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All correspondence should be addressed to:
Mediterranean Society of Myology - Cardiomyology and Medical Genetics - Primo Policlinico - Piazza Miraglia - 80138 Naples, Italy - Tel. +39 081 566 5300 - Fax +39 081 566 5101.
Luisa Politano, via Ferrarecce 57, 81100 Caserta, Italy; email: actamyologica2@gmail.com

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ORIGINAL ARTICLES

Gender effect on onset, prevalence and surgical treatment of cataract in patients with Myotonic Dystrophy type 1

Marianna Scutifero[1], Michele Lanza[2], Roberta Petillo[1], Maddalena De Bernardo[3], Luigia Passamano[1], Nicola Rosa[3], Luisa Politano[1]

[1] Cardiomyology and Medical Genetics, University Hospital of Campania "Luigi Vanvitelli", Naples, Italy; [2] Eye Department, University of Campania "Luigi Vanvitelli", Naples, Italy; [3] Department of Medicine and Surgery, University of Salerno, Salerno, Italy

Myotonic Dystrophy type 1 (DM1) is the most common muscular dystrophy in adults, affecting 1:8000 individuals. It is a multi-systemic disorder involving muscle, heart, endocrine and respiratory apparatus and eye. The eye symptoms can include ptosis, external ophthalmoplegia, epiphora, and early onset cataracts. Cataracts occur at a much earlier age (usually between 30 and 40) than the general population, where females are usually affected more than men. We studied gender differences in cataract prevalence and treatment age in 243 DM1 patients (134 M; 109 F), aged 18 to 70 years, who were subsequently screened at routine follow-up. For each patient, information was collected on age, sex, CTG expansion, age of cataract onset, and age at cataract surgery, when available. Seventy-three patients, 30 females and 43 males, had cataracts, at a mean age of onset of 41.14 ± 12.64 in females, and 40.36 ± 10.03 in males. Sixty-nine of them underwent cataract surgery, males at an earlier age than females (42.8 ± 9.8 years versus 47.3 ± 12.6 years) and in 52.5% of cases before the age of 40, compared to 17.2% of females. The difference was statistically significant. The assumption that females in general and those with DM1 in particular develop cataracts more frequently and earlier than males is not confirmed, at least in this study. A possible explanation for these results could be related to non-advanced age, the protective role of estrogen and the lower prevalence of smoking in the study population.

Key words: Myotonic Dystrophy type 1, Steinert disease, gender, cataract, cataract surgery, prevalence

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Correspondence
Luisa Politano
Cardiomyology and Medical Genetics, University Hospital of Campania "Luigi Vanvitelli", piazza Miraglia 2, 80138 Naples, Italy. E-mail: luisa.politano@unicampania.it
ORCID: https://orcid.org/0000-0002-0925-7158

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Introduction

Myotonic Dystrophy type 1 (DM1) is the most common muscular dystrophy in adults, affecting 1:8000 individuals. It is a multi-systemic disorder involving muscle, heart, endocrine and respiratory apparatus, brain and eye [1-3]. Muscle disease is characterized by myotonia and various degrees of muscle weakness [1-3]. The heart damage [4-5] combines arrhythmias and/or cardiac conduction disorders [6-8], often necessitating of device or heart implantation [7-12]. The disorder may also affect the function of many endocrine glands, with an increased risk for insulin resistance or diabetes, erectile dysfunction, impaired fertility, benign and malignant thyroid

tumors [13-16]. The respiratory involvement is frequent with a progressive decline of vital capacity values and sleep disorders [17-19]. DM1 is often associated with cognitive impairment and developmental behavioural disorders [20-22].

The pattern of inheritance is autosomal dominant, although complicated by the phenomenon of anticipation, where symptoms appear earlier and are more severe in successive generations [23]. Phenotypes range from individuals who are only mildly affected in late adulthood, to severely affected children with the congenital form of the disease [24,25]. DM1 is caused by a CTG expansion in the 3′-untranslated region (UTR) of the dystrophia myotonica protein kinase (DMPK) gene on chromosome 19q13.3 [26]. Small expansions (50 to 80 repeats) may be transmitted for several generations with minor changes. These alleles display greater instability when passing through the male germline [27,28]. Accordingly, the jump from small expansion with minor symptoms to large expansion with classical DM1 is more likely to occur with paternal transmission. In contrast, the massive intergenerational expansions to 1,000 or more repeats are more likely to occur with maternal transmission [28,29]. This explains the near exclusive maternal transmission of congenital DM1 [29]. Anticipation is not an inevitable phenomenon. Occasionally the expanded repeat undergoes an intergenerational contraction (< 5% of transmissions) [30,31].

Myotonic Dystrophy is a unique form of muscular dystrophy which is associated with a variety of ocular manifestations [32,33]. The eye is severely affected and symptoms can include ptosis, external ophthalmoplegia, epiphora, pupillary light-near dissociation, early onset cataracts, pigmentary retinopathy, bilateral optic nerve atrophy and low intraocular pressure (IOP). It has been shown that IOP is related to the detachment of the ciliary body [34] rather than to differences in central corneal thickness or corneal biomechanical properties [35,36]. The lens is particularly affected in DM1 and an early appearance of cataract is often the most common and reliable symptom of the disease, since it is frequently the first occasion for patients to seek medical attention [37].

In patients with DM1 cataract occurs at a much earlier age (usually in the 30s-40s) compared to general population and can also appear even in the lenses of teenagers. Females are usually more affected than men [38,39]. This could be due to a longer survival, and to age-dependent cataract incidence.

We studied the gender differences in cataract prevalence and treatment age in DM1 population.

Patients and methods

A population of 243 patients with DM1 (134 M; 109F), aged 18-70 years (mean 43.3 ± 14.2) and regularly followed at the Cardiomyology and Medical Genetics of the University Hospital of Campania "Luigi Vanvitelli", were subsequently screened at the routine follow-up. For each patient, information was collected on age, sex, CTG expansion, age of cataract onset, and age at cataract surgery, when available.

Consent to the use of data in an aggregate manner was obtained upon admission to the University Hospitals as per established practice.

Statistical analysis

Data are shown as mean ± standard deviation. We performed Student T test for non-paired data, and chi-square test to investigate differences in mean and percentage between the two groups.

Results

The clinical characteristics of the patients enrolled in the study are shown in Table I. Seventy-three out of 243 patients with DM1 (30%) developed cataracts. Of them, 30 were females and 43 were males. The mean age of onset of cataracts was 41.14 ± 12.64 in females and 40.36 ± 10.03 in males. The differences were not statistically different (p = 1.29; Student t test for non-paired data).

Sixty-nine patients (94.5%) had cataract surgery, at an average age of 44.7 ± 11.2 years. However, males underwent the surgery at an earlier age than females (mean age 42.8 ± 9.8 *versus* 47.3 ± 12.6). Furthermore, by dividing the patients - males and females - according to the age of cataract surgery more or less 40 years, we noticed that

Table I. Demographic characteristics of the study population.

	Total	Males	Females	P-value
Patients with DM1 examined (N)	243	128	115	n.s
Mean age in years		39.97 ± 14.85	40.77 ± 14.78	n.s
CTG expansion (Mn ± SD)		495.78 ± 477.95	466.25 ± 416.74	n.s
Smoking (N; % of patients)		57; 45	6; 5	p < 0.001
Insuline resistance/overt type 2 diabetes (N; % of patients)		52; 40.6	45; 39.1	n.s

Table II. Cataract onset and surgery in the study population.

	Males	Females	P-value
Number of patients with DM1 with cataract	43	30	n.s
Mean age in years	40.36 ± 10.03	41.14 ± 12.64	n.s
Patients with cataract surgery (N; %)	40; 93	29; 96.7	n.s
Mean age in years	42.8 ± 9.8	47.3 ± 12.6	< 0.05
Cataract surgery < 40 years (N; %)	21; 52.5	5; 17.2	< 0.001

21/40 (52.5%) males underwent surgery before the age of 40 compared to only 5/29 (17.2%) females. The difference was statistically significant ($p < 0.001$, chi-squared test) (Tab. II).

Discussion

Cataract is a common cause of visual impairment in the elderly [40], and surgery is often effective in restoring vision [39]. Cataract is a multifactorial disease associated with age, female sex, genetic predisposition, smoking, diabetes mellitus, drug intake and environmental exposure to UVB radiation [41-43]. In a study on cataract prevalence and prevention in Europe, Prokofyeva et al. [44] found that the overall prevalence of cataract was higher in Germany and Italy compared to the rest of Europe. They showed an increase with age in 2/3 of cases diagnosed over the age of 70. Sex-specific cataract prevalence was higher in women than in men, although not all of the reviewed studies were consistent in this aspect. Sex-specific cataract prevalence in a Spanish study [45] was higher in men over 64 years of age than in women at the same age, and a case-control study [46] from Athens, Greece, found only borderline significance of female sex to the risk of cortical cataract.

Furthermore, several European epidemiological studies [47-49] showed that former and current smoking [47], a history of cardiovascular disease [48], family history of ophthalmic disease, and higher exposure to sunlight [49] lead to increased risk of cataract, whereas only one study [50] showed an association of increased cataract risk with diabetes duration of 10 years or longer, or with asthma and chronic bronchitis. Importantly, that literature review showed that chlorpromazine, corticosteroids and multivitamin/mineral formulation intake increased the cataract risk depending on dose, treatment application, and duration [51,52].

