Established in 1982 as Cardiomyology

ACTA MYOLOGICA
(Myopathies, Cardiomyopathies and Neuromyopathies)

Official Journal of
Mediterranean Society of Myology
and
Associazione Italiana di Miologia

Founders: Giovanni Nigro and Lucia Ines Comi

Four-monthly

EDITORIAL BOARD

Ekram Abdel-Salam, Cairo
Corrado Angelini, Padova
Enrico Bertini, Roma
Serge Braun, Paris
Kate Bushby, Newcastle upon Tyne
Kevin P. Campbell, Iowa City
Marinos Dalakas, Athens
Feza Deymeer, Instanbul
Salvatore Di Mauro, New York
Denis Duboc, Paris
Victor Dubowitz, London
King W. Engel, Los Angeles
Michel Fardeau, Paris
Irena Hausmanowa-Petrusewicz, Warszawa
Fayçal Bentati, Tunis
Byron A. Kakulas, Perth
Frank Lehmann-Horn, Ulm
Carlo Minetti, Genova

Clemens Müller, Würzburg
Francesco Muntoni, Londra
Carmen Navarro, Vigo
Gerardo Nigro, Napoli
Anders Oldfors, Göteborg
Eijiro Ozawa, Tokyo
Heinz Reichmann, Dresden
Serenella Servidei, Roma
Piraye Serdaroglu, Instanbul
Yeuda Shapira, Jerusalem
Osman I. Sinanovic, Tuzla
Michael Sinnreich, Montreal
Andoni J. Urtizberea, Hendaye
Gert-Jan van Ommen, Leiden
Lord John Walton of Detchant, Oxford
Steve Wilton, Perth
Klaus Zerres, Aachen
Janez Zidar, Ljubljana
CONTENTS

INVITED REVIEW

Muscle fatigue, nNOS and muscle fiber atrophy in limb girdle muscular dystrophy
Corrado Angelini, Elisabetta Tasca, Anna Chiara Nascimbeni and Marina Fanin ........................................ 119

ORIGINAL ARTICLES

The effect of atrial preference pacing on atrial fibrillation electrophysiological substrate in Myotonic Dystrophy type 1 population
Vincenzo Russo, Gerardo Nigro, Federica Di Meo, Andrea Antonio Papa, Nadia Della Cioppa, Riccardo Proietti, Maria Giovanna Russo, Raffaele Calabrò and Luisa Politano .................................................. 127

Psychological and practical difficulties among parents and healthy siblings of children with Duchenne vs. Becker muscular dystrophy: an Italian comparative study
Lorenza Magliano, Maria Grazia D’Angelo, Giuseppe Vita, Marika Pane, Adele D’Amico, Umberto Balottin, Corrado Angelini, Roberta Battini and Luisa Politano; Telethon GUP10002 Working Group .................................................. 136

Charcot-Marie-Tooth 4B2 caused by a novel mutation in the MTMR13/SBF2 gene in two related Portuguese families
Luís Negrão, Luciano Almendra, Joana Ribeiro, Anabela Matos, Argemiro Geraldo and Jorge Pinto-Basto .................................................. 144

CASE REPORT

Ventricular fibrillation induced by coagulating mode bipolar electrocautery during pacemaker implantation in Myotonic Dystrophy type 1 patient
Vincenzo Russo, Anna Rago, Federica Di Meo, Nadia Della Cioppa, Andrea Antonio Papa, Maria Giovanna Russo and Gerardo Nigro .................................................................................. 149

LETTER TO THE EDITOR

Facio-scapulo-humeral muscular dystrophy and its connection with facio-scapulo-peroneal muscular dystrophy 4q35-linked: some historical remarks
Valery Kazakov, Dmitry Rudenko, Vladislav Kolynin and Tima Stuchevskaya .................................................. 152

PROCEEDINGS OF LGMD DAYS MEETING: Prognosis and Treatment in LGMD
Lido di Venezia, Italy – October 15-17, 2014

Program .................................................................................................................................................. 157
Abstracts ............................................................................................................................................. 158
Author Index ........................................................................................................................................ 165

OBITUARY

Erich Kuhn
Reinhardt Rüdel ....................................................................................................................................... 166

NEWS FROM AROUND THE WORLD

MSM .................................................................................................................................................. 168
GCA .................................................................................................................................................. 168
AIM .................................................................................................................................................. 168
LGMD-Euro-Net ................................................................................................................................. 168
WMS .................................................................................................................................................. 168

FORTHCOMING MEETINGS ........................................................................................................... 169

Volume XXXIII - CONTENTS ........................................................................................................... 170
Volume XXXIII - AUTHOR INDEX .................................................................................................... 172
Volume XXXIII - SUBJECT INDEX .................................................................................................... 175
Volume XXXIII - LIST OF REFEREES CONSULTED IN 2014 ....................................................... 176

Instructions for Authors ...................................................................................................................... 178
Muscle fatigue, nNOS and muscle fiber atrophy in limb girdle muscular dystrophy

Corrado Angelini¹, Elisabetta Tasca¹, Anna Chiara Nascimbeni² and Marina Fanin²

¹IRCCS Fondazione “San Camillo” Hospital, Lido di Venezia, Italy; ²Department of Neurosciences, University of Padova, Italy

Muscle fatigability and atrophy are frequent clinical signs in limb girdle muscular dystrophy (LGMD), but their pathogenetic mechanisms are still poorly understood.

We review a series of different factors that may be connected in causing fatigue and atrophy, particularly considering the role of neuronal nitric oxide synthase (nNOS) and additional factors such as gender in different forms of LGMD (both recessive and dominant) underlying different pathogenetic mechanisms.

In sarcoglycanopathies, the sarcolemmal nNOS reactivity varied from absent to reduced, depending on the residual level of sarcoglycan complex: in cases with complete sarcoglycan complex deficiency (mostly in beta-sarcoglycanopathy), the sarcolemmal nNOS reaction was absent and it was always associated with early severe clinical phenotype and cardiomyopathy. Calpainopathy, dysferlinopathy, and caveolinopathy present gradual onset of fatigability and had normal sarcolemmal nNOS reactivity. Notably, as compared with caveolinopathy and sarcoglycanopathies, calpainopathy and dysferlinopathy showed a higher degree of muscle fiber atrophy. Males with calpainopathy and dysferlinopathy showed significantly higher fiber atrophy than control males, whereas female patients have similar values than female controls, suggesting a gender difference in muscle fiber atrophy. In female patients, the smaller initial muscle fiber size associated to endocrine factors and less physical effort might attenuate gender-specific muscle loss and atrophy.

Key words: LGMD, nNOS, sarcoglycan

Introduction

Limb Girdle Muscular Dystrophies (LGMD) are a group of disorders of skeletal muscle showing a wide clinical and genetic heterogeneity (for classification see: www.musclegenetable.fr). The physio-pathological mechanism underlying LGMDs is different for each form, and for most of them it is only poorly understood. According to the disease mechanism, the LGMDs may be grouped as follows (1): defects of dystrophin-glycoprotein complex (DGC) (LGMD2C, 2D, 2E, 2F, 2P, 2T), enzyme defects affecting glycosylation of α-dystroglycan (DG) (LGMD2I, 2K, 2M, 2N, 2O), sarcomeric defects (LGMD1A, 2G, 2J), enzyme defects affecting sarcomere remodelling (LGMD2A, 2H), defects of signal transduction (LGMD1C), defects affecting membrane repair (LGMD2B, 2L), defects of the nuclear membrane (LGMD1B, 1F).

