The role played by the treatment with idebenone is probably negligible and the involvement of oxidative metabolism in BAG3-related MFM has not been demonstrated to date. However, a favorable effect on a possible secondary mitochondrial dysfunction cannot be excluded. A possible explanation of the pathologic mechanisms underlying dilated cardiomyopathy in BAG3-related MFM is reported in a recent study of Mc Dermott-Roe et al. [12]. Their data imply a pathologic mechanism in which BAG3-RH improperly engages HSC/HSP70: this impairs the formation of multimeric chaperone complexes required for essential protein quality control including, but likely not limited to, myofibrillar maintenance. The observation that fiber disorganization was only apparent when cells were forced to use autophagy suggests that BAG3 variant expressivity is influenced by age-related dynamics in protein quality control subsystem usage. This provides a potential explanation for the delayed onset of BAG3-associated dilated cardiomyopathy and heart failure, often characterized by an aggressive clinical course. Moreover, male sex, low left ventricular EF (< 50%) and increased left ventricular end-diastolic diameter at first evaluation seem associated with an adverse prognosis during follow-up [13]. At 11 years our patient had acute respiratory failure and he began NIV. The worsening of the respiratory function is reported as an early symptom also in the other cases, and BAG3 myopathy has been demonstrated to meet pathologic criteria for hereditary myopathy with early respiratory failure (HMERF), an adult-onset autosomal-dominant myopathy, which typically presents with respiratory muscle weakness in patients who are still ambulant [13]. On the other hand, in BAG3-related MFM multiple contractures and rigid spine may also be present early on and usually worsen with time. These findings may justify the restrictive respiratory failure that is reported in some cases as early onset symptom. Limb muscle weakness was symmetric and mild in our patient, more evident proximally, with mild deficit in distal districts, and unlike other reported cases, ambulation was still preserved with support at the follow-up, while scoliosis and respiratory involvement were severe.

In conclusion, it is important to hypothesize a neuromuscular disorder caused by BAG3 mutations in the presence of early onset HOC (first decade of life) and/or peripheral neuropathy and MFM for a correct diagnosis and to monitor cardiac and respiratory functions, which have usually a bad prognosis. Our case provides further evidence of progressive multisystem clinical involvement of BAG3-related MFM. Natural history studies of BAG3-related MFM are necessary especially in case pharmacological treatments are to be identified, for example com-

**Table I.** Cases reports in the literature.

| Reference | Age at onset (years) | Features at onset | Cardiomyopathy | Contractures | Weakness | Peripheral neuropathy | Respiratory failure | Outcome | BAG3 mutation |
|---|---|---|---|---|---|---|---|---|---|
| Odgerel et al., 2010 | 5 | Not reported | Restrictive-hypertrophic | Not reported | Yes | Axonal neuropathy | Yes | Sudden death at 9 years | P209L |
| Odgerel et al., 2010 | 12 | Not reported | Restrictive-hypertrophic heart transplantation | Not reported | Yes | Axonal neuropathy | Yes | Not ambulant | Pro209L |
| Odgerel et al., 2010 | 12 | pes cavus, weakness, cardiopathy | Restrictive hypertrophic | Scoliosis and rigide spine | Distal weakness and neck weakness | Axonal neuropathy | Yes | Death at 20 years | P209L de novo |
| Odgerel et al., 2010 | 5 | Gait disturbance | Restrictive-hypertrophic heart transplantation | Not reported | Proximal weakness | Axonal neuropathy | Yes | Death at 15 years | P209L de novo |
| Lee HC et al., 2012 | 6 | Gait disturbance | Restrictive hypertrophic | Multiple contractures and rigide spine | Mild proximal weakness | Axonal neuropathy | Not reported | Ambulant at 12 years | P209L de novo |
| D'avila et al., 2016 | 11 | Contractures | Hypertrophic and arhytmia | Rigide spine | Proximal weakness | Axonal neuropathy | Yes | Not ambulant | P209L de novo |
| Selcen et al., 2009 | Toddler | Toe walker | restrictive heart transplant | Toe walker | Severe weakness | Not reported | Yes | not reported | Not reported |
| Selcen et al., 2009 | 13 | Scoliosis rigide spine, fatigability | hypertrophic | Scoliosis and rigide spine | Distal and proximal weakness | Axonal demyelinating neuropathy | Yes | not reported | Not reported |
| Selcen et al., 2009 | Toddler | Toe walker | Restrictive | Scoliosis, rigide spine and toe walker | Progressive proximal weakness | Not reported | Yes | Death at 13 years | Not reported |
| Jaffer et al., 2012 | Toddler | Toe walker | Restrictive-hearth transplantation | Multiple contractures and rigide spine | Distal and proximal weakness | Axonal neuropathy | Not reported | Not ambulant | P209L de novo |
| Jaffer et al., 2012 | Toddler | Toe walker | Restrictive | Multiple contractures, scoliosis and rigide spine | Distal and proximal weakness | Axonal neuropathy | Yes | Ambulant at 13,5 years | P209L de novo |
| Kostera Pruszczyk et al., 2015 | 12 | Toe walker and foot deformity | Restrictive (subclinical) long QT | Rigide spine and multiple contractures | Subclinical weakness | Axonal demyelinating neuropathy | No | Ambulant at 15 years | Not reported |