Myotonic cataract is a posterior sub-capsular cataract, detectable as red and green iridescent opacities on slit lamp examination. Its characteristic multi-colored "Christmas tree" appearance (Fig. 1) is present in nearly all affected individuals, so that, in the absence of any evident clinical features, identification of typical sub-capsular opacities in subjects at risk for Myotonic Dystrophy, can be an indicator of a minimally affected gene carrier before the characterisation of the DMPK mutation [39].

Several factors may influence the gender differences observed in the cataract onset and prevalence. Hormonal differences between women and men represent one of the most commonly cited factors [53] as advanced age at menarche, younger age at menopause and a shortened fertile period were significantly associated with an increasing incidence in cataract surgery in several studies. These data suggest that estrogen deficiency may contribute to the cataract development and that estrogen may play a protective role in the human lens [53]. Several observations may support this suggestion: (i) women using postmenopausal estrogen seem to have less cataracts compared to postmenopausal women non using estrogen; (ii) the antioxidant properties of various estrogen, used in hormone replacement therapy, have been shown to protect lens proteins from the oxidative damage, that has been implicated in the pathogenesis of some forms of cataracts; (iii) alfa-estrogen mRNA has been found in human lens epithelial cells, suggesting a possible mechanism for a direct estrogen effect on lens; (iv) estrogen has been shown to protect against TGF-beta-induced cataract in a rat model of cataractogenesis [54]. However, evidences for this hypothesis remain controversial, and it appears that sex hormone levels could be regarded as a risk factor for cataractogenesis more than as a key factor.

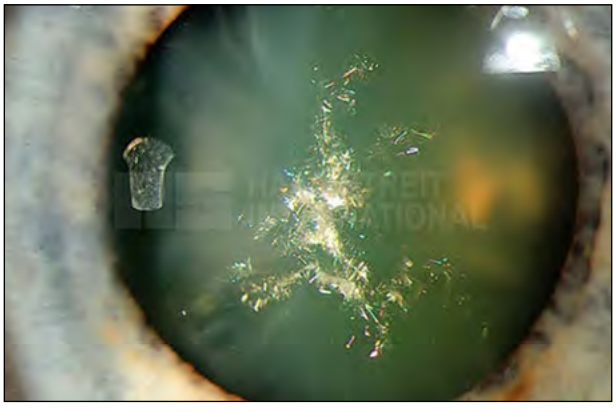

Figure 1. Myotonic cataract presenting as red and green iridescent opacities on slit lamp examination, with the characteristic multi-colored "Christmas tree" appearance.

A second influencing factor could be the insulin resistance [13,14], an endocrine abnormality often associated with DM1, or the presence of an overt type 2 diabetes [55]. Muscle insulin sensitivity is reduced by about 70% in patients with DM1, compared to those of controls [1]. Dysregulation of alternative splicing of the insulin receptor (IR) pre-mRNA in skeletal muscles has been recently indicated as one of the causes [56-58].

Diabetes type 2 is a chronic systemic disorder affecting nearly one in eight adults worldwide. Ocular complications, such as cataract can lead to significant visual impairment. Patients with diabetes have an increased incidence of cataracts which mature earlier compared to the rest of the population, and cataract surgery is a common and safe procedure to treat such a complication [59-60]. However, no differences were found in the prevalence of insulin resistance or overt type 2 diabetes in our patients.

Conclusions

The assumption that females in general and those with DM1 in particular, develop cataracts more frequently and earlier than males is not confirmed, at least in this study. A possible explanation for these results could rely on the non-advanced age, as women are more affected in older ages, the protective role of estrogen (none of females was in menopause) and the lower prevalence of smoking among females. However, further studies are necessary to better clarify this particular aspect in DM1 population.

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Conflict of interest statement

The Authors declare no conflict of interest.

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Author' contribution

MS, LPa, RP: performed the clinical investigations; ML, MDB, NR: performed eye exams and cataract surgery; LP: conceived, wrote and supervised the manuscript. All Authors have approved the current version of the manuscript.

Ethical consideration

This study was performed in line with the principles of the Declaration of Helsinki. Consent to the use of data in an aggregate manner was obtained from patients upon admission to the University Hospital, as per established practice.

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Congenital myopathy associated with a novel mutation in *MEGF10* gene, myofibrillar alteration and progressive course

Carolina Croci[1], Monica Traverso[2], Serena Baratto[2], Michele Iacomino[3], Marina Pedemonte[2], Francesco Caroli[3], Marcello Scala[1], Claudio Bruno[4], Chiara Fiorillo[1,2]

[1] Department of Neurosciences, Rehabilitation, Ophthalmology, Genetics, Maternal and Child (DINOGMI), University of Genoa, Genoa Italy; [2] Paediatric Neurology and Neuromuscular Disorders Unit, IRCCS Istituto "G. Gaslini", Genoa Italy; [3] Medical Genetics Unit, IRCCS Istituto "G. Gaslini", Genoa, Italy; [4] Center of Translational and Experimental Myology, IRCCS Istituto "G. Gaslini", Genoa, Italy

Early-onset myopathy, areflexia, respiratory distress, and dysphagia (EMARDD) is caused by homozygous or compound heterozygous mutation in the *MEGF10* gene (OMIM#614399). Phenotypic spectrum of EMARDD is variable, ranging from severe infantile forms in which patients are ventilator-dependent and die in childhood, to milder chronic disorders with a more favorable course (mild variant, mvEMARDD). Here we describe a 22 years old boy, offspring of consanguineous parents, presenting a congenital myopathic phenotype since infancy with elbow contractures and scoliosis. The patient developed a slowly progressive muscle weakness with impaired walking, rhinolalia, dysphagia, and respiratory involvement, which required noninvasive ventilation therapy since the age of 16 years. First muscle biopsy revealed unspecific muscle damage, with fiber size variation, internal nuclei and fibrosis. Myofibrillar alterations were noted at a second muscle biopsy including whorled fibres, cytoplasmic inclusion and minicores. Exome sequencing identified a homozygous mutation in *MEGF10* gene, c.2096G > C (p.Cys699Ser), inherited by both parents. This variant, not reported in public databases of mutations, is expected to alter the structure of the protein and is therefore predicted to be probably damaging according to ACMG classification. In conclusion, we found a new likely pathogenic mutation in *MEGF10*, which is responsible for a progressive form of mvEMARDD with myofibrillar alterations at muscle biopsy. Interestingly, the presence of *MEGF10* mutations has not been reported in Italian population. Early diagnosis of MEGF10 myopathy is essential in light of recent results from in vivo testing demonstrating a potential therapeutic effect of SSRIs compounds.

Key words: congenital myopathy, *MEGF10*, myofibrillar, respiratory defect

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Correspondence
Chiara Fiorillo
Paediatric Neurology and Neuromuscular Disorder Unit, IRCCS Istituto "G. Gaslini", largo Gaslini 5, 16147 Genoa, Italy. Tel.: +39 010 56363566.
E-mail: chiara.fiorillo@edu.unige.it

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Introduction

Early-onset myopathy, areflexia, respiratory distress, and dysphagia (EMARDD) is a congenital myopathy caused by homozygous or compound heterozygous mutation in the *MEGF10* gene (OMIM #614399) [1,2]. Patients with EMARDD often present severe childhood weakness and hypotonia with respiratory distress caused by diaphragmatic paralysis where-

by they become ventilator-dependent, and die in infancy. Within MEGF10 myopathies, milder chronic disorders with a more favourable course and later onset have been described and classified as mild variant or mvEMARDD. These forms can manifest only very mild muscle weakness and mild respiratory dysfunction. Of note, minicores on muscle biopsy are described albeit not in all mvEMARDD patients [3-6].

In 2011, pathogenic variants in the multiple epidermal growth factor-like domains 10 (MEGF10) gene were found to be causative of EMARDD in five independent index patients [1]. Since then, just over 20 patients have been reported with variants in *MEGF10* [6]. MEGF10 is a single transmembrane protein with 17-EGF-like domains in the extracellular portion and multiple tyrosine phosphorylation residues in the cytoplasmic domain. It has been proposed that MEGF10 mediates cell-cell adhesion, acts as a phagocytosis receptor for apoptotic cells [1,7,8], and mediates signalling such as cell proliferation and differentiation [9,10]. In skeletal muscle, MEGF10 is mainly expressed in the satellite cell, which is a mononuclear muscle stem cell [10]. Thus, MEGF10 appears to be a key regulator of muscle development and repair. Expression of the human MEGF10 transcript is restricted to the adult and foetal brain, spinal cord, and skeletal muscle. High concentrations are also present in the neuromuscular junction [1].

In this study, we report the first Italian patient harbouring a novel homozygous *MEGF10* variant. This subject showed early disease onset with progressive course and muscle biopsy revealed the presence of myofibrillar alterations.

Methods

Tissue and blood samples were obtained after written informed consent from the patient parents and stored in the Muscle Pathology Laboratory for diagnostic and research purposes as approved by Local Ethic Committee.

Exome sequencing (ES) was performed as previously described[11] and the computational analysis of candidate variants was performed according to allele frequency, conservation of affected residues, and predicted impact on protein function and structure [12]. The candidate pathogenic variant in *MEGF10* was confirmed by Sanger sequencing and segregation was performed in the healthy parents. Web-based softwares were used to predict the pathogenicity of mutations, as reported in the Results section.