The sarcolemma of skeletal muscle fibers is characterized by the presence of the DGC, which is composed of cytoskeletal proteins (dystrophin, syntrophins), the DG complex, and the sarcoglycan (SG) complex. The DGC has also signalling roles, due to its interaction with other proteins, including neuronal Nitric Oxide Synthase (nNOS), which is anchored at the sarcolemma by binding of α1-syntrophin (2-8). The production of Nitric Oxide (NO), which is a messenger molecule that rapidly transduces signalling events in a calcium-dependent manner, is able to regulate muscle development, contractility and blood flow.
Caveolin-3 appears to be not directly associated with the DGC; caveolins act as scaffolding proteins to organize and concentrate specific caveolin-interacting lipids and proteins. Caveolin-3 has been shown to directly bind nNOS and has a possible interaction with dysferlin. Similar to proteins involved in the DGC, it is recognized that sarcomeric proteins (myotilin, titin, telethonin) have not only important structural roles, but also signalling roles, such as those involved in muscle cell proliferation, fusion, maintenance, regeneration and repair. Dysferlin is involved in the repair of plasmalemma lesions, since it mediates vesicle trafficking and membrane fusion in muscle cells, binding its C2 domain to phospholipids in a calcium-dependent manner.

In this review paper we discuss the results reported in earlier studies from our group and the literature, which independently investigated the role of nNOS, muscle fatigue and muscle fibre atrophy in various forms of LGMD, in order to offer a comprehensive view of their individual role and their relationship in this group of disorders.

Muscle fatigue and nNOS in muscular dystrophies

Dystrophic patients have difficulties to support an excessive or long-term physical activity, and frequently complain of fatigue during the exercise of moderate or short-lived intensity (9). The increase of physical exhaustion for the energetic expense for an exercise is the cause of acute fatigue, whereas the inability to maintain a certain level of force is the cause of chronic fatigue. Muscle fatigue can be due to coupling excitement-contraction, to lack of availability of substrates or blood flow and lack of adaptation of vasodilatation by NO (Fig. 1), and to the possible modifications of the intracellular environment and disruption of contractile apparatus (3).

A secondary deficiency of nNOS has been suggested to contribute to fibre degeneration in muscular dystrophies, because the loss of nNOS would reduce the normal protective action of NO against local ischemia during contraction (vascular hypothesis) and increase the cellular susceptibility to superoxides (oxidative stress hypothesis) (Fig. 1). Indeed, absent nNOS at the sarcolemma was observed not only in muscle from α1-syntrophin knock-out mice, but also from DMD and Becker muscular dystrophy (BMD) patients where dystrophin gene deletions removed a region which is crucial for the interaction between nNOS and α1-syntrophin (10). The loss of nNOS in DMD muscle may result in aberrant regulation of adrenergic vasodilatation, since dystrophin loss was demonstrated to impair the regulation of vasoconstrictor response (11), and dystrophin-deficient mdx mice as well as nNOS null mice are unable to control muscle blood flow during exercise (12). Without proper vascular dilatation and subsequent blood flow, muscles suffer from focal necrosis and are susceptible to fatigue. The potential of nNOS to improve mdx muscle pathology suggested NO-related therapies may be beneficial for treatment of dystrophinopathy.

nNOS in sarcoglycanopathies

Crosbie et al. thoroughly investigated the nNOS expression in the animal models of sarcoglycanopathies (including both the BIO 14.6 hamster with delta-SG deficiency and mice with targeted disruption of alpha-SG, beta-SG, and delta-SG genes) and in patients with sarcoglycanopathies (13), where they clearly demonstrated that nNOS is reduced in sarcoglycan-deficient muscle, and that the deficiency at the sarcolemma was more pronounced in patients with complete SG complex deficiency (beta-sarcoglycanopathy), suggesting a possible direct correlation between the levels of nNOS expression at the sarcolemma and the overall level of SG complex.

This hypothesis was further validated in another study (14), in which 14 muscles from patients affected with different forms of sarcoglycanopathies (4 alpha-SG, 7 beta-SG, 2 gamma-SG, 1 delta-SG) have been investigated for the expression of both the cytosolic nNOS (by western blotting) and its sarcolemmal localization (by immunohistochemistry). This latter study (14) showed that the sarcolemmal nNOS reaction varied from absent to reduced, depending on the integrity of the SG complex.
Fatigue, nNOS, fiber atrophy in LGMD

(Fig. 2, Table 1), demonstrating that the integrity of the SG complex is essential for the sarcolemmal localization of nNOS. Indeed, a perturbation in the structural integrity of the DGC may alter syntrophin’s PDZ domains, which have been shown to directly interact with nNOS (15).

**Figure 2.** Sarcolemmal immunolabelling of nNOS in muscle biopsies from patients with sarcoglycanopathies (A-E) and control (F). The reaction was absent in LGMD2E patients with complete SG complex defect (C) and partial in LGMD2E patients with partial SG complex defect (D), indicating that the reduction of nNOS level depends directly on the residual level of SG complex. Scale bar = 50 μm. Original magnification: 200x.

**nNOS and dilated cardiomyopathy in sarcoglycanopathies**

In the study by Fanin et al. (14), the sarcolemmal nNOS expression correlated with the clinical severity, as
described for some cases also in previous reports (14, 16-18) and muscle fatigue: absence or severe reduction of sarcolemmal nNOS expression was associated with a severe and childhood-onset form of muscular dystrophy and in most cases also with dilated cardiomyopathy (Table 1).

Mice lacking either γ-SG or δ-SG display progressive focal cardiomyocyte degeneration that ultimately leads to reduced cardiac function and death (19). This model of cardiomyopathy closely parallels what is seen in humans with SG and dystrophin gene mutations (20-22). Furthermore, null mice for β-SG and δ-SG [but not for α-SG (17)] presented a disruption of the vascular smooth muscle SG complex (23-25). The perturbed vascular function induces ischemic injury in cardiac and skeletal muscle (23), suggesting that this mechanism could contribute to the development of cardiomyopathy and exacerbate skeletal myopathy.

It is well known that vascular spasm is an important contributor to cardiac pathology (19). Elevated levels of intracellular calcium, disturbances of the NOS pathway, and increased activity of protein kinase C, have been implicated in increased contractility and/or spasm of the microvasculature.

Therefore, the observation that sarcolemmal nNOS can be absent or mislocalized in sarcoglycanopathy muscle (14, 26, 27) provides a possible link between this pathogenetic mechanism and the development of cardiomyopathy in sarcoglycanopathies, offering further insights for therapeutic interventions.

NO stimulates soluble guanylate cyclase (sGC) to produce cyclic guanosine monophosphate (cGMP), and in the absence of dystrophin the NO-sGC-cGMP pathway is disrupted. The nucleotide phosphodiesterases (PDEs) hydrolyze the cGMP and regulate their downstream signalling. PDE5 expression in cardiomyocytes is low at baseline and increases in response to ischemia or pressure overload from heart failure. Impaired blood flow in muscle and heart in mdx dystrophin-deficient and NOS-deficient mice was rescued by inhibition of PDE5 (28).

Unfortunately, in Duchenne and Becker dystrophy patients a clinical trial with PDE5 inhibitor (Sidenafil) did not improve cardiomyopathy, since 30% of patients progressed to ventricular dilatation (29).

Long term dietary supplementation of L-arginine (a NOS substrate) was not a viable therapy for dystrophinopathy (30), but the use of antioxidants that attenuate the superoxide attack and restore the bioactive NO level,