▶

**Table I.** Cases reports in the literature.

| Reference | Age at onset (years) | Features at onset | Cardiomyopathy | Contractures | Weakness | Peripheral neuropathy | Respiratory failure | Outcome | BAG3 mutation |
|---|---|---|---|---|---|---|---|---|---|
| Konersman et al., 2015 | Not reported | Cardiopathy | restrictive heart transplant | Rigide spine | Severe weakness | Sensory-motor neuropathy | Not reported | Not ambulant | P209L de novo |
| Seung Ju Kim et al., 2018 | 11 | Gait disturbance and rigid spine | No | Rigide spine and multiple contractures | not reported | Axonal neuropathy | yes | Not reported | P209L de novo |
| Noury et al., 2018 | Not reported | Not reported | No | Rigide spine | not reported | Axonal sensory -motor neuropathy | Not reported | Not reported | Not reported |
| Current report | 4 | Cardiopathy | Restrictive-hypertrophic | Multiple contractures and rigide spine | Proximal weakness and mild distal weakness | Axonal neuropathy | Yes | Ambulant with support | P209L de novo |

pounds resulted able to removing protein aggregates such as metformin [14].

*Ethical consideration*

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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None of the authors has any conflict of interest to disclose.

*Author contributions*

GS wrote the manuscript. AP and MG visited the patient and collected clinical informations. AP coordinated the contributes of each co-Author to the paper. MLV performed muscle biopsy hystological and immunofluorescence analyses. MC performed muscle ultrastructural analyses. AT and VN performed NGS genetic analyses. LR performed cardiolgical assessment.

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# Early treatment with Ataluren of a 2-year-old boy with nonsense mutation Duchenne dystrophy

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**Duchenne muscular dystrophy (DMD) is an X-linked myopathy caused by mutations, in most cases deletions and duplications, in the dystrophin gene. Point mutations account for 13% and stop codon mutations are even rarer. Ataluren was approved for the treatment of DMD caused by nonsense mutations in 2014, and several clinical trials documented its efficacy and safety. However, few real-life experience data is available, especially in pediatric age. We report the case of a 2-year- ambulant child affected by DMD caused by the stop-codon mutation c.10801C > T, p.Gln3601X in exon 76, who was early treated with Ataluren at a dosage of 40 mg/kg/die, and presented a rapid improvement in both muscle strength and cognitive and social skills.**

**Key words:** ataluren, nmDuchenne dystrophy, stop codon point mutations, early treatment

## Introduction

Duchenne muscular dystrophy (DMD) is an X-linked recessive muscular disease caused by mutations in the dystrophin gene, which is the most common muscle disorder in childhood. In most cases, the disease causing mutations are deletions and duplications, but in about 10-15% of cases, DMD is caused by nonsense mutations (nmDMD) in the gene that encodes for dystrophin, resulting in a premature stop codon in the mRNA that affects the production of a full-length functional protein [1]. Clinically, the disease is characterised by progressive muscle weakness and atrophy due to the absence of a functional dystrophin, which results in premature death due to heart and respiratory failure [1]. Until few years ago, the treatment of DMD was mainly limited to corticosteroid therapy, which only mitigates the rate of muscle degeneration [2]. In July 2014, the European Medicines Agency (EMA) approved Ataluren (Translarna® by PTC Therapeutics) for the specific treatment of nmDMD in walking patients aged 5 years and older. Ataluren enables ribosomal readthrough of mRNA containing premature stop codons allowing cellular machinery to bypass nonsense mutation in the genetic material, continue the translation process, and restore the production of a full-length functional protein [3]. In July 2018, the European Commission (EC) authorized the prescription of ataluren in younger nmDMD patients aged two to five years[4]. The decision was supported by the results obtained in the clinical study 030, in which ataluren demonstrated a positive risk-benefit ratio in Duchenne patients of this age group.