Muscle biopsies were obtained from patient quadricep muscle after local anaesthetic injection and snap frozen in isopentane. Routine histological procedures were performed according to standard protocols and included Hematoxylin and eosin (H&E), Modified Gomori Trichrome, Cytochrome Oxidase (COX), Succinate Dehydrogenase (SDH), Nicotinamide adenine dehydrogenase (NADH), Adenosine triphosphatase (ATPase), Adenosine triphosphatase (ATPase), Periodic Acid Schiff (PAS), Oil red O.

Case report

This patient is a 22-year-old boy, born to healthy consanguineous parents without family history of neuromuscular diseases. The patient was born at 33 weeks gestation by planned caesarean delivery due to foetal tachycardia, after a pregnancy complicated by threatened miscarriage requiring drug therapy and rest. Apgar to minute 1 was 3, so he underwent primary resuscitation for asphyxia and was admitted to the NICU for 48 hours with good recovery. He had weak suction and presented with a congenital myopathic phenotype with elbow contractures and scoliosis since the first months of life.

No motor developmental delay or intellectual disability were observed, but he developed a slowly progressive muscle weakness with impaired walking, rhinolalia, dysphagia, and respiratory involvement, which required nocturnal non-invasive ventilation therapy since the age of 16 years. Brain and spine MRI, ECG, echocardiogram and serum creatine kinase (CK) level test were performed, resulting normal. Electrophysiological investigation documented motor and sensory conduction parameters within normal limits, with the exception of a reduction in amplitude of the muscle evoked potential. A first muscle biopsy at age of 5 years, revealed unspecific muscle damage, with fibre size variation, internal nuclei and fibrosis. In a second muscle biopsy performed years later, myofibrillar alterations were noted, including whorled fibres, cytoplasmic inclusion and minicores (Fig. 1).

Follow-up neurological examination showed axial hypotonia, generalised muscular hypotrophy and weakness (Fig. 2), preserved facial expressions, cleft palate, and global areflexia. Joint retractions at the elbows and ankles bilaterally were also observed. The patient was able to walk without support with equinus-varo-supinate foot stance, hyperlordosis, and tilting of the pelvis. Sensation was not impaired. In the following years, there was a slow worsening of the neuromuscular picture, requiring the use of a manual wheelchair for longer trips. The patient also underwent bilateral Achilles tendon tenotomy and vertebral arthrodesis surgery. Since age 17, he developed swallowing difficulties and recurrent pulmonary infections due to aspiration of food.

Exome sequencing led to the identification of the homozygous variant (NM_001256545.2): c.2096G > C (p. Cys699Ser) in the *MEGF10* gene. This variant is absent

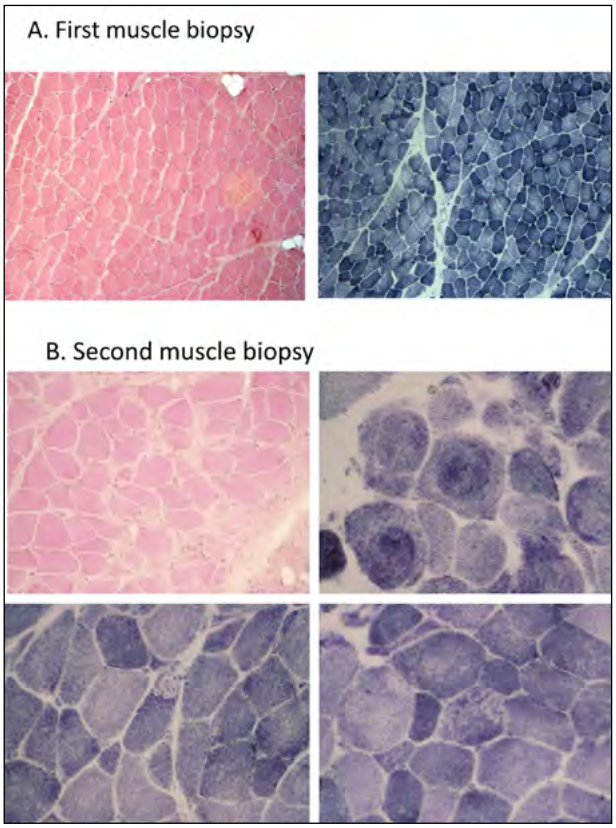

Figure 1. Muscle biopsy with HE and NADH staining. A) first muscle biopsy revealed unspecific muscle damage, with fibre size variation, internal nuclei and fibrosis; B) in a second muscle biopsy performed years later, myofibrillar alterations including whorled fibres, cytoplasmic inclusion and minicores, were evident.

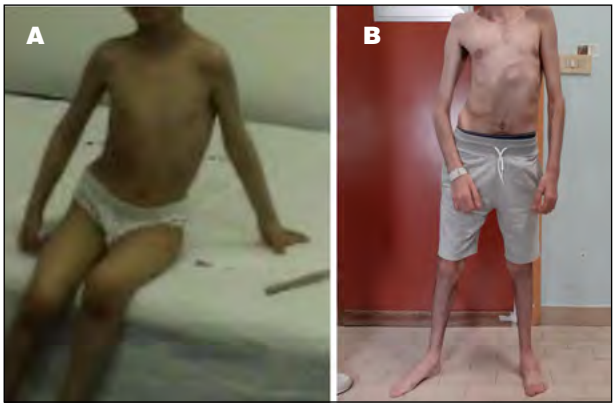

Figure 2. A) patient at the age of six; B) patient at 17 years old. A progression of generalised muscular hypotrophy, scoliosis and hyperlordosis is evident; joint retractions at the elbows and tibio-tarsus bilaterally are also present. Ambulation is still possible for less than 10 meters.

in genomic databases (such as ClinVar and gnomAD), affects a conserved residue (GERP score = 5.85), and is predicted to be pathogenic by several in silico tools, including CADD (27.9), Mutation Taster (disease causing, score = 1), SIFT4G (damaging, score = 0.001), and Polyphen-2 HVAR (damaging, score = 0.99). This amino acid change is further classified as likely pathogenic according to the ACMG criteria (PM2, PP3). The variant is not predicted to alter the splicing (SpliceAI scores = 0). Sanger sequencing was also performed and confirmed that the patient was homozygous for the variant, and both parents were carriers.

Discussion

We describe a novel mutation in *MEGF10* in a patient presenting with a progressive form of myopathy characterised by generalised muscle weakness, scoliosis and respiratory involvement, in whom a muscle biopsy showed features suggestive of myofibrillar myopathy.

To date, 23 patients with MEGF10 myopathy have been reported in the literature and these cases have been recently reviewed by Fujii et al. [6]. We summarised the clinical characteristics of the current and reported patients, according to the classification in one of the two forms (classic early onset EMARDD or mild variant mvEMARDD) (Fig. 3). The age of onset was established as the age at which patients manifested respiratory distress or progressive muscle weakness.

Initially, mutations in the *MEGF10* gene were associated with a severe phenotype of congenital myopathy termed EMARDD, characterized by generalised muscle weakness, breathing difficulties, joint contractures and scoliosis [1,2]. More recently, it became apparent that *MEGF10* mutations may results in a wide spectrum of disease in terms of clinic pathological features, ranging from severe infantile to milder chronic disorders with a more favourable course [1-6,10,13]. In a study reported for EMARDD and mvEMARDD, it was shown that there is considerable overlap in clinical findings between the EMARDD and minicore myopathy diagnostic groups, although certain features are more frequently reported according to clinical/genetic diagnosis [5].

Genotype–phenotype correlations have been also postulated [6,10]. Indeed, individuals with more damaging variants (homozygous null mutations/frameshift mutations) [1,2] show the EMARDD phenotype with respiratory failure starting in infancy and non-specific changes on muscle biopsy. In contrast, patients displaying a less severe phenotype, with later respiratory failure and minicores on muscle biopsy (mvEMARDD or with minicore myopathy) [3,10] harbour homozygous or compound heterozygous missense variants affecting the cysteine in

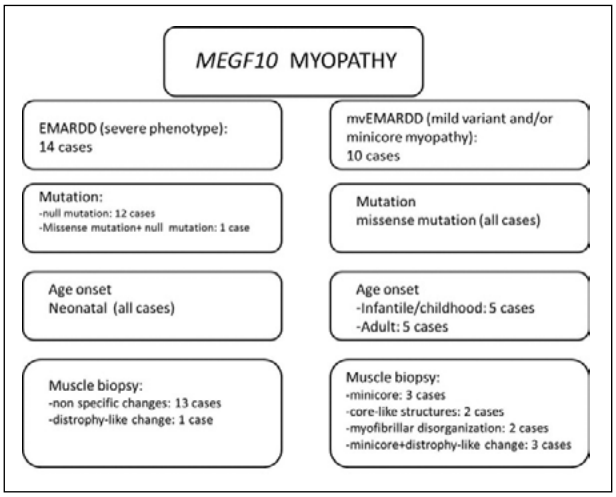

Figure 3. Clinical and genetic characteristics of patients with *MEGF10* mutations reported in literature according to the classification in early onset severe form and mild variant form.

the EGF-like domain [10]. Additionally, missense changes are statistically associated with a later onset and benign course, whereas truncating variants are expected to cause an early onset severe phenotype [6].