### Table 1. Clinical and experimental data in different types of LGMD.

<table>
<thead>
<tr>
<th>Disease type</th>
<th>N. cases</th>
<th>Mean age at biopsy (years)</th>
<th>Skeletal muscle phenotype</th>
<th>Dilated cardio-myo-pathy</th>
<th>SG complex deficiency</th>
<th>Cytosolic nNOS (% of control)</th>
<th>Sarcolemmal nNOS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sarcoglycanopathies</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LGMD2C</td>
<td>2</td>
<td>20.5</td>
<td>Childhood-onset</td>
<td>None</td>
<td>Partial in 2</td>
<td>66.5</td>
<td>Reduced in 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>LGMD in 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LGMD2D</td>
<td>4</td>
<td>16.7</td>
<td>Childhood-onset</td>
<td>None</td>
<td>Complete in 1/4, partial in 3/4</td>
<td>78.5</td>
<td>Absent in 1, reduced in 3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>LGMD in 3, hyperCKemia in 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LGMD2E</td>
<td>7</td>
<td>14.6</td>
<td>Childhood-onset</td>
<td>Present in 4</td>
<td>Complete in 5/7, partial in 2/7</td>
<td>42.5</td>
<td>Absent in 5, reduced in 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>LGMD in 7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LGMD2F</td>
<td>1</td>
<td>34.0</td>
<td>Childhood-onset</td>
<td>Present in 1</td>
<td>Partial in 1</td>
<td>52.0</td>
<td>Reduced</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>LGMD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other types of LGMD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LGMD1C</td>
<td>8</td>
<td>26.7</td>
<td>Childhood-onset</td>
<td>None</td>
<td>None</td>
<td>98.7</td>
<td>Reduced in 8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>LGMD in 2, adult-onset LGMD in 2, distal myopathy in 2, rippling in 1, hyperCKemia in 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LGMD2A</td>
<td>8</td>
<td>36.5</td>
<td>Childhood-onset</td>
<td>None</td>
<td>None</td>
<td>95.2</td>
<td>Normal in 3, reduced in 5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>LGMD in 2, adult-onset LGMD in 5, hyperCKemia in 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LGMD2B</td>
<td>2</td>
<td>46.5</td>
<td>Distal myopathy in 2</td>
<td>None</td>
<td>None</td>
<td>146.0</td>
<td>Reduced in 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

More detailed clinical and laboratory data from the same patients series are reported in Fanin et al. (14).
might be useful approaches for the treatment of these disorders.

**nNOS in calpainopathy, dysferlinopathy, caveolinopathy**

In a study which investigated 18 muscles from calpainopathy, dysferlinopathy and caveolinopathy patients (14), nNOS was found to be present at normal levels in the cytosol (by western blotting) (Table 1) and correctly localized to the sarcolemma by immunohistochemistry, although with variable intensity. In all these disorders, this variability might be in part due to the presence of scattered regenerating fibers which show absent sarcolemmal nNOS staining on serial sections (14). These data suggested that the interactions between the corresponding mutant proteins (calpain-3, dysferlin, caveolin-3) and nNOS are not crucial for the nNOS signaling pathway.

**Muscle fibers atrophy in LGMDs and gender differences in LGMDs**

Besides muscle fatigue, LGMD patients experience a loss of muscle mass and the associated muscle weakness. Consequently, patients have an increased risk of suffering from co-morbidities related with reduced physical activity (e.g. reduced mobility, joint contractures). Muscle atrophy results from an imbalance between dynamic anabolic and catabolic reactions, where the increased myofibrillar protein breakdown exceeds the protein synthesis. Muscle atrophy is an active process which is controlled by specific signalling pathways and transcriptional programs involving many atrophy-promoting genes or atrogenes (31, 32). Three proteolytic systems are involved in the maintenance of the sarcomeric function: the cytosolic calpain system, the ubiquitin-proteasome system, and the autophagic-lysosomal pathway. Although they are all activated in muscle atrophy, the key player in the degradation of myofibrillar proteins is the ubiquitin-proteasome system.

In contrast to muscle strength, which depends on several factors (e.g. exercise, bulk), fiber atrophy seems to be a more precise marker of muscle atrophy because it reflects an abnormal process in the contractile apparatus of muscle. Several studies have investigated muscle fiber size in LGMD using well-known morphometric parameters, such as diameter, cross sectional area, atrophy factor, hypertrophy factor (33). In particular, the degree of muscle fiber atrophy was measured in calpainopathy (34), in dysferlinopathy (35) and in other forms of LGMD including sarcoglycanopathies and caveolinopathy (36).

Calpainopathy and dysferlinopathy muscles were those in which the atrophic process was more relevant, since fiber diameter and fiber cross sectional area were significantly reduced, and atrophy factor was significantly increased as compared to controls (Fig. 3). This observation confirms previous studies that investigated the molecular pathways involved in muscle atrophy program in calpainopathy and dysferlinopathy (34, 35), and has important clinical consequences since possible rehabilitative, pharmacological and nutritional interventions directed at reducing muscle atrophy and wasting could contribute in slowing the disease course.

**Gender differences in muscle fiber atrophy in LGMDs**

Previous studies comparing men and women in response to disuse atrophy suggested that there are gender differences in strength loss and atrophy, and one of these studies compared also muscle fiber size in diseased and control muscles of the same gender (36). Male patients affected with calpainopathy and dysferlinopathy have significantly lower values of fiber diameter, cross sectional area and higher values of atrophy factor than male controls, whereas female patients have values not significantly different from female controls (Fig. 3). Earlier studies in LGMD patients suggested that there are gender differences both in strength and atrophy. Among potential factors differentiating women and men, the are gonadal hormone levels (i.e. oestrogen and testosterone) which are known to influence muscle mass (37). Another factor enhancing the degree of atrophy may be the initial muscle mass (38), suggesting that a smaller initial muscle size, rather than endocrine factors attenuate gender-specific muscle loss in women (39).

A study of calpainopathy muscle showed that the degree of muscle fiber atrophy significantly correlated with the clinical-functional severity of the disease (34), and therefore a higher degree of fiber atrophy in males should correspond to a higher degree of clinical muscle impairment. The possibility that male patients with various LGMD may be more severely affected than females has already been explored: among calpainopathy patients, a more rapid progression was observed in males than in females (40-42), but no gender differences were evident in the age at onset or loss of ambulation in other large series of calpainopathy patients (43-45). A more severe phenotype in males than in female was reported in an animal model of dysferlinopathy but not in dysferlinopathy patients (42); similar observations were done also in a large family with gamma-sarcoglycanopathy (46) but not in other series of sarcoglycanopathy patients (42), in LGMD2G (42), and in other myopathies. Furthermore, a male gender predominance is frequently observed in LGMDs, including LGMD2L (47), and one possible expla-
Figure 3. Histograms showing the comparison between the mean values of fiber diameter, cross sectional area, atrophy factor and hypertrophy factor in the different groups of LGMD patients and in the control group. Black bars = total cases of both genders, gray bars = male patients, white bars = female patients. Significant difference (p < 0.05) is indicated as: * individual disease group versus control group, § males versus females within the same disease group, # one gender of a disease group versus the same gender of the control group.
nation is that females might be less severely affected and therefore less likely to be ascertained.

Any intervention aimed to reduce muscle atrophy could improve the course of the disease. Several factors have been described as reducing the otherwise elevated expression of atrogenes and therefore as potentially counteracting muscle atrophy: muscle exercise training (48, 49), treatment with Bortezomib and Fenofibrate (50, 51), branched-chain amino acids (52), L-carnitine (53) and long-chain ω-3 fatty acid (54).

**Conclusions**

In the recent years, the research on LGMD, besides the successful and continuous work dedicated to the identification of new causative genes, was focused on the investigation of molecular pathways involved in their pathogenetic cascade.

The sarcoglycanopathies share with DMD the disruption of the linkage between the extracellular matrix and the intracellular cytoskeleton, and the consequent loss of sarcolemma integrity and stability, which is also essential for the localization of nNOS. In sarcoglycanopathies the deficiency of nNOS is an adverse modulating factor in the course of muscular dystrophy and dilated cardiomyopathy; indeed, SG complex deficiency in the vascular smooth muscle might lead either to structural changes or to an impairment of metabolic and NOS signalling pathways in tissues involved in the microvascular dysfunction, making cardiomyocytes more susceptible to intermittent ischemia.

While the structure and function of DGC and nNOS does not seem to be affected in other forms of LGMD, in calpainopathy and dysferlinopathy a significant atrophy of muscle fibers is a peculiar characteristic, which originate from the activation of specific intracellular degenerative pathways and slowly progresses leading to chronic pathological changes of muscle.

There is evidence suggesting that male patients with such LGMD are more likely to undergo muscle fibre atrophy than female patients, and may be therefore more likely to suffer from the consequent muscle weakness and clinical disability.

A specific rehabilitative program in LGMD, taking into account muscle fatigue and atrophy, should be planned, avoiding both eccentric exercise and possible local muscle ischemia. It may consist of moderate endurance training and stretching (55).