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In Italy, PTC has notified the Italian Medicines Agency AIFA of the activation of the expanded access therapeutic use program, following the Ministerial Decree 07/09/2017, for the use of ataluren in nmDMD ambulatory patients, aged between 2 and 5 years.

To date, numerous articles in the literature demonstrate both the efficacy and safety of Ataluren [5,6]; however, few real-life experience studies are available, especially in children. Herein, we report the results in the outcomes of a walking child affected by nmDMD who started the treatment with ataluren, at the age of 2.

## Case report

A 3-month- old- baby came to our observation for the finding of increased values of Creatinekinase (CK, 5941 UI/L), CK-MB (243 ng/ml) and myoglobin (1857 ng/ml). The neurological examination was normal for age. Cardiological and pneumological investigations showed no alterations. NGS (Next Generation Sequencing) identified the stop-codon point mutation c.10801C > T; p.Gln3601X in exon 76, consistent with a diagnosis of nmDMD. In the follow-up, the mother reported delay in the acquisition of motor (autonomous ambulation acquired at 21 months) and language milestones. At the age of 21 months, the neurological examination revealed evidence of Gower's manoeuvre, but no calf pseudohypertrophy. The North Star Ambulatory Assessment (NSAA), administered to measure functional motor abilities, showed a total score of 10/34. The Bayley Scales of Infant and Toddler Development-Third Edition [7], used for the neurocognitive evaluation, showed that the child had lower composite scores across all domains (see Table I). Laboratory tests confirmed elevated serum CK levels (15813 UI/L). Cardiological investigation showed only a bland patent foramen ovale. No therapy was prescribed. When the child turned 2 years old, an early treatment with ataluren was initiated, at a dosage of 750 mg/day (40 mg/kg/day) according to EMA SmPC guideline [8]. Eight months later, the neurological examination still showed waddling gait, slight proximal muscle weakness, reduced deep tendon reflexes, partial Gower maneuver and slight delay in global neurodevelopment. However, the patient's muscle strength, upper limb movements and motor skills in walking, jumping and running were significantly improved. NSAA showed a total score of 19/34. No change in heart function was observed, nor deterioration of respiratory function. Serum CK levels were persistently high (20753 UI/L). After 16 months from the beginning of the ataluren therapy, the child appears participant and able to walk and rise up on his own with negative Gower's sign. Gower's sign is a classic maneuver observed in children with DMD, that indicates weakness of the proximal lower limb muscles. Its negativization indicates a clear improvement in proximal lower limb muscle strength, not expected in the natural history of children with DMD. He still shows waddling gait but not reduced strength in the 4 limbs during repetitive or prolonged movements. Muscle trophism is good. He has discrete dynamic equilibrium, good bimanual manipulation of objects and only a slight weakness in the execution of fine movements. NSAA shows a further improvement in the total score: 21/34. Respiratory function assessed by dynamic night pulse-oximetry does not show alterations. Cardiological visit and echocardiogram are normal. Cognitive, motor and language skills are also improved (Tab. I).

## Discussion

We report the clinical follow-up of a child with nmDMD starting treatment with ataluren at 2 years. After 16 months of treatment, the patient showed an improvement in both motor and cognitive skills compared to the baseline evaluation. The disease progression in young boys affected by Duchenne muscular between age 3 and 6 years (± 3 months), using the NSAA scale was documented by Coratti et al. [9] in 153 DMD boys (573 assessments) younger than 6 years (mean: 4.68, SD: 0.84) with a genetically proven DMD diagnosis. They showed that NSAA scores progressively increased with age, the largest increase being between age 3 and 4 years. A further increase until age of 6 was steadily observed. They also observed that, irrespective of age and pharmacological treatment, DMD boys having a mutation between exon 44 and 62 presented reduced NSAA score by 0.64 points compared to those having a mutation before exon 44. Furthermore, having a mutation after exon 63 reduced NSAA score by 4.67 points compared to those having a mutation before exon 44 and of 4.03 points compared to mutations between exon 44 and 62. Our patient, of about 3.5 years, achieved an NSAA score of 21/34, much higher than the average observed at the same age in the Coratti cohort, both naive (13.64) and treated with steroids (16.33). The improvement is even more remarkable if we keep in mind