Although our patient carried a missense variant, he displayed an early-onset myopathy with progressive course. Hypotonia at birth was followed by scoliosis, retraction and respiratory involvement, requiring respiratory support at age 6 and swallowing problems since at age 17. Two consecutive muscle biopsies confirmed the progression of the disease over the years. The first muscle biopsy performed at age 5 years revealed unspecific muscle damage, whereas the second biopsy performed one year later showed more definite and significant alterations, suggestive of a myofibrillar myopathy with presence of core like areas, inclusions, and whorled fibre (Fig. 1). Interestingly, another reported patient with late onset MEGF10 myopathy phenotype had only nonspecific findings at the first and second biopsies, and then exhibited cores at the third biopsy [13]. Thus, we propose that minicores that mark the late onset group, can actually be a consequence of a longstanding myofibrillar pathological processes starting in infancy.

Myofibrillar alterations have been already reported in late onset mvEMARDD [3,4]. However, not all the mvEMARDD patients present minicores [5,6] and these features are not typical for early-onset forms. This seems to confirm the hypothesis that minicores may only occur at a later stage. Indeed, myofibrillar myopathies (MFM) are a group muscle disorders usually with adult onset of distal weakness. Onset in infancy or childhood is very uncommon. In addition, the association of MEGF10 defect and deterioration of the myofibrillar network has not been formally validated and functional studies are needed to explore the putative role of MEGF10 in maintenance of the myofibrillar network.

From a genetic perspective, *MEGF10* variants have never been reported in subjects of Italian ancestry. In our patient, we identified the novel homozygous variant p. Cys699S affecting a very conserved cysteine residue in a crucial EGF-like functional domain. Cysteine substitutions in the extracellular EGF-like domains of MEGF10 are the most commonly reported missense variants [1,3-5,10] and they have been shown to result in a significant decrease in the tyrosine phosphorylation activity in the intracellular domain [9,10]. These findings thus support the pathogenic relevance of the p.Cys699Ser variant in our patient. Furthermore, the degree of functional impairment of MEGF10 has been demonstrated to depend on which EGF-like domain is mutated [9,14]. This suggests that different EGF-like domains may be relatively more important for MEGF10 signalling than others [5]. Functional studies are warranted to confirm the role and pathogenetic association of *MEGF10* mutation in our patient.

It has been reported that MEGF10 regulates the proliferation and differentiation of muscle stem cells by promoting the activation of satellite cell proliferation and, simultaneously, inhibiting the myoblast differentiation [1,10,15]. Centrally located nuclei are also a substantial feature in the histopathological picture of our case. Albeit unspecific, central nuclei can be linked to a possible involvement of the regeneration pathway which is so far the most corroborated patho-mechanism of the MEGF10 defect. MEGF10 also interacts with the intracellular domain of NOTCH. The action of MEGF10 on myoblasts appears to be mediated, at least in part, by interactions with components of the Notch signalling pathway. The NOTCH pathway in general and NOTCH1 in particular, is a key regulator of satellite cell and myoblast physiology [16]. Notch1 interacts with both MEGF10 [17] and the serotonin pathway [18] and defects in these interactions may participate in the pathogenesis of EMARDD [17]. Recent findings indicate that sertraline, an SSRI, ameliorates the phenotype of MEGF10 myopathy in different disease model, including myoblasts (mouse-derived and human-derived), Drosophila and zebrafish, revealing its potential as a novel therapy for MEGF10 myopathy [13].

Conclusions

In summary, our report proposes a continuum in the phenotypes associated with *MEGF10* variants, which include a clinical and histopathological progression of the disease and represent a novelty from the dichotomy between the early onset classic severe EMARDD and mvE-

MARDD. We also suggest that myofibrillar alterations are a common finding, and that *MEGF10* gene should be tested in suspected MFM patients with childhood onset of progressive myopathy, scoliosis and retraction, and respiratory defect. Accumulation of further cases will be important to confirm the current genotype-phenotype correlations and for early diagnosis, which will play a relevant role in the management of patients and the use of potential therapeutic strategies.

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Conflict of interest statement

The Authors declare no conflict of interest.

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Author contributions

CC: acquisition and analysis of data, drafting of the manuscript; MT, MI, FC: acquisition and analysis of genetic data; SB: acquisition and analysis of muscle biopsy data; MP, CB: acquisition and analysis of clinical data; MS: drafting the manuscript; CF: conception and design of the study, drafting the manuscript.

Ethical consideration

This study has been approved by local Ethic Committee within a project of WES in Neuromuscular Disorders. Patient and family signed informed consent for research use of clinical data and publication of photographic material.

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CASE REPORT

Switching therapies: safety profile of Onasemnogene abeparvovec-xioi in a SMA1 patient previously treated with Risdiplam

Michele Tosi[1,2], Michela Catteruccia[1], Claudio Cherchi[3], Irene Mizzoni[1,2], Adele D'Amico[1]

[1] Unit of Neuromuscular and Neurodegenerative Disorders, Department of Neurosciences, Bambino Gesù Children's Hospital, IRCCS, Rome, Italy; [2] Genetics and Rare Diseases, Research Division, Bambino Gesù Children's Hospital, IRCCS, Rome, Italy; [3] Unit of Paediatric Pulmonology and Respiratory Intermediate Care, Academic Department of Paediatrics, Bambino Gesù Children's Hospital, IRCCS, Rome, Italy

Three disease-modifying drugs (Nusinersen, Risdiplam and Onasemnogene abeparvovec) have been approved for SMA type I. Onasemnogene abeparvovec (GRT) can be administered in naïve patients or patients who are already being treated with Nusinersen or Risdiplam. Safety data on GRT in naïve patients or previously treated Nusinersen have been extensively described whereas any case of switch therapy from Risdiplam to GRT has been reported yet. We report on a SMA type I patient treated with Risdiplam by 2 months and switched to GRT at 5 months. She manifested the more common and awaited side effects that resolved in 3 months. The follow-up after 9 months from GRT infusion showed normal blood count, renal and cardiac function. She had great improvement in motor outcome, and no respiratory and bulbar problems as well as normal neurocognitive profile. This case suggests that the GRT may be safe also in patients previously treated with Risdiplam.

Key words: Risdiplam, Onasemnogene abeparvovec, safety, switching therapies

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Correspondence
Adele D'Amico
Unit of Muscular and Neurodegenerative Disorders, Bambino Gesù Children's Hospital, piazza S. Onofrio 4, 00165 Rome, Italy. Tel.: +39 06 68592105. Fax: +39 06 68592024. E-mail: adele2.damico@opbg.net

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Introduction

Spinal muscular atrophy (SMA) is an autosomal recessive neuromuscular disease, caused by homozygous mutations of the survival motor neuron 1 (*SMN1*) gene, leading to degeneration of alpha motor neurons which results in progressive proximal muscle weakness and paralysis [1]. The disease severity is classified in three main phenotypes from type I to type III on the basis of age of onset and highest motor function achieved [1]. In recent years, three disease-modifying drugs have been approved [2]. Nusinersen, an anti-sense oligonucleotide (ASO) that acts as an SMN2 splicing modifier, was the first therapy approved for the treatment of all types of SMA in 2017 [2]. Onasemnogene abeparvovec (GRT), a gene therapy product devised to insert a functional SMN1 copy in motor neurons using an adeno-associated viral vector (AAV9), was approved in 2020 for treatment of SMA patients (with different indications by FDA and EMA) [2]. Risdiplam, the latest approved therapy for all types of SMA, is a small pyridazine derivative molecule, which modifies the splicing of pre-mRNA from the

SMN2 gene promoting the expression of full-length mRNA and higher levels of functional SMN protein [2].

While mid- and long-term (at least 12 months) follow-up from SMA patients treated with Nusinersen or GRT are available as well as safety data on their combinations or switch [3,4], to date, there is no safety data about the switch from Risdiplam to GRT. Here we report on a SMA type 1 baby treated with Risdiplam from the age of 2 months who switched to GRT at 5 months. To the best of our knowledge, this is the first report describing the safety profile and the clinical outcome of a switching from Risdiplam to GRT.

Case report

The baby was the first child born of unrelated parents coming from an eastern European country. The pregnancy was uneventful. The vaginal delivery was at term and the neonatal period was reported as normal until the age of 30 days, when parents noted a reduction in leg movements. Soon after the neurological examination, a genetic test was performed demonstrating the absence of SMN1 with two copies of SMN2, thus confirming the diagnosis of a SMA type 1. At the age of two months, the baby started treatment with Risdiplam at a dose of 1.6 ml/day that was gradually implemented, according to weight in the following months.

Our first neurological evaluation was performed at the age of 3 months. The child was alert, reactive, showing good visual tracking and interaction with the examiner. In supine traction, the child still did not control her head but she was able to balance her head quite well when sitting with support. She had minimal distal leg movements, and she had subgravity movements at forearms and hands. She presented diaphragmatic breathing without a chest deformity and bulbar function was completely preserved. She fed herself by mouth, without difficulties in swallowing or drooling. The CHOP-INTED score was 32/64. Compound muscle action potential (CMAP) registered from ulnar nerve showed a low voltage (0.4 mV, normal values > 1.7 for age 1-5 months [5]). In the following months, parents enquired the access to GRT because they planned to move to their country of origin where Risdiplam was unavailable. Thus, extensive blood tests including cells blood count, serum transaminases, serology for hepatotropic viruses and antibody titer for AAV9 were performed, resulting in normal range. The child met all the eligibility criteria, and at the age of 5 months, after a washout period of 3 days from Risdiplam, she received the treatment with Onasemnogene abeparvovec (7.7×10^{14} vg, based on a weight of 6.8 kg). As recommended she started treatment with prednisolone at 1 mg/kg/day the day before the GRT infusion [6].