**References**


The effect of atrial preference pacing on atrial fibrillation electrophysiological substrate in Myotonic Dystrophy type 1 population

Vincenzo Russo1, Gerardo Nigro1, Federica Di Meo1, Andrea Antonio Papa1, Nadia Della Cioppa1, Riccardo Proietti2, Maria Giovanna Russo1, Raffaele Calabrò1 and Luisa Politano3

1 Chair of Cardiology, Second University of Napoli, Monaldi Hospital, Napoli, Italy; 2 Cardiology Department, “Luigi Sacco” Hospital, Milano, Italy; 3 Cardiomyology and Medical Genetics, Department of Experimental Medicine, Second University of Napoli, Italy

P-wave dispersion is a non invasive indicator of intra-atrial conduction heterogeneity producing substrate for reentry, which is a pathophysiological mechanism of atrial fibrillation. The relationship between P-wave dispersion (PD) and atrial fibrillation (AF) in Myotonic dystrophy type 1 (DM1) patients is still unclear. Atrial Preference Pacing (APP) is an efficient algorithm to prevent paroxysmal AF in patients implanted with dual-chamber pacemaker. Aim of our study was to evaluate the possible correlation between atrial preference pacing algorithm, P-wave dispersion and AF burden in DM1 patients with normal cardiac function underwent permanent dual-chamber pacemaker implantation. We enrolled 50 patients with DM1 (age 50.3 ± 7.3; 11 F) underwent dual-chamber pacemaker implantation for various degree of atrioventricular block. The study population was randomized following 1 months stabilization period to APP algorithm features programmed OFF or ON. Patients were assessed every 3 months for the first year, and every 6 months thereafter up to 3 years. At each follow-up visit, we counted: the number of premature atrial beats, the number and the mean duration of AF episodes, AF burden and the percentage of atrial and ventricular pacing.

APP ON Group showed lower number of AF episodes (117 ± 25 vs. 143 ± 37; p = 0.03) and AF burden (3059 ± 275 vs. 9010 ± 630 min; p < 0.04) than APP OFF Group. Atrial premature beats count (44903 ± 30689 vs. 13720 ± 7717 beats; p = 0.005) and P-wave dispersion values (42,1 ± 11 ms vs. 29,1 ± 4,2 ms, p = 0.003) were decreased in APP ON Group. We found a significant positive correlation between PD and AF burden (R = 0.8, p = 0.007). Atrial preference pacing algorithm, decreasing the number of atrial premature beats and the P-wave dispersion, reduces the onset and perpetuator factors of AF episodes and decreases the AF burden in DM1 patients underwent dual chamber pacemaker implantation for various degree of atroventricular blocks and documented atrial fibrillation.

Key words: atrial fibrillation, Myotonic Dystrophy, atrial preference pacing

Introduction

Myotonic dystrophy type 1 (DM1), or Steinert disease, is a serious autosomal-dominant hereditary disease with an estimated incidence of 1 in 8,000 births. It is caused by an abnormal expansion of an unstable trinucleotide repeat in the three-prime untranslated region of DMPK gene on chromosome 19. The cardiac involvement is noticed in about 80% of cases, and it often precedes the skeletal muscle one (1-3). Heart failure (HF) often occurs late in the course of the disease as a consequence of cardiac myopathy due to progressive scar replacement (4-6). Arrhythmias and/or conduction defects are frequent, occurring in 50-65% of patients with DM1 (7). Heart block is the first and most clinically significant cardiac disease in this group of patients and it is related to fibrosis of the conduction system and fatty infiltration of the His bundle (8). Paroxysmal atrial arrhythmias (atrial fibrillation, atrial flutter, atrial tachycardia) frequently occur in DM1 patients (9). P-wave dispersion is a non invasive
indicator of intra-atrial conduction heterogeneity producing substrate for reentry, which is a pathophysiological mechanism of atrial fibrillation (10, 11). The role of P-wave dispersion (PD) as independent risk factor for atrial fibrillation (AF) development in DM1 patients is still unclear. Atrial Preference Pacing (APP) is an efficient algorithm to prevent paroxysmal AF in patients implanted with dual-chamber pacemaker and significantly reduces the atrial fibrillation episodes and burden (defined as the quantity of AF-minutes/day- retrieved from the device data logs) in DM1 patients (12-14). Aim of our study was to evaluate the possible correlation between atrial preference pacing algorithm, P-wave dispersion and AF burden in DM1 patients with conserved systolic and diastolic function underwent permanent dual-chamber pacemaker implantation.

Materials and methods

Patients selection

From a large cohort of 240 DM1 patients, referred to Cardiomyology and Medical Genetics, Department of Experimental Medicine of Second University of Naples, we enrolled 60 DM1 patients underwent to dual chamber pacemaker with atrial preference pacing (APP) algorithm for various grade of atrioventricular blocks and with documented atrial fibrillation detected by 12-lead surface electrocardiogram (ECG) or 24-h ECG Holter monitoring. The diagnosis of Steinert disease, firstly based on family history and clinical evaluation, had been subsequently confirmed by genetic test in all patients, to evaluate the CTG triplet expansion. We excluded from the study all DM1 patients with patent foramen ovale, atrial septal aneurysm, severe mitral stenosis or regurgitation, coronary bypass or valvular heart surgery, sick sinus syndrome, or inducible ventricular tachycardia. Subjects with a history of hypertension (systolic and diastolic blood pressure >140/90 mmHg), diabetes mellitus or impaired glucose tolerance, obesity, electrolyte imbalance, systolic and diastolic dysfunction, connective tissue disorders, hepatic, renal, thyroid diseases, and sleep disorders were excluded from the study. The study was conducted according to the declaration of Helsinki.

Study protocol

DM1 eligible patients underwent dual-chamber pacemaker implantation were randomized following 1 month stabilization period to APP algorithm features programmed OFF or ON. Pharmacological therapy was required to remain stable. Patients were assessed every 3 months for the first year, and every 6 months thereafter up to 3 years. At each follow-up visit the study population underwent medical history, physical examination, 12-lead surface ECG, 2D color Doppler echocardiogram and device interrogation. Patients interrupted the follow-up, before completing the 3 years, in the case of severely symptomatic AT/AF requiring major changes in therapy.

Pacemaker implantation and programming

All DM1 patients were implanted with a dual-chamber PM system (Medtronic Adapta ADDR01, Medtronic Inc., Minneapolis, MN, USA). The right ventricular lead (Medtronic 4074 CapSure Sense) was positioned in the apex, under fluoroscopic guidance; the bipolar atrial screw-in lead (Medtronic 5076 CapSure-Fix) was positioned in the right atrial appendage or on the right side of the inter-atrial septum in the region of Bachmann’s bundle, according to optimal site, defined as the location with lowest pacing and highest sensing thresholds. To minimize confounding variables with different electrode materials and inter-electrode pacing, an identical model lead was used in all patients. Similarly, PMs with identical behaviour and telemetric capabilities were used to assure accuracy in comparing measurements among patients. To minimize atrial lead oversensing, the sensitivity configuration was bipolar. All devices were programmed in DDD mode with a lower rate of 60 bpm and an upper rate of 120 bpm. Mode switches were programmed for atrial rates >200 bpm, persisting for more than 12 ventricular beats. Managed Ventricular Pacing algorithm (MVP, Medtronic Inc., Minneapolis, MN, USA) was enable in order to promote the intrinsic conduction and reduce the possible influence of high percentage ventricular pacing on atrial fibrillation incidence. Atrial Preference Pacing (APP, Medtronic Inc., Minneapolis, MN, USA) was enable according to the prospective programming compliance criteria. The devices used in this study were programmed to detect the episodes of atrial tachycardia, and to record summary and detailed data, atrial and ventricular electrograms (EGMs) included.