**Table I.** Bayley Scales of Infant and Toddler Development – Third Edition composite scores.

| | Cognitive | Language | Motor |
|---|---|---|---|
| Pre-therapy | 80 (percentile 9°) | 65 (percentile 1°) | 73 (percentile 4°) |
| 16 months after | 85 (percentile 16°) | 83 (percentile 13°) | 79 (percentile 8°) |

the mutation site (exon 76) for which, again according to the data of Coratti et al, a lower score of 4.67 points is expected. Our findings are in line with previous studies demonstrating efficacy of Ataluren in pediatric patients with nmDMD10. However, at our knowledge, this is the first time that the efficacy of the drug is documented in DMD boys less than 3 years. This observation has important clinical repercussions because the precocity of the treatment can radically modify the natural history of the disease [10].

Interestingly, serum CK levels were consistently high during the follow-up, with a peak after 8 months of treatment (20753 IU/L). This suggests that serum CK levels do not correlate with symptom's severity. Furthermore, the increase in CK levels could be explained with the increase in muscle mass and the improvement in motor performance. In conclusion, our data confirm the importance of an early diagnosis with gene analysis and sequencing, as an early initiation of the Ataluren treatment can help to prevent muscle degeneration and achieve better motor-cognitive outcomes in children with nmDMD. Further studies in larger cohorts are needed, to better delineate the potential of Ataluren in very young nmDMD patients.

*Ethical consideration*

All procedures were in accordance with the standards of the bioethical committee and the Declaration of Helsinki.

*Acknowledgement*

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*Funding*

None.

*Conflict of interest*

The Authors declare that they have no financial or other conflicts of interest in relation to this research and its publication.

*Author contributions*

All Authors participated in data collection, review and modification of the project. IB and CM have provided substantial contributions to the analysis and interpretation of the data, to the critical review and to the drafting of the manuscript. All Authors approved the submission of the final manuscript and agreed to be responsible for all aspects of the work

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# NEWS FROM AROUND THE WORLD

## AIM

In the period between October and December 2021 many of the activities were aimed at perfecting the organization of the XXI congress of the Association which took place in Milan, from 1 to 4 December in a mixed mode (online and in presence). The proceedings of the congress are published online in Acta Myologica, the official journal of the Association, and are available at the following website: www.actamyologica.it.

The Association promoted and sponsored the masterclass on *Congenital Myopathies* which took place on 12 November 2021 in digital mode and involved neurologists, pediatricians, child neuropsychiatrists, neonatologists, geneticists. Two webinars of great interest on the "*Management of Complexity in Neuromuscular Pathologies*" and "*The respiratory aspect in the neuromuscular patient in clinical and home care*" were respectively posted on the virtual platform since the 6 December 2021.

From 6 December 2021, the six national and the four regionals webinars were also been available online as asynchronous distance educations (further information is available at https://www.aim-fad2021.it/).

Prof. Carmelo Rodolico
Secretary of Italian Association of Myology

## MSM

Due to pandemics, the 14th Meeting of the Mediterranean Society of Myology (MSM) is moved to 2022. Proposals to organize and host the event are welcome.

## WMS

The 26th WMS congress took place, as a virtual meeting between 20 and 24 September. The 5-day congress week has been an opportunity to catch up on the latest developments in neuromuscular diseases from around the world. Controversial debates, oral lectures and electronic poster presentations were planned through the virtual platform and a series of inspiring industry symposia on a dedicated day. The usual WMS 2021 Virtual Pre-Congress Teaching Course was held on the neuromuscular field. To learn more, please visit the congress website: https://www.wms2021.com

# FORTHCOMING MEETINGS

## 2021

**December 19-21**

Stanford University 3rd Annual Spinal Muscular Atrophy Continuing Medical Education Conference on Clinical Decision-Making in the Midst of an Unfoldin. Virtual edition. Information: website: *https://stanford.cloud-cme.com*

## 2022

**January 28-30**

254th ENMC Workshop: Formation of a European network to initiate a European data collection, along with development and sharing of treatment guidelines for adult SMA patients. Information: website: *https://www.enmc.org*