There were no immediate adverse effects. Two days after the infusion, the patient presented fever (T max 38.6°C) and loss of appetite. Five days after infusion, blood chemistry tests showed the presence of hyper-transaminasemia (AST 170 U/L, ALT 131 U/L, normal range > 33 U/l), thrombocytopenia (62.000/uL, normal range 150.000-450.000/uL) and hyper-ferritinemia (4809 ng/ml, normal range 13-150 ng/ml), thus prednisolone was doubled at 2 mg/kg/day. Her liver enzymes and platelet count have gradually normalized, and we proceeded with progressive steroid's reduction until complete suspension after 95 days. Liver ultrasound was always normal.

The last clinical evaluation was performed 9 months after the gene therapy administration. The baby was 14 months old and she presented significant improvement in motor function. She acquired a stable sitting position, she maintained the kneeling position with anterior support and she was able to stand unaided with upper limbs support. Her CHOP-INTEND score was 59/64 and HFMSE score 18/66. No respiratory problems bell-chest or paradoxical breathing were observed. The neurocognitive and speech profile was normal. The blood tests showed normal blood count, renal and cardiac function. She fed by mouth and no swallowing problems were reported, nor chewing fatigue or drooling were observed. She had a good body-weight growth. The ulnar CMAP < 1 mv, showed an increased amplitude (0.6 mV).

Discussion

To the best of our knowledge, this is the first report of a therapeutic switch from Risdiplam to Onasemnogene abeparvovec in a SMA type 1 child.

Monotherapy using Nusinersen, Risdiplam or GRT results in motor milestone achievements, and prolonged survival in symptomatic infants with SMA type 1.

The safety profiles of these modifying therapies are quite well known. The most common side effects related to intrathecal injection of Nusinersen, observed in clinical trials and in clinical practice, are: headache, post-puncture syndrome, back pain, nausea, vomiting, rash, and pyrexia; whereas the most reported risdiplam-related adverse events in clinical trials (FIREFISH and SUNFISH) are fever, diarrhea, mouth and aphthous ulcers, arthralgia, urinary tract infection, constipation, that are seemingly associated with progression of or complications related to the underlying condition, rather than the drug [3].

Common and almost awaited side effects related to GRT are vomiting, loss of appetite, thrombocytopenia and liver enzymes elevation [7]. These complications are most often mild and self-limiting; however some safety concerns related to GRT include severe liver failure, caused by hepatotoxicity secondary to a hyperinflammatory re-

action and thrombotic microangiopathy, a rare, acute, and life-threatening condition, characterized by microangiopathic hemolytic anemia with thrombocytopenia. These fatal complications occurred in a very low percentage of treated patients [8].

Following the approval of Onasemnogene abeparvovec, increasingly often, families ask for combined treatments or therapeutic switches. Clinical trials data on combination therapies are lacking whereas in the last two years several observational studies have been published reporting safety and efficacy profiles of SMA type 1 patients who switched from Nusinersen to GRT or viceversa [9-12].

Risdiplam has been the latest treatment approved for SMA and, up to date, no switching from Risdiplam to GRT has been still reported in SMA type 1.

This has been our first clinical experience of switching from Risdiplam to GRT in a young SMA type 1 patient. The switch was well tolerated; the baby experienced the expected side effects (high transaminases, fever, and thrombocytopenia) that resolved in 3 months under prednisolone treatment. We are certainly aware that the age and the weight as well as the clinical status at the time of infusion have played a crucial role in the good clinical outcome in our patient. However, this case shows that the switch from Risdiplam to Onasemnogene abeparvovec is safe, as already demonstrated for Nusinersen.

The clinical decision regarding the opportunity in switching and the timing of administration of a second therapy is always challenging. Currently, there are no published guidelines or clinical recommendations regarding the switch of therapies in SMA type 1 patients, so it is left to the decision of the clinician if, how and when to proceed.

It is known that Risdiplam and Nusinersen have different mechanisms of action than GRT, but it is unclear whether the combination of these therapies can really increase the SMN expression levels above the monotherapy approach. Moreover, the long-term effects of their concurrent administration in terms of efficacy and safety are not yet known.

In our opinion, it is extremely important to continue collecting safety and efficacy data to answer these questions.

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Conflict of interest statement

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Author contributions

MT, MC: performed data analysis and their interpretation, drafted the manuscript; CC, IM, AC: collect clinical data and evaluate the patient; ADA: planned the study, performed data analysis and their interpretation, revised and submitted the manuscript.

Ethical consideration

This study was performed in line with the principles of the Declaration of Helsinki. The study was approved by Ethics Committee of Bambino Gesù Hospital.

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Good response to the late treatment with ataluren in a boy with Duchenne muscular dystrophy: could the previous mild course of the disease have affected the outcome?

Ludovica Pasca[1,2], Alice Gardani[1], Matteo Paoletti[3], Daniele Velardo[4], Angela Berardinelli[1]

[1] Department of Child Neurology and Psychiatry, IRCCS Mondino Foundation, Pavia, Italy; [2] Department of Brain and Behaviour Neuroscience, University of Pavia, Pavia, Italy; [3] Department of Neuroradiology, IRCCS Mondino Foundation, Pavia, Italy; [4] IRCCS Foundation Ca' Granda Ospedale Maggiore Policlinico; Neurology Unit, Milan, Italy

Duchenne muscular dystrophy (DMD) is a severe, progressive X-linked recessive disorder, caused by the absence of the dystrophin protein. A resolutive therapy for DMD is not yet available. The first approved drug for DMD patients with nonsense mutations is ataluren, approved for the treatment of children aged ≥ 2 yrs, that seems effective in slowing the disease progression. An earlier introduction of ataluren seems to give better results. We report the case of a 14-year-old DMD patient with a nonsense mutation in exon 70, still ambulant, who started taking ataluren at 12 years and remained stable for the following two years. The patient was on steroid since the age of 6, with beneficial effects. At two-years follow-up, an optimal disease evolution was observed, associated with a constant decrease of creatine kinase blood levels. Despite the late start of the treatment, ataluren seems to have significantly contributed to the stabilization of the functional status in this patient though it cannot be excluded that the result may have been influenced by the previous favorable course of the disease. However, further studies should be planned in patients with similar age treated with ataluren to better evaluate the treatment's results compared to the natural course of the disease.

Key words: Duchenne muscular dystrophy, target therapy, nonsense mutation, ataluren, time function tests

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Correspondence
Angela Berardinelli
Department of Child Neurology and Psychiatry, IRCCS Mondino Foundation, via Mondino 2, 27100 Pavia, Italy.
E-mail: angela.berardinelli@mondino.it

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Introduction

Duchenne muscular dystrophy (DMD) is a disabling, life-shortening X-linked neuromuscular disease [1]. Causative mutations (deletions, duplications, point mutations) involve the *DMD* gene, which codes for dystrophin, a large protein responsible for muscle membrane stabilization and signaling mediation [1]. About 13% of DMD patients have a nonsense mutation that converts an amino acid into a premature stop codon in the dystrophin mRNA, generating a nonfunctional protein [2]. Typical disease trajecto-

ry involves delayed walking, worsening motor skills, loss of ambulation, and premature death due to cardiorespiratory failure. In the last decade, an improved standard of care focused on the preservation of the ambulatory function [2] has positively modified the natural history of DMD. Corticosteroids are still the gold standard treatment to increase muscle strength [1] nevertheless, they cannot target the molecular cause of the disease. For DMD patients with nonsense mutations, a novel treatment approach has been essayed with ataluren (PTC124), able to restore the function of dystrophin by introducing an amino acid at the stop codon site to continue the mRNA translation. Ataluren is an orally bioavailable drug evaluated in two randomized, double-blind, placebo-controlled trials in Phase IIb and Phase III [3-5].

In DMD patients, motor function changes with age, anthropometric and myometric variables, genetic predisposition, training and steroid use [1]. Therefore, baseline disease trajectories and reliable measures of outcome are crucial to interpret new drugs' efficacy.

We report the case of a DMD patient with a nonsense mutation, who started ataluren at 12 years and 9 months, and maintained ambulation and an optimal functional status at two-years follow-up.

Case report

The patient, currently aged 14 years, reached independent ambulation at 15 months showing speech delay. He received a DMD diagnosis at one year of age, after an incidental registration of elevated creatin kinase (CK) values (13.369 U/L). Muscle biopsy showed a severe dystrophic muscle picture. The immunohistochemical staining for dystrophin was absent with COOH and rod domains monoclonal antibodies, while a faint signal was detected with NH2 domain antibody. The WB analysis showed the absence of the dystrophin band, while traces of an immunoreactive band were detected with the rod-domain antibody. Genetic analysis showed the nonsense mutation *c.10141C > T; R3381X* in exon 70 of the *DMD* gene. The stop codon generated by the nonsense mutation is TGA and the 3' adjacent nucleotide is A (TGAA tetranucleotide).