Electrocardiographic measurements

All subjects underwent a routine standard 12-lead surface ECG recorded at a paper speed of 50 mm/s and gain of 10 mm/mV in the supine position and were breathing freely but not allowed to speak during the ECG recording. To avoid diurnal variations, we generally took the ECG recordings at the same time (9:00-10:00 a.m.). The analysis was performed by one investigator only without knowledge of subject’s clinical status. ECGs were transferred to a personal computer by an optical scanner and then magnified 400 times.
by Adobe Photoshop software (Adobe Systems Inc., San Jose, CA, USA). P-wave duration measurement was manually performed with the use of computer software (Configurable Measurement System). Intra-observer coefficients of variation for P-wave variables were found to be less than 5% and not significant. In each electrocardiogram lead, the analysis included three consecutive heart cycles wherever possible. ECG with measurable P-wave in less than ten leads were excluded from analysis. The onset of P-wave was defined as the junction between the isoelectric line and the start of P-wave deflection; the offset of the P-wave was defined as the junction between the end of the P-wave deflection and the isoelectric line (15). If starting and endpoints were not clear, the derivations including these points were taken as excluding criteria from the study. Maximum and minimum P-wave durations were measured. Maximum P-wave duration was defined as the longest P-wave duration, and minimum P-wave duration was defined as the shortest P-wave duration. PD was defined as the difference between the maximum and minimum P-wave durations.

Echocardiography measurements

All echocardiographic examinations were performed using a standard ultrasound machine with a 3.5-MHz phased-array probe (M3S). All patients were examined in the left lateral and supine positions by precordial M-mode, 2-dimensional and Doppler echocardiography. One lead ECG was recorded continuously. Left ventricular end-diastolic diameter (LVEDD), left ventricular end-systolic diameter (LVESD), interventricular septum thickness (IVST) and left ventricular posterior wall thickness (LVPWT) were measured from M-mode in the parasternal long-axis views according to the standards of the American Society of Echocardiography. Left ventricular mass (LVM) was calculated by using Devereux’s formula, and was indexed for body surface area and height. Left atrium diameter (LAD) was measured during systole along the parasternal long-axis view from the 2-dimensional guided M-mode tracing; LA length was measured from the apical 4-chamber view during systole. The maximum LA volume (LAV) was calculated from apical 4- and 2-chamber zoomed views of the LA using the biplane method of disks. Ejection fraction was measured using a modified Simpson biplane method. Each representative value was obtained from the average of 3 measurements. Pulsed-wave Doppler examination was performed to obtain the following indices of LV diastolic function: peak mitral inflow velocities at early (E) and late (A) diastole and E/A ratio. Average values of these indices obtained from 5 consecutive cardiac cycles were used for analysis.

Device interrogation and data analysis

All DM1 patients underwent device interrogation to evaluate sensing/pacing parameters, leads impedance and battery voltage. The devices used in this study were programmed to detect the episodes of atrial tachycardia and to record summary and detailed data, atrial and ventricular electrograms (EGMs) included.

We counted:
- the number of premature atrial beats;
- the number and the mean duration of AF episodes occurred;
- AF burden – defined as the quantity of AF (minutes/day) retrieved from the device data logs;
- the percentage of atrial and ventricular pacing in synchronous rhythm during the collection period.

Atrial tachycardia episodes, identified by regular atrial activity, were excluded from the analysis.

Statistical analysis

Continuous variables are expressed as mean ± standard deviation. Statistical analysis was performed using Student’s t-test for unpaired data. p values < 0.5 were considered to be statistically significant. Analyses were performed using the statistical package SPSS 11.0 software for Windows SPSS Inc. (Chicago, IL, USA). The relationships between PD and AF burden were evaluated by linear regression analysis.

Endpoints

Primary endpoints were the number, mean duration and burden of AF episodes and their correlation to PD between APP ON Group and APP OFF Group during 36-months follow-up. Secondary endpoints were the overall number of premature atrial beats and the percentage of atrial and ventricular pacing in synchronous rhythm during the observation period.

Results

Patients population

From the cohort of 60 DM1 patients, 10 patients were excluded due to: far-field ventricular sensing despite refractory periods reprogramming (three cases) after implantation; atrial undersensing (four cases) and persistent AF during follow-up (three cases). Finally, the study group included 50 patients with DM1 (age 50.3 ± 7.3; 11 F), who underwent dual-chamber PM implantation in our division for first-degree atrioventricular block with a pathological infra-Hissian conduction (18 patients), symptomatic type 1 (12 patients), and type 2 (20 patients) second-degree block.
The study population was randomized and treated according to the protocol. No statistically significant difference in the electrical parameters (P-wave amplitude, pacing threshold, and lead impedance) and medication intake was found at implantation between the patients with right atrial appendage (RAA) and Bachmann’s bundle (BB) lead placement. Table 1 shows the baseline characteristics of the study population.

### Atrial preference pacing and atrial fibrillation

A statistically significant difference was found in the number of AF episodes between no treatment group (APP OFF) and active treatment (APP ON) group in DM1 population study during the follow-up period. The number of AF episodes in APP ON Group was lower than those registered in APP OFF Group (117 ± 25 vs. 143 ± 37; p = 0.03). No statistically significant difference was found in AF episodes mean duration between the two groups (47 ± 17 vs. 43 ± 13 min; p = 0.4).

AF burden was lower in APP ON Group than in APP OFF Group (3059 ± 275 vs. 9010 ± 630 min; p < 0.04) (Fig. 1). In APP OFF group and APP ON group, the atrial pacing percentage were 0 and 98%, respectively, while the ventricular pacing percentage did not show statistically significant difference (27 vs. 29%; p = 0.2).

Atrial premature beats count was significantly greater in APP OFF group than in APP ON group (44903 ± 30689 vs. 13720 ± 7717 beats; p = 0.005). There was no significant difference in the atrial pacing capture, sensing threshold, and atrial lead impedances at implant and at 36-month follow-up. Lead parameters remained stable over time and there were no lead-related complications. All data are shown in Table 2.

### P-wave Duration and Dispersion

APP OFF Group showed increased maximum P-wave duration (109,4 ± 10,9 ms vs. 69,8 ± 8.2 ms, p = 0.03) and P-wave dispersion values (42,1 ± 11 ms vs. 29,1 ± 4,2 ms, p = 0.003), compared to APP ON group (Fig. 2). No statistically significant difference was found in heart rate (79,5 ± 6,3 bpm vs. 80,8 ± 5,4 bpm, p = 0.3) and minimum P-wave duration (73,7 ± 11,8 ms vs. 69,4 ± 8,1 ms, p = 0.4). All data are shown in Table 3. We found a significant positive correlation between PD and AF burden (R = 0.8, p = 0.007) (Fig. 3).

### Tables

#### Table 1. Baseline characteristics of the study population.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients (n)</td>
<td>50</td>
</tr>
<tr>
<td>Age (years)</td>
<td>50.3 ± 7.3</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>39/11</td>
</tr>
<tr>
<td>Atrioventricular block I grade</td>
<td>18</td>
</tr>
<tr>
<td>Atrioventricular block II grade type 1</td>
<td>12</td>
</tr>
<tr>
<td>Atrioventricular block II grade type 2</td>
<td>20</td>
</tr>
<tr>
<td>QRS duration (ms)</td>
<td>93 ± 13</td>
</tr>
<tr>
<td>LVEDD (mm)</td>
<td>42.7 ± 9</td>
</tr>
<tr>
<td>LVESD (mm)</td>
<td>27.24 ± 2.8</td>
</tr>
<tr>
<td>IVST (mm)</td>
<td>9.7 ± 1.3</td>
</tr>
<tr>
<td>LVPWT (mm)</td>
<td>9.9 ± 1.5</td>
</tr>
<tr>
<td>Ejection fraction (%)</td>
<td>60.39 ± 4</td>
</tr>
<tr>
<td>E wave (cm/s)</td>
<td>82.3 ± 15.5</td>
</tr>
<tr>
<td>A wave (cm/s)</td>
<td>57.9 ± 9.5</td>
</tr>
<tr>
<td>E/A ratio</td>
<td>1.5 ± 0.5</td>
</tr>
</tbody>
</table>