**February 11-12**

262nd ENMC Workshop: Standards of Care for the Dysferlinopathies. Information: website: *https://www.enmc.org*

**February 13-17**

International Conference on Human Genetics. Cape Town, South Africa. Information: website: *https://www.ichg2022.com*

**February 24-27**

World Congress on osteoporosis, osteoarthritis and musculoskeletal diseases. Berlin, Germany. Information: website: *https://wco-iof-esceo.org*

**March 11-13**

260th ENMC Workshop: Congenital Myasthenic syndromes. Information: website: *https://www.enmc.org*

**March 25-27**

258th ENMC Workshop: Leigh syndrome. Information: website: *https://www.enmc.org*

**April 28-May 2**

14th European Paediatric Neurology Society Congress, Glasgow, UK. Information: website: *https://www.epns.org*

**May 13-15**

263rd ENMC Workshop: Focus on female carriers of dystrophinopathy: refining recommendations for prevention, diagnosis, surveillance and treatment. Information: website: *https://www.enmc.org*

**June 10-12**

257th ENMC Workshop: The 3rd ENMC workshop on Dystroglycan and the Dystroglycanopathies. Information: website: *https://www.enmc.org*

**June 15-17**

TREAT-NMD Conference 2022. Vancouver Convention Centre 1055 Canada Pl, Vancouver, BC V6C 0C3, Canada. Information: website: *https://treat-nmd-conference.org*

**June 17-19**

261st ENMC Workshop. Management of safety issues arising following AAV gene therapy. Information: website: *https://www.enmc.org*

**June 24-26**

253rd ENMC workshop. Skeletal muscle laminopathies – natural history and clinical trial readiness. Information: website: *https://www.enmc.org*

**June 25-28**

8th EAN Congress. Vienna, Austria. Information: website: *https://www.ean.org*

**July 5-9**

17th International Congress on NeuroMuscular Diseases (ICNMD). Brussel, Belgio. Information: website: *https://www.icnmd.org*

**September 13-15**

7th Congress of Myology. Nice Acropolis, France. Information: website: Institut de Myologie *https://www.institut-myologie.org*

**September 15-17**

Mitochondrial Medicine Meeting. Nice Acropolis, France. Information: website: Institut de Myologie *https://www.institut-myologie.org*

**October 11-15**

27th Congress of World Muscle Society. Halifax, Canada. Information: website: *https://worldmusclesociety.org*

## 2023

**July 1-4**

9th EAN Congress. Budapest, Hungary. Information: website: *https://www.ean.org*

**October 3-7**

28th Congress of World Muscle Society. Charleston, USA. Information: website: *https://worldmusclesociety.org*

## 2024

**June 29 - July 2**

10th EAN Congress. Helsinki, Finland. Information: website: *https://www.ean.org*

**October 8-12**

29th Congress of World Muscle Society. Prague, Czech Republic. Information: website: *https://worldmusclesociety.org*

## 2025

October 7-11

30th Congress of World Muscle Society. Vienna, Austria. Information: website: *https://worldmusclesociety.org*

**For application or renewal to MSM**

**MEDITERRANEAN SOCIETY OF MYOLOGY* (MSM)**



APPLICATION/RENEWAL FORM

**Application/Renewal for** **1yr** ☐ **2 yrs** ☐

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Fax: 39 081 5665101 E-mail: actamyologica@gmail.com • luisa.politano@unicampania.it
Fax or Mail to the above address. Type or print.

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Last First

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Institution _____

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* Amount payable: 1 year Euro 100
2 years Euro 180

❑ I enclose copy of the bank transfer to:

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Account holder: MSM-Mediterranean Society of Myology
IBAN code: IT36 F030 6909 6061 0000 0160 879
BIC/SWIFT code (for foreign countries): BCITITMM

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# INSTRUCTIONS FOR AUTHORS

***Acta Myologica*** publishes articles related to research in and the practice of primary myopathies, cardiomyopathies and neuromyopathies, including observational studies, clinical trials, epidemiology, health services and outcomes studies, case report, and advances in applied (translational) and basic research.

Manuscripts are examined by the editorial staff and usually evaluated by expert reviewers assigned by the editors. Both clinical and basic articles will also be subject to statistical review, when appropriate. Provisional or final acceptance is based on originality, scientific content, and topical balance of the journal. Decisions are communicated by email, generally within eight weeks. All rebuttals must be submitted in writing to the editorial office.