At the age of 6 years, daily deflazacort was started (0.9 mg/kg/day) without major side effects. At 10 years, though ejection fraction and heart volumes were within the normal limits, angiotensin-converting-enzyme inhibitor therapy was introduced to delay the left ventricle dysfunction. Motor skills and performance remained stable, showing a positive profile as demonstrated by 6MWT and time function tests values (TFTs) (Fig. 1). The patient practiced physical rehabilitation since the time of diagnosis and regularly utilized ankle- and leg-foot orthoses. A cognitive evaluation with Weschler III test showed an Intelligence Quotient (IQ) of 75. At 12 years and 3 months, a muscle MRI was performed that showed hypertrophy of triceps surae and a mild to moderate fat substitution of adductor magnus, vastus lateralis and intermedius (Fig. 2).

The treatment with ataluren was started at 12 years and 9 months, at the dosage of 40 mg/kg/day. At that time, he was still ambulant with a waddling gait, lumbar lordosis and showed a positive Gowers' sign. He was in the pre-pubertal period with short stature (129 cm, less than 3rd percentile) and a body mass index (BMI) of 26; mild cushingoid features, tightness of the Achilles tendons, and proximal muscle weakness were present. Echocardiography revealed normal kinetic and dimensional parameters. Spirometric examination showed normal vital capacity and expiratory volumes. CK values were about 12.000/13.000 U/L in several determinations.

Soon after the ataluren introduction, the patient reported a prompt subjective improvement in muscle strength. The 6MWT values registered in the two years after the start of the drug were superimposable to those achieved in the previous years, with the best performance obtained at one year follow-up (covered distance: 540 m). Also the North Star Ambulatory Assessment (NSAA) score and the other TFTs remained stable over the last 2 years. Interestingly, the CK blood levels decreased to 6000-7000 U/L, at last follow-up visits (Fig. 1).

Discussion

The only approved drugs for DMD concern deletions amenable for exon 53 skipping (Viltolarsen, in the USA and Japan), and deletions amenable for exon 51 skipping (Eteplirsen). Ataluren was recently approved for the treatment of nonsense mutations. Several randomized clinical trials [3-5] showed that ataluren is well-tolerated and able to improve dystrophin expression, reducing the rate of muscle degeneration as measured using 6MWT. In Italy, ataluren is approved by the Italian Drug Agency (AIFA) for DMD boys since two years of age. Recent studies show that ataluren can be of benefit in non-ambulatory as well as in ambulatory patients [6]. However, for a better interpretation of the data in clinical practice, different outcome measures are necessary according to the different stages of the disease.

Furthermore, a positive relation between earlier drug introduction and better results has also been described [7].

Our patient has shown a good response to the steroid treatment and physiotherapy, compared to DMD pairs. According to the model of Mercuri et al. [8], he relies on the stable/improvement disease class. Moreover, according to the most recent studies [7], our patient was not among those most likely to benefit from treatment with ataluren, as 6MWD was 439 m at baseline. Nonetheless, the response

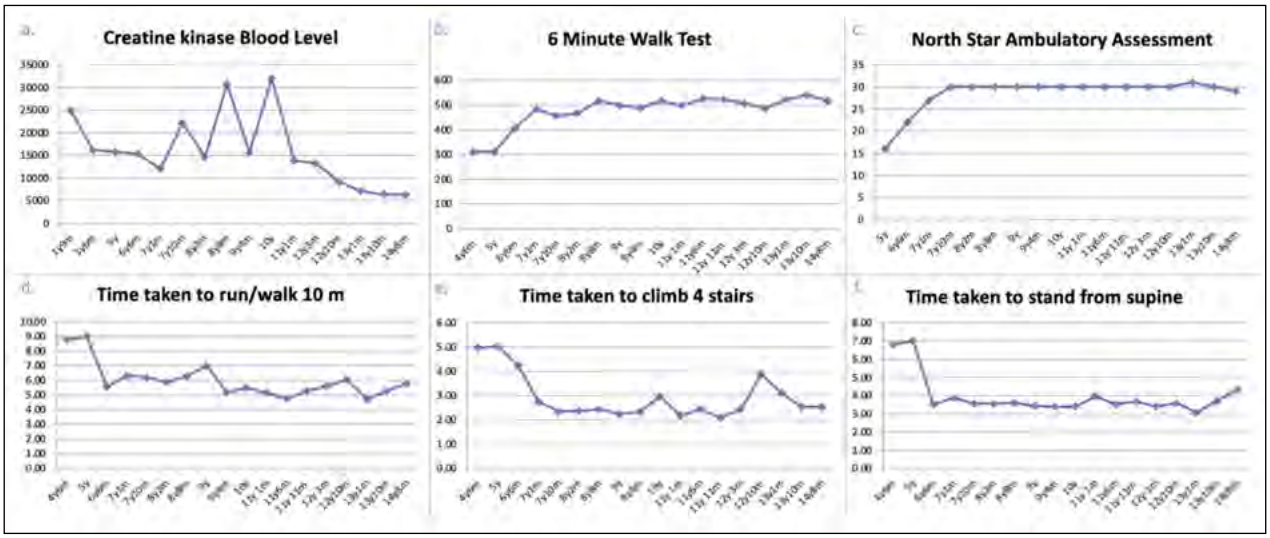

Figure 1. Time function tests and CK levels during follow-up. A) creatine kinase value (U/L) at different follow-up visits; B) 6 minute walk test results (meters) at different follow-up visits; C) North Star Ambulatory Assessment (score) at different follow-up visits; D) time taken to run/walk 10 meters (seconds) at different follow-up visits; E) time taken to climb 4 stairs (seconds) at different follow-up visits; F) time taken to stand from supine (seconds) at different follow-up visits.

to ataluren contributed to the stabilization of the previously shown functional profile and even to the improvement of some motor performances. Compared to the population of nonsense and deletion DMD patients described by Hamuro et al. [9], our patient shows a disease course that can be considered exceptional (6MWT > 500 m at > 14 years of age). Furthermore, in our patient, both values of 6MWT and of 10 m walking test – which measure the walking function – are significantly related each other, a result usually more evident in boys with preserved functional activity. Finally, the improvement in motor performance seems to have a corresponding biochemical trend in a decrease of CK levels (more than half), observed in the last two years of treatment compared to the values recorded at the start of treatment. This pattern may reflect a treatment effect as described in the study by Finkel et al. [3], in which the majority of subjects treated with ataluren showed a decrease in CK values without any correlation with the magnitude of the response.

Given the increasing availability of trials aimed at establishing new drugs's efficacy in DMD, reliable tools are needed to measure the outcomes and more predictable trajectories of natural diseases. DMD patients having a nonsense mutation do not appear to show a significantly different disease course than patients with other mutations. In the study of Pane et al. [10], boys with *DMD* gene duplications generally performed better than those with other mutations, and those skipping exon 44 had better baseline results and less drastic changes than those eligible for skipping of exon 45 or 53 [10].

Our patient has a point mutation involving exon 70, for which predictable responses to active therapeutic management have not yet been reported. Considering the mild natural disease evolution, the results obtained could be explained not only by the steroid treatment or the ataluren association, but by the set of therapeutic interventions implemented. The significant results observed in particular in the 6MWT after the ataluren introduction, are likely due to the greater reliability of this test in measuring muscle endurance compared to the MRC scale.

The good response to ataluren could also be explained by predisposing genetic factors not yet fully explored. For instance, the stop+4 model may help in predicting functional changes, as shown by the recent papers of Wangen et al. [11]. In this report while no difference in 6MWT were observed in patients with point mutations according to the type of stop codon, frame status of exons involved and protein domain affected, a significant difference ($p < 0.05$) was observed when considering the stop codon together with the 3' adjacent nucleotide ("stop+4 model"), as patients with stop codon TGA and 3' adjacent nucleotide *G* (TGAG) have a more rapid decline [12].