#### Table 2. Differences in the number of AF episodes, AF episodes mean duration, AF burden, atrial and ventricular pacing percentage, atrial premature beats and lead parameters between the two groups.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>APP ON Group</th>
<th>APP OFF Group</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AF episodes numbers(n)</td>
<td>117 ± 25</td>
<td>143 ± 37</td>
<td>0.03</td>
</tr>
<tr>
<td>AF episodes mean duration (min)</td>
<td>47 ± 17</td>
<td>43 ± 13</td>
<td>0.4</td>
</tr>
<tr>
<td>AF burden (min)</td>
<td>3059 ± 275</td>
<td>9010 ± 630</td>
<td>0.04</td>
</tr>
<tr>
<td>Atrial pacing percentage (%)</td>
<td>98</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Ventricular pacing percentage (%)</td>
<td>27</td>
<td>29</td>
<td>0.2</td>
</tr>
<tr>
<td>Atrial premature beats</td>
<td>13720 ± 7717</td>
<td>44903 ± 30689</td>
<td>0.005</td>
</tr>
<tr>
<td>Atrial pacing threshold (V)</td>
<td>0.7 ± 3</td>
<td>0.9 ± 2</td>
<td>0.6</td>
</tr>
<tr>
<td>Atrial sensing threshold (mV)</td>
<td>5 ± 3</td>
<td>7 ± 2</td>
<td>0.6</td>
</tr>
<tr>
<td>Atrial lead impedance (ohm)</td>
<td>582 ± 18</td>
<td>622 ± 12</td>
<td>0.7</td>
</tr>
<tr>
<td>Ventricular pacing threshold (V)</td>
<td>0.8 ± 3</td>
<td>0.6 ± 3</td>
<td>0.6</td>
</tr>
<tr>
<td>Ventricular sensing threshold (mV)</td>
<td>15 ± 5</td>
<td>17 ± 4</td>
<td>0.7</td>
</tr>
<tr>
<td>Ventricular lead impedance (ohm)</td>
<td>769 ± 45</td>
<td>889 ± 37</td>
<td>0.5</td>
</tr>
</tbody>
</table>
The effect of atrial preference pacing on atrial fibrillation electrophysiological substrate in Myotonic Dystrophy type 1 population

**Figure 1.** Difference in atrial fibrillation burden between APP ON Group and APP OFF Group (3059 ± 275 vs. 9010 ± 630 min; p < 0.04).

**Figure 2.** Difference in P-wave dispersion between APP ON Group and APP OFF Group (29.1 ± 4.2 ms vs. 42.1 ± 11 ms, p = 0.003).
Discussion

Non invasive electrocardiographic risk indexes

QTc dispersion (QTcD) and JTc dispersion (JTcD) have been proposed as non invasive methods to measure the heterogeneity of ventricular repolarization (16). Increased dispersion of ventricular repolarization is considered to provide an electrophysiological substrate for life-threatening ventricular arrhythmias in several clinical conditions such as dilated cardiomyopathy (17, 18), obesity (19, 20), congenital disease (21, 22), beta thalassemia major (23) and cardiomyopathies (1-4, 24-26).

P-wave dispersion is a non invasive indicator of intra-atrial conduction heterogeneity producing substrate for reentry, which is a pathophysiological mechanism of atrial fibrillation. PD has been evaluated in some clinical conditions such as obesity (27), beta-thalassemia major (28, 29), Emery-Dreifuss muscular dystrophy (30). In a recent study (31), we showed a statistically significant increase in PD and P max in DM1 patients with AF compared to DM1 patients with no arrhythmias, confirming that P-wave dispersion may be a simple electrocardiographic parameter for identify high risk atrial fibrillation in DM1 patients.

Heart Rate Variability (HRV) is a reliable index to assess sympathovagal balance, used to stratify arrhythmic risk in several clinical conditions (32-39) and cardiomyopathies (40-42). However previous studies on autonomic modulation of heart rate in DM1 patients have obtained conflicting results (43-45).

Pacing in DM1 Patients

We have previously shown that: a) AF episodes increase in DM1 patients with a high percentage of right ventricular pacing and a lower percentage of atrial stimulation (46); b) right atrial septal stimulation in the Bachmann’s bundle region is a safe and feasible proce-

| Table 3. Differences in electrocardiographic findings between the two groups. |
|----------------------------------------|----------------|----------------|---------|
|                                       | APP ON Group  | APP OFF Group  | p value |
| Heart rate (bpm)                      | 80.8 ± 5.4    | 79.5 ± 6.3     | 0.3     |
| Max P-wave duration (ms)              | 69.8 ± 8.2    | 109.4 ± 10.9   | 0.03    |
| Min P-wave duration (ms)              | 69.4 ± 8.1    | 73.7 ± 11.8    | 0.4     |
| P-wave dispersion (ms)                | 29.1 ± 4.2    | 42.1 ± 11      | 0.003   |

![Figure 3. Correlation between P-wave dispersion and atrial fibrillation burden in DM1 patients (R = 0.8, p = 0.007).](image)
The effect of atrial preference pacing on atrial fibrillation electrophysiological substrate in Myotonic Dystrophy type 1 population

dure (47), with less atrial pacing and sensing defects than the right atrial appendage stimulation (48), though it does not seem to provide significant benefits for prevention of paroxysmal atrial fibrillation (49).

Atrial preference pacing algorithm may prevent the onset of atrial fibrillation through the following mechanisms: a) prevention of the relative bradycardia that triggers paroxysmal AF; b) prevention of the bradycardia-induced dispersion of refractoriness; c) suppression or reduction of premature atrial contractions that initiate the re-entry and predispose to AF; and d) preservation of atrio-ventricular synchrony, which may prevent switch-induced changes in atrial repolarization, predisposing to AF. According to our previous studies, the APP is an efficient algorithm for preventing AF episodes (50-52) and for reducing AT/AF burden in DM1 patients implanted with dual-chamber pacemaker.

Main findings

The current study investigated the effect of atrial preference pacing (APP) on AF burden in a three year follow-up period and the possible correlation between P-wave dispersion and AF burden, in myotonic dystrophy type 1 patients with conserved systolic and diastolic function who underwent dual chamber pacemaker implantation. Our data demonstrate that atrial preference pacing algorithm significantly reduces the number and the mean duration of AF episodes and AF burden and decreases the P-wave duration and dispersion in DM1 patients. Our results showed that P-wave dispersion is significantly higher in DM1 patients with increased AF burden. Therefore, we suggest that PD is an important factor affecting AF burden and that atrial preference pacing is responsible of AF burden reduction, through two mechanisms: reduction of premature atrial contractions and prevention of the bradycardia-induced dispersion of refractoriness.

Limitation of study

PD reflects only the intra-atrial conduction heterogeneity, but it not provides other atrial electrophysiological properties. Errors in PD measurement done with manual evaluation, may be a potential bias for observed conflicting results. However according to Dilaversis et al. (10), scanning and digitizing ECG signals from paper records using an optical scanner, is a feasible and accurate method for measuring P-wave duration.

Conclusions

Our study supports the hypothesis that the intra-atrial conduction heterogeneity, assessed by P-wave dispersion measurement, plays an important role in the AF initiation and perpetuation in DM1 patients with normal cardiac function. Atrial preference pacing algorithm, decreasing the number of atrial premature beats and the P-wave dispersion, reduces the onset of AF episodes and decreases the AF burden in DM1 patients underwent dual-chamber pacemaker implantation.