**Starting from 2020, a publication fee of 200 Euros is required. The Corresponding Author must fill in the appropriate form and send it with the corrected proofs. 50% off is offered for members of Associazione Italiana di Miologia (AIM) and/or Mediterranean Society of Myology (MSM) in good standing with dues. A copy of the payment receipt for the current year is mandatory to prove the membership).**

**On-line submission**

Manuscript submission must be effected on line: www.actamyologica.it according to the following categories:

*Original articles* (maximum 5000 words, 8 figures or tables). A structured abstract of no more than 250 words should be included.

*Reviews, Editorials* (maximum 4000 words for Reviews and 1600 words for Editorials). These are usually commissioned by the Editors. Before spontaneously writing an Editorial or Review, it is advisable to contact the Editor to make sure that an article on the same or similar topic is not already in preparation.

*Case Reports, Scientific Letters* (maximum 1500 words, 10 references, 3 figures or tables, maximum 5 authors). A summary of 150 words may be included.

*Letters to the Editor* (maximum 600 words, 5 references). Letters commenting upon papers published in the journal during the previous year or concerning news in the myologic, cardio-myologic or neuro-myologic field, will be welcome. All Authors must sign the letter.

*Rapid Reports* (maximum 400 words, 5 references, 2 figures or tables). A letter should be included explaining why the author considers the paper justifies rapid processing.

*Lectura*. Invited formal discourse as a method of instruction. The structure will be suggested by the Editor.

*Congress Proceedings* either in the form of Selected Abstracts or Proceedings will be taken into consideration.

Information concerning new books, congresses and symposia, will be published if conforming to the policy of the Journal.

The manuscripts should be arranged as follows: 1) Title, authors, address institution, address for correspondence; 2) Repeat title, abstract, key words; 3) Text; 4) References; 5) Legends; 6) Figures or tables. Pages should be numbered (title page as page 1).

*Title page.* The AA are invited to check it represents the content of the paper and is not misleading. A short running title is also suggested.

*Key words*. Supply up to six key words. Wherever possible, use terms from Index Medicus – Medical Subject Headings.

*Text.* Only international SI units and symbols must be used in the text. Tables and figures should be cited in numerical order as first mentioned in the text. Patients must be identified by numbers not initials.

*Illustrations.* Figures should be sent in .jpeg or .tiff format. Legends should be typed double-spaced and numbered with Arabic numerals corresponding to the illustrations. When symbols, arrows, numbers, or letters are used to identify parts of the illustrations, each should be explained clearly in the legend. For photomicrographs, the internal scale markers should be defined and the methods of staining should be given.

If the figure has been previously published a credit line should be included and permission in writing to reproduce should be supplied. Color photographs can be accepted for publication, the cost to be covered by the authors.

**Patients in photographs are not to be recognisable**

*Tables*. Tables should be self-explanatory, double spaced on separate sheets with the table number and title above the table and explanatory notes below. Arabic numbers should be used for tables and correspond with the order in which the table is first mentioned in the text.

*References.* Indicate all Authors, from 1 to 3. If their number is greater than 3, indicate only the first 3, followed by “et al.” . Arabic numbers in the text must be superscript. References in the list must be numbered as they appear in the text, with the reference number superscript. **DOI number must be included with each reference** (when available). If not available, indicate the PMID number.

Examples of the correct format for citation of references:

Journal articles: Shapiro AMJ, Lakey JRT, Ryan EA, et al. Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. N Engl J Med 2000;343:230-238. doi.org/10.14639/0392-100X-1583

Books and other monographs: Dubowitz V. Muscle disorders in childhood. London: WB Saunders Company Ltd; 1978.

Please check each item of the following checklist before mailing:

- Three-six index terms, short title for running head (no more than 40 letter spaces) on the title page.
  Name(s) of the author(s) in full, name(s) of institution(s) in the original language, address for correspondence with email address on the second page.
- Summary (maximum 250 words).
- References, tables and figures cited consecutively as they appear in the text.
- Figures submitted actual size for publication (i.e., 1 column wide or 2 columns wide).
- Copyright assignment and authorship responsibility signed (with date) by all Authors.
- References prepared according to instructions.
- English style.
- Patients in photographs not recognisable.