Limitations of the study

We are aware that the results obtained in a single case report cannot be extended to the population of nonsense DMD patients. Furthermore, the lack of a second muscle biopsy (refused by the patient's parents) which might show the state of dystrophin after the ataluren in-

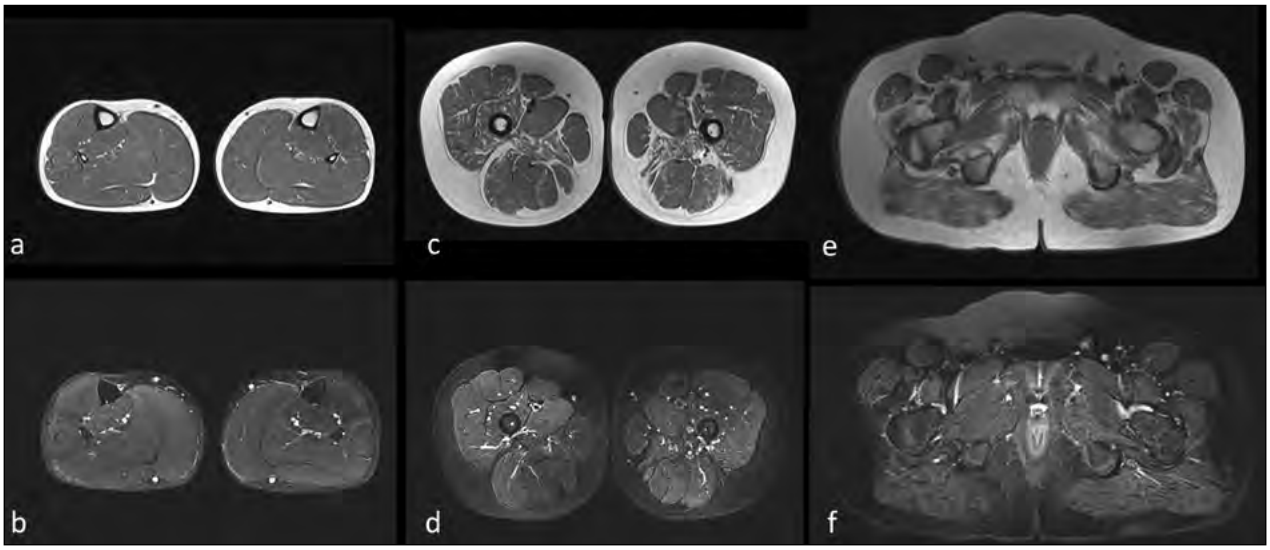

Figure 2. Muscle MRI. Muscle MRI findings performed at the age of 12 years and 3 months. (A,C,E) Axial turbo spin echo T2 weighted (w), and (B,D,F) short tau inversion recovery (STIR) axial images at the level of the lower leg (A,B), thigh (C,D) and pelvic girdle (E,F) are shown. No significant fat substitution or edema is evident at the level of the leg muscles, in which, by contrast, a mild triceps surae hypertrophy can be detected (A,B). At the level of the thigh (C,D) the adductor magnus, vastus lateralis and intermedius show signs of fat substitution bilaterally, without associated muscle edema. At the level of the pelvic girdle the involvement of the glutei is evident with fat substitution (E) and no corresponding edema (F).

troduction, and the relative long-term follow-up required to better describe the evolution pattern in the patient, can be considered as limitations of the study. However, while there is now supporting evidence for an early introduction of ataluren in patients with DMD, indications regarding ataluren introduction at older ages are lacking.

We are convinced that a deep clinical and genetic characterization of patients with exceptional course of disease and possibly good response to ataluren may be of interest for both clinical and research purposes, as well as that further studies on larger cohorts, exploring modifying genes and other genetic determinants are necessary to clarify the stratification criteria for DMD, and to identify patients who can most benefit from the ataluren treatment.

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Conflict of interest statement

The Authors declare no conflict of interest.

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Author contributions

AB, LP: conceptualization; AB, LP: methodology; AB, LP, AG, DV, MP: investigation; AB, LP, AG, DV, MP: data curation; AB, LP: writing-original draft preparation; AB, LP, AG, DV, MP: writing-review and editing. All Authors have read and agreed to the published version of the manuscript.

Ethical consideration

The informed consent to the treatment was collected from the patient's parents.

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Juvenile Myasthenia Gravis in a 14-year-old adolescent masked by mood disorder: the complex balance between neurology and psychiatry

Rossella D'Alessandro[1], Anna Salvalaggio[1], Martina Vacchetti[1], Tiziana E. Mongini[2], Federica S. Ricci[1]

[1] Section of Child and Adolescent Neuropsychiatry, Department of Public Health, and Paediatric Sciences, Università degli Studi di Torino, Turin, Italy; [2] Neuromuscular Unit, Department of Neurosciences RLM, University of Turin, Turin, Italy

Juvenile Myasthenia Gravis (JMG) is a neuromuscular disease, often characterized at onset by fatigue and fluctuating weakness. We report a case of a girl affected by severe mood disorder, in which the diagnosis of JMG and its treatment were challenged by the concomitant psychiatric condition. A 14-year-old girl, with a history of severe mood disorder and emotional dysregulation, had been treated with benzodiazepines, sertraline, and antipsychotics, reporting generalized fatigability, weakness, and drowsiness, first ascribed to her psychiatric condition and therapy. After a suicide attempt, she was hospitalized and a neurological assessment revealed a fluctuating ptosis and facial weakness, that improved with rest. The diagnosis of JMG was confirmed by repeated nerve stimulation test, and by the response to pyridostigmine. Antibodies anti-AChR and anti-MuSK were negative. JMG diagnosis may be harder in adolescents with psychiatric comorbidity. Moreover, the neurological condition limits the choice of the appropriate psychopharmacotherapy.

Key words: juvenile Myasthenia Gravis, psychiatric comorbidities, mood disorder

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Correspondence
Rossella D'Alessandro
Section of Child and Adolescent Neuropsychiatry, Department of Public Health and Paediatric Sciences, University of Turin, Regina Margherita Hospital, piazza Polonia 94, 10126 Turin, Italy. Tel.: +39 011 3135248. Fax: +39 011 3135387. E-mail: rosselladalessandro@live.com
ORCID: https://orcid.org/0000-0002-6358-5937

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Introduction

Paediatric Myasthenic Syndromes (PMS), caused by defects of transmission at the neuromuscular junction, may manifests in three main distinct forms, with different pathophysiological mechanisms: the congenital myasthenic syndromes (CMS), a heterogeneous group of genetically inherited disorders of the neuromuscular transmission; the transient neonatal myasthenia, resulting from placental transfer of maternal antibodies to infants from mothers with autoimmune myasthenia; and the juvenile myasthenia gravis (JMG), an autoimmune acquired disorder in which antibodies are directed at the postsynaptic membrane of the neuromuscular junction, leading to varying degrees of muscle weakness and fatigability [1]. The disease presentation is usually under 19 years-old [1-4].

All forms of PMS lead to muscle fatigue and weakness of varying degrees. Especially in JMG, fluctuations in weakness are a hallmark of this disease. The weakness can involve ocular, bulbar, respiratory, and skeletal

muscles. Clinical diagnosis is confirmed by electromyography (EMG) and response to therapy, while serology is less helpful in children [2-4].

In some cases, initial myasthenic symptoms may be underestimated, especially in presence of other medical conditions causing fatigability and exhaustion [5].

Depressive features can also be a key confounder in JMG; as a consequence, the simultaneous onset of the two disorders adds to the risk of diagnostic confusion [6].

The main objective of this presentation is to describe how a psychiatric condition, namely a severe mood disorder, challenged the diagnosis of JMG in a 14 year-old girl. This case highlights the importance of a careful neurological assessment in young psychiatric patients presenting confounding symptoms and signs, with an integration of neurological and psychiatric perspectives, causing a relevant impact on differential diagnosis and pharmacological options.

Case report

A 14 year-old girl was admitted in the psychiatric department for a suicide attempt with ingestion of metal objects. She had already been hospitalized twice during the previous year, because of eating dysregulation, recurrent panic attacks with depressive symptoms, and general emotional dysregulation. Her family history was positive for psychiatric disorders and substance addiction (her father had an history of alcohol abuse and depression), in the context of a poor family environment, complicated by a complex parental separation. Family and personal history was silent for neuromuscular diseases: in particular, perinatal conditions were reported as regular, and the development of motor milestones was normal. She had been treated with high-dose benzodiazepines (delorazepam, lorazepam), sertraline, and an atypical antipsychotic (aripiprazole) for six months, with poor results on depressive symptoms and worsening of general fatigue, weakness, and drowsiness, considered in part as side effects of the therapy and in part as a manifestation of her psychiatric condition. Nocturnal respiratory distress was also described with mild desaturation and diagnosed as panic attacks.

At admission, the neurological assessment revealed a myasthenic face, with asymmetric eyelid ptosis and facial muscle weakness, which were fluctuating during the day, and improving with rest. The patient showed a mild generalized weakness, and there were no other neurological signs nor bulbar involvement.

Suspecting a myasthenic syndrome, neurophysiological and laboratory exams were performed. EMG showed a pathological decrement in the compound motor action potential after the 4 th stimulation (18%, 26%) of the right facial nerve (orbicularis oculi muscle). Both anti-AchR and anti-MuSK antibodies resulted negative. A chest TC was performed and excluded the presence of thymoma. . A DNA sample was collected for genetic analysis in order to detect possible congenital myasthenic syndromes (still on-going). A clinical and neurophysiological diagnosis of seronegative JMG syndrome was made. First-line symptomatic therapy with low-dose pyridostigmine (15 mg, 5 times per day) was initiated, leading to a progressive reduction of eyelid ptosis, generalized fatigue, and drowsiness with positive subjective outcome. A multidisciplinary team composed by child neurologists and psychiatrists assessed different therapeutical options: steroids were avoided because of her psychiatric comorbidities, and for the good response to the symptomatic therapy alone (pyridostigmine).

From the psychiatric point of view, the therapy was re-assessed, avoiding drugs associated to the risk of myasthenic symptoms worsening, such as benzodiazepines. Sertraline was also suspended for the lack of efficacy, and aripiprazole was confirmed as a stabilizer agent, together with the diagnosis of severe mood disorder with emotional dysregulation. After reaching an adequate control of myasthenic symptoms, with an evident improvement of her neurologic condition, a better anxiety control and lower emotional lability were also observed.