References


Psychological and practical difficulties among parents and healthy siblings of children with Duchenne vs. Becker muscular dystrophy: an Italian comparative study

Lorenza Magliano, Maria Grazia D’Angelo, Giuseppe Vita, Marika Pane, Adele D’Amico, Umberto Balottin, Corrado Angelini, Roberta Battini and Luisa Politano; Telethon GUP10002 Working Group

TELETHON GUP10002 WORKING GROUP: Melania Patalano, Alessandra Sagliocchi, Federica Civati, Erika Brighina, Gian Luca Vita, Sonia Messina, Maria Sframeli, Maria Elena Lombardo, Roberta Scalise, Giulia Colia, Maria Catteruccia, Angela Berardinelli, Maria Chiara Motta, Alessandra Gaiani, Claudio Semplicini, Luca Bello, Guia Astrea, Antonella Zaccaro, Marianna Scutiferro

1 Department of Psychology, Second University of Naples (SUN), Naples, Italy; 2 NeuroMuscular Unit, Department of NeuroRehabilitation, IRCCS “E. Medea”, Bosisio Parini (LC), Italy; 3 Department of Neurosciences, University of Messina, Messina, Italy; 4 Department of Paediatric Neurology, Catholic University, Rome, Italy; 5 Unit of Neuromuscular and Neurodegenerative Diseases, Bambin Gesù Children’s Hospital, Rome, Italy; 6 Child Neuropsychiatry Unit, Department of Brain and Behavioral Sciences, University of Pavia, Pavia, Italy; 7 Department of Neurosciences, University of Padova, Padua, Italy and IRCSS San Camillo, Lido, Venice, Italy; 8 Developmental Neuroscience, IRCCS Stella Maris, University of Pisa, Pisa, Italy; 9 Cardiomyology and Medical Genetics, Department of Experimental Medicine, Second University of Naples (SUN), Naples, Italy

This study explored the burden in parents and healthy siblings of 4-17 year-old patients with Duchenne (DMD) and Becker (BMD) muscular dystrophies, and whether the burden varied according to clinical aspects and social resources.

Data on socio-demographic characteristics, patient’s clinical history, parent and healthy children burden, and on parent’s social resources were collected using self-reported questionnaires administered to 336 parents of patients with DMD (246) and BMD (90). Parents of patients with DMD reported higher burden than those of patients with BMD, especially concerning feeling of loss (84.3% DMD vs. 57.4% BMD), stigma (44.2% DMD vs. 5.5% BMD) and neglect of hobbies (69.0% DMD vs. 32.5% BMD). Despite the burden, 66% DMD and 62.4% BMD parents stated the caregiving experience had a positive impact on their lives. A minority of parents believed MD has a negative influence on the psychological well-being (31.0% DMD vs. 12.8% BMD), and social life of unaffected children (25.7% vs. 18.4%).

In the DMD group, burden correlated with duration of illness and parent age, and burden was higher among parents with lower social contacts and support in emergencies. In DMD, difficulties among healthy children were reported as higher by parents who were older, had higher burden and lower social contacts. In both groups, burden increased in relation to patient disability. These findings underline that the psychological support to be provided to patients with MD, should take into account clinical features of the disease.

Key words: Duchenne muscular dystrophy, Becker muscular dystrophy, parents, healthy siblings, burden, social network

Introduction

Muscular Dystrophies (MDs) are degenerative, rare muscle diseases leading to progressive restriction of functional autonomy (1). Although curative therapy is not yet available, the improvement of standard care has led to a considerable increase in patients’ life expectancy (2).
Duchenne Muscular Dystrophy (DMD) – the most severe form of MD – is due to X-linked dystrophin gene mutations and affects about one in 5,000 males (1). Typically, symptoms of DMD manifest between 2 and 5 years of life, ambulation is lost by 12 years, and death mostly occurs in the second or third decade of life (1). Becker MD (BMD), the allelic milder form, affects about one in 20,000 males. In BMD, muscle symptoms usually onset in the second decade, walking autonomy is preserved up to the fifth or sixth decade, and life expectancy is not significantly reduced, unless cardiomyopathy occurs (3).

Most patients with MD, even those affected by severe forms, live at home and receive daily assistance from their relatives. Home care facilitates patients’ maintenance of an acceptable daily routine for as long as possible, while caregivers receive a multifaceted experience (4-6).

Consequences of family caregiving in chronic diseases are commonly named “family burden” and subdivided into “practical” and “psychological” burden (7). Practical burden refers to problems such as disruption of family relationships, constraints in social, leisure, and work activities, and financial difficulties. Psychological burden describes the reactions that family members experience, e.g. feeling of loss, sadness, tension, and feeling unable to cope with the situation. Family burden has been scarcely explored in MDs, differently from cancer (8), dementia (9) and mental disorders (10). Available data reveal that caregivers of patients with MDs may perceive moderate to high levels of stress, and have frequent feelings of guilt, sadness and depression related to the patient’s condition (6, 11, 12). Moreover, the caregivers frequently face financial difficulties due to costs of care and constraints of work activities, neglect other family members and reduce their own social activities (4, 6, 7, 12-14). Mothers, low-income families, unemployed relatives, and relatives of patients with high disability and severe MD present higher levels of burden (6). Conversely, relatives who have adequate coping skills, high self-esteem, and a supportive social network perceive lower burden and identify more valuable benefits from the caregiving experience (12, 14). These findings can be interpreted within the framework of Lazarus and Folkman’s transactional model (15), which postulates that an individual’s adaptation to an event is a process based on primary and secondary cognitive appraisal. In regards to MD, primary appraisal has to do with the perception of the stress and consequences associated with MD, while secondary appraisal implies the development of strategies to cope with the difficulties of caring. In this model, adaptation is significantly influenced by internal factors (i.e. key-relative’s attitude toward the patient) and external resources (i.e. availability of social and professional support) (14).

Home care for a child with MD involves the whole family, including minor unaffected siblings. However, little information on the psychological adjustment and the practical consequences of family care for healthy children is available. Studies on unaffected siblings of children with other severe pediatric illnesses suggest that emotional distress and behavioral problems may be significantly high in healthy siblings, and in part related to adult relatives’ burden (16-18). In a study addressing psychological adjustment in 46 minor siblings of DMD children (19), 52% and 44% of siblings reported “great” or “some” deal of involvement in their brother’s care, respectively, while 37% and 35% stated they missed special activities and/or daily activities due to a patient’s care. Furthermore, a higher proportion of healthy siblings reached the high-risk threshold for emotional problems, and – as rated by parents – more than twice (19%) the high-risk cut-off for a psychiatric disorder, compared to the normative sample. Psychological symptoms were found to be weakly/moderately related to a patient’s age and closeness in age to the affected sibling, adult relatives’ burden, and family communication skills. However, DMD was also found to be associated with positive psychological adjustment in the family (20).

Research on MDs burden has several weaknesses. The few available studies have only investigated family burden in DMD (5, 12) and were carried out in North-America (6-13), limiting the generalization of the results in contexts with different health care policies (21). The poor data have probably slowed down the development and dissemination of targeted interventions to support patients and families in the routine, as the allocation of economic and staff resources.

In 2012, within the framework of the Telethon UILDM Italian National Program for Clinical Research in Muscular Diseases, we performed a national survey on the condition of families of patients with DMD, BMD, or Limb-Girdle MD (LGMDs). Data on 502 families of patients aged 4 to 25, revealed that feelings of loss and sadness were present in 77% and 74% of relatives, respectively, while constraints to leisure activities were present in 59%. Burden was higher among relatives of patients with lower functional abilities, who were older in age, and suffering from DMD, and among those who were more involved in a patient’s daily care or who perceive lower social support. Psychological benefits were acknowledged by 88% of the relatives, particularly those who perceived a higher level of professional and social support (7, 14).

Based on the national data bank mentioned above, this paper is focused on the difficulties experienced by 246 parents of minors with DMD and 90 parents of minors with BMD, and the parents’ perception of difficulties in minor unaffected children. In particular, the study
aims to verify whether: the burden is higher in relatives of patients with DMD than in those of patients with BMD, even in early and intermediate stages of the diseases; the difficulties experienced by the parents and by healthy siblings – as perceived by the parents – are higher among parents who are older and have lower social resources and with affected children having longer duration of illness, higher disability and older age.