Discussion

JMG represents a challenging diagnosis, which can result even harder in adolescents with psychiatric comorbidity, due to the difficulty of differentiating neurologic and psychiatric symptoms. To date, little is still known about the relationship between myasthenia and associated psychiatric disorders; however, it is known that psychiatric symptoms, such as fatigue, lack of energy and shortness of breath may coincide with those of the neurological disease, leading to a misdiagnosis. The correct diagnosis is missed in up to 46% of myasthenic patients during the 1st year of disease manifestation and depressive symptoms are a key confounder accounting for 20% of initial misdiagnoses [5,6].

In the literature, psychiatric comorbidities are reported as quite common in myasthenic patients, with a prevalence of mood disorders around 45% and a prevalence of anxiety disorders around 58% [7]. Moreover, it is important to remark that many pharmacological treatments, including psychiatric ones, can cause myasthenic-like symptoms, or unmask a latent form of myasthenia, or exacerbate it or also induce de novo myasthenia [8]. Therefore, psychiatric treatments must be carefully planned because of the risk of worsening myasthenic symptomatology. In particular, antipsychotics impair neuromuscular trans-

mission at presynaptic and postsynaptic levels; lithium, for instance, causes reduction in acetylcholine synthesis and release reduction in number of receptors, and sedatives like benzodiazepines, inducing central respiratory depression, which can be very dangerous for patients with bulbar symptoms or borderline respiratory reserve [9]. In the presented case, immediate worsening of general fatigue and weakness was reported following initiation of high dose of benzodiazepines, initially interpreted as mood deflection.

On the other hand, regarding JMG as a chronic, debilitating, life-threatening disease with unpredictable progression, patients affected may have psychiatric consequences in terms of coping and adaptation to the disease [7,10]. Thus, subject with JMG may be at increased risk of psychiatric disorders.

In the presented case, the diagnosis was suspected after a careful neurological clinical assessment, which is always recommended in the evaluation of psychiatric patients, particularly at onset or in the presence of a modification in symptoms. The clinical diagnosis was confirmed by the positive repetitive nerve stimulation test, which was performed on the orbicularis oculi muscle; the single-fiber electromyography (sfEMG), which is considered the gold standard for seronegative JMG, was not carried out due to discomfort and the length of the test, poorly tolerated in young patients [2]. At the time of the first assessment, immunological analysis did not detect the presence of anti-AChR and MuSK antibodies. This finding is consistent with literature reports, as delayed seroconversion can be years after onset and particularly in pre-pubertal children [3]. Therefore, it is suggested to repeat this test in seronegative young patients at 6 monthly intervals.

The final therapeutic strategy was discussed by a multidisciplinary team, and included pyridostigmine and aripiprazole, avoiding benzodiazepine, sertraline and steroids, with a good outcome both on neurological and psychiatric manifestations [1].

Conclusions

Mood and anxiety disorders symptoms may be a key confounder in JMG, leading to a diagnostic delay. Information on the relationship between JMG and psychiatric symptoms are limited, and misdiagnosis and under-treatment are actual issues in these patients. Although JMG is a rare disease if compared to juvenile psychiatric disorders, 'red flags' symptoms such as fluctuating weakness and fatigability should always prompt a neurological assessment. Moreover, clinically relevant guidelines for managing myasthenic patients in presence of psychiatric comorbidities are hardly available. This case outlines the need of further studies, especially about the safety of different therapeutical options for the comorbidities.

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Conflict of interest

The Authors declare no conflict of interest.

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Author contributions

AS, MV: data collection; AS, MV, RD: conceptualization, methodology, data curation, original draft preparation, writing; FSR, TEM: review and editing and supervision; FSR, TEM: validation

Ethical consideration

The study was conducted in accordance with the Declaration of Helsinki. The approval of the Ethics Committee was not necessary as no procedures other than those routinely performed were employed.

Parents gave their informed consent for the publication of this case report.

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NEWS FROM AROUND THE WORLD

AIM

The Association promoted the Webinar "SMA and dysphagia", held on 18 July 2022 and the *Workshop On Neuromuscular Diseases* which taken place in Brescia from 22 to 24 September.

The XXII National Congress of the Italian Association of Myology will be held at the Auditorium R. Gervasio in Matera, from 19 to 22 October 2022. It is possible to proceed with the registration for the event, by 17 July with a reduced fee. To learn more, please visit the congress website: *https://congressoaim2022.it*

MSM

Due to pandemics, the 14th Meeting of the Mediterranean Society of Myology (MSM) is moved to the 2023. Proposals to organize and host the event are welcome.

WMS

The 27th International Annual Congress of the World Muscle Society will take place on 11th-15th October 2022, in Halifax, Nova Scotia, Canada. The congress venue is the Halifax Convention Centre at 1650 Argyle Street, in the heart of this Atlantic seaport.

Electronic presentations and posters will be made available via the Society's platform as well as apps for delegates wishing to participate virtually. Hard work is being done to ensure that the registration process is as flexible as possible in these ever-changing and uncertain times.

To learn more, please visit the congress website: *https://www.wms2021.com*

FORTHCOMING MEETINGS

2022

September 13-15

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

September 15-17

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

October 11-15

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

October 19-22

22nd Congress of the Italian Association of Myology. Matera, Italy. Information: website: *www.miologia.org; segreteria.aim@fclassevents.com*

December 7-8

The 4th International Conference on Rare Diseases. Vienna, Austria. Information: website: *www.bioevents.net*

2023

January 9-11

12th World Gene Convention. Sapporo, Japan. Information: website: *https://www.bitcongress.com/WGC2023*

April 22-28

75th AAN Annual Meeting. Boston, MA. USA. Information: website: *https://www.aan.com*

May 16-19

4th International Meeting on Laminopathies. Madrid, Spain.

4th International Meeting on Laminopathies
Madrid, 16-19 May, 2023

ORGANIZERS
- Ignacio Pérez de Castro (ISCIII, Spain)
- Vicente Andrés (CNIC, Spain)
- Gisèle Bonne (Sorbonne Université - INSERM, France)
- Giovanna Lattanzi (CNR, Italy)
- David Araújo-Vilar (Universidad de Santiago de Compostela, Spain)

The **4th International Meeting on Laminopathies** will bring together a wide range of experts in the field of these rare diseases caused by mutations in genes encoding proteins of the nuclear envelope. Laminopathies can affect single tissues (mainly striated muscle, adipose tissue and peripheral nerve) or multiple organs. Hence, most laminopathies can be classified in 4 different disease groups: muscular dystrophies, lipodystrophies, peripheral neuropathies and progeroid disorders. Numerous mutations have been associated with laminopathies, but the mechanisms underlying disease initiation and progression remain poorly characterized. Accordingly, there is a lack of specific and effective treatments for laminopathies. The meeting, to be celebrated in Madrid on May 16-19 2023 on a **face-to-face** format, will be open to researchers, physicians and patients from around the world with the main goal of **synergistically exchange knowledge and ideas** to better understand laminopathies and **help develop new therapies**. The meeting will include sessions focused on basic-molecular research, disease-modeling, as well as therapeutic and clinical aspects of laminopathies. **Patients** will be part of the meeting in a specific session co-organized with scientists. The number of participants (~200) and format of the event will promote the dialogue between all the main stakeholders involved in the study of laminopathies. In summary, the 4th International Meeting on Laminopathies will be a multidisciplinary scientific meeting focused on helping researchers and clinicians to find effective therapies and eventually the cure for laminopathies.

TOPICS
- **Clinical aspects of laminopathies** (natural history, care and treatments, registers, biobanking) including Progeroid syndromes, Lipodystrophies, Peripheral nerve disorders and Skeletal/Cardiac striated muscle laminopathies.
- **Biomarkers** with prognostic value and to measure response to treatments.
- **Mechanisms underlying laminopathies,** from transcriptional regulation, metabolism, differentiation processes, nuclear envelope signaling.
- **Disease Models**, including 3D cell models, microfluidic devices, flies, mice, pigs..
- **Therapies**, both drug-based and advanced therapies.
- **Patient Advocacy Organizations (PAO) sessions** too be defined by patients.

SPECIAL FEATURES
Novelty => Communication of unpublished results.
Integration => favoring the participation of under-represented countries (free registration*)
Scientific career => helping early career researchers (1/3 of the talks; free registration**; best talk award) and PhD students (selection of talks from poster submissions; best talk and poster award; reduced registration fee***)

*Underrepresented countries: Armenia, Bulgaria, Croatia, Czech Republic, Estonia, Georgia, Hungary, Latvia, Lithuania, Malta, Poland, Romania, Serbia, Slovakia, Slovenia and Turkey
**Free registration for early career researchers with talk selected for oral presentation
***50% discount on registration fees for PhD students (100% if presenting abstract)

May 20-23

Heart Failure 2023 and World Congress on Acute Heart Failure. Prague, Czech Republic. Information: website: *https://www.escardio.org/Congresses-&-Events/Heart-Failure*

May 25-26

International Conference on Neuroscience and

Neurology in Tokyo, Japan. Information: website: *https://www.neuralscience.scientexconference.com*

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

April 13-19

76th AAN Annual Meeting. Denver, CO. USA. Information: website: *https://www.aan.com*

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

April 5-11

77th AAN Annual Meeting. San Diego, CA. USA. Information: website: *https://www.aan.com*

October 7-11

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

For application or renewal to MSM

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L. Politano, *Treasurer*

APPLICATION/RENEWAL FORM

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

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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 700 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 name 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).
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- References prepared according to instructions.
- English style.
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