Methods

Design of the study

The study was carried out in Italy in eight tertiary neuromuscular centers from January to December 2012. In each center, key-relatives (i.e., the relative spending more daily time in contact with the patient and being more involved in his/her care) of 4-25 year patients who had a diagnosis of DMD, BMD, or LGMD, and lived with at least one relative 18-80 years-old, were consecutively contacted and asked to participate. In occasion of the patient’s clinical scheduled control, key-relatives were interviewed – after written informed consent – by a trained researcher on: a) main socio-demographic characteristics of the family and clinical history of the patient through an ad-hoc designed schedule; b) patient’s functional abilities, according to Barthel Index (BI) (22); c) treatments and support received by the patient and his/her family through the Muscular Dystrophy Care Schedule (MD-CS). Furthermore, they were invited to fill in the Family Problems Questionnaire (FPQ) (21) and the Social Network Questionnaire (SNQ) (21). Among the 502 key-relatives who participated in the study, those having one 4 to 17 year-old child with DMD or BMD, were subsequently extrapolated for the aims of this paper.

The protocol of the study was approved by the Ethic Committee of the Second University of Naples (coordinating centre) and accepted by the Ethical Committee of each center.

Instruments description

BI assesses patient’s global degree of independence in daily activities on a 0-100 score (from 0 “totally dependent” to 100 “totally independent”). In this study, the inter-rater reliability in BI scoring, measured by Cohen’s kappa coefficient, ranged from 1 to 0.90 for 9 BI items and was equal to 0.67 for the lasting one. MD-CS collects information on pharmacological, socio-rehabilitative, and psychotherapeutic interventions received by the patient, and professional and welfare support provided to the family in the last six months. FPQ is a 34-item tool exploring: a-b) psychological and practical burden; c-d) social and professional support to families in patient’s emergencies; and e) relative’s positive attitude toward the patient. Two additional items explore respondent’s perception of psychological and social consequences in minor children. SNQ is a 15-item tool exploring: a) quality and frequency of social contacts; b-c) practical and emotional support; and d) quality of an intimate relationship. FPQ and SNQ are self-reported and contain items rated on a 4-level scale from 1 “never” to 4 “always”. Mean subscale scores, ranging from 1 to 4, are also computed. FPQ and SNQ have been initially developed for schizophrenia and validated in five languages (English, Italian, Portuguese, Greek, and German) within the framework of a EC study on schizophrenia (21) (Cohen’s kappa coefficient: 0.50 to 1 in 79% of FPQ items, and in 69% of SNQ items; Cronbach’s alpha: 0.61 to 0.88 of FPQ subscales, and 0.56 to 0.75 of SNQ subscales; factor analysis: 45% explained variance in FPQ, and 56% in SNQ ). The main psychometric properties of FPQ and SNQ were further explored in samples of relatives of patients with physical diseases (10) and found to be consistent with those of the schizophrenia group (FPQ subscales alpha ranging between 0.91 and 0.65; explained variance 74%; SNQ subscales alpha value ranging between 0.75 and 0.59; percentage of the explained variance 72%). In the whole GAP10002 study sample (7), Cronbach’s alpha on FPQ and SNQ subscales were found consistent with previous measurements (0.63 to 0.86 in FPQ subscales and 0.68 to 0.79 in SNQ subscales). In this paper, items from FPQ a-c subscale items (alpha from 0.66 to 0.87 in this sample) and additional items on siblings difficulties (alpha: 0.72), and SNQ a) subscale items (alpha: 0.69) have been reported. Furthermore, for this study, a burden total score was also computed (alpha: 0.86).

Statistical analysis

χ² and analysis of variance were used, as appropriate, to test differences in nominal and ordinal variables between DMD and BMD samples. χ² was also used to compare the two samples with regards to FPQ burden items. Analysis of variance was used to compare the two samples in their mean scores of parents’ burden. In each group, correlations of parents’ burden with perceived difficulties in unaffected siblings, were explored by Spearman’s r correlation. The same test was used to explore the relationships of parents’ burden and perceived difficulties in healthy children with parents’ social contacts and social support in emergencies, parents’ age, and patient’s age, BI and duration of illness. Because of the large number of analyses, only results at the p < 0.05 with Bonferroni correction are reported, to reduce the probability of type I errors (false positives).
Results

Among the 246 children with DMD and 90 with BMD, the majority attended school and had healthy siblings (Table 1). Children with DMD were younger ($\bar{F} = 13.9$, $\text{df} = 1, 334$, $p < .05$), had lower levels of functional autonomy ($\bar{F} = 95.7$, $\text{df} = 1, 334$, $p < .05$) and more frequently received economic welfare benefits ($\chi^2 = 177$, $\text{df} = 1, p < .05$) than patients with BMD. Two-hundred-eight patients with DMD (84.6%) and 90 (43.3%) with BMD ($\chi^2 = 39.3$, $\text{df} = 1, p < .05$) were in drug treatment (corticosteroids: 168 (68.3%) of DMD vs 7 (7.8%) of BMD; bone metabolism drugs: 92 (37.4%) vs. 7 (7.8%); cardiological drugs: 80 (32.5%) vs. 22 (24.4%); gastric drugs: 48 (19.5%) vs. 4 (4.4%); neurological drugs: 5 (2.0%) vs. 1 (1.1%); pulmonary drugs: 4 (1.6%) vs. 1 (1.1%)) while 203 (82.5%) patients with DMD and 26 (28.9%) with BMD ($\chi^2 = 87.3$, $\text{df} = 1, p < .05$) attended rehabilitation programs. Most participating parents were mothers, and had a middle to high educational level (Table 1). DMD parents spent more daily hours in patient caregiving than BMD parents ($\bar{F} = 43.2$, $\text{df} = 1, 334$, $p < .05$). On average, burden was significantly higher among parents of children with DMD [1.8 (0.5), vs. 1.4 (0.4), $\bar{F} = 44.7$, $\text{df} = 1, 334$, $p < .05$]. In particular, the feeling of loss was reported by 84.3% of parents in the DMD group vs. 57.4% of parents in the BMD group (Table 2). Perception of a stigma in a public setting was reported by 44.2% of DMD parents, while it was almost nonexistent in BMD group (5.5%). Moreover, 59.3% DMD vs. 30.3% BMD parents agreed with the statement “I felt that I would not be able to bear the situation longer” and 55% DMD vs. 29.2% BMD parents believed that if the patient was not sick, everything would be fine in their family. As far as practical consequences of caregiving (Table 3), differences between DMD and BMD group were particularly relevant in regard to the need to awaken during the night (47.3% vs. 17.7%), a neglect of hobbies (69.0% vs. 32.5%), difficulties in work/household activities (55.5% vs. 18.9%), taking holidays (38.9% vs. 12.0%), and financial difficulties (42.0% vs. 17.8%).

Forty-one (31.0%) DMD and 5 (12.8%) BMD parents believed that the patient’s condition negatively influenced the psychological well-being of unaffected children, while 34 (25.7%) and 7 (18.4%) respectively felt a negative influence on the sibling’s social life (Table 4).

Despite difficulties, most key relatives (66.0% DMD and 62.4% BMD parents) considered their caregiving experience to have a positive impact on their lives. Both DMD and BMD parents mentioned “personal grown” (73.6% vs. 63.1%, e.g., “I learned that difficulties of life help you to grow”), “resilience” (15.9% vs. 18.5%, e.g., “I learned to have more strength to fight for the people I love”), and “altruism” (15.9% vs. 15.4%, e.g., “I get involved in helping people in a condition similar to mine”) among the psychological benefits.

Furthermore, the majority of parents (70% DMD and 73.1% BMD) claimed to have at least two trustworthy

| Table 1. Characteristics of patients with DMD and BMD and their parents. |
|------------------------|------------------------|------------------------|
|                        | DMD (N = 246)          | BMD (N = 90)           |
| **Patients**           |                        |                        |
| Age, mean (SD) years  | 10.0 (3.7)             | 11.9 (3.6)             |
| School attendance, N (% yes) | 233 (94.7)        | 89 (98.9)             |
| Minor healthy siblings, N (% yes) | 134 (54.5)      | 45 (50.0)             |
| Age of older healthy sibling, mean (SD) years | 9.6 (4.4)     | 9.5 (4.7)             |
| Duration of symptoms, mean (SD) years | 6.8 (3.9)   | 7.5 (4.1)             |
| BI, mean (SD)*        | 65.6 (28.0)           | 95.4 (11.3)           |
| **Parents**           |                        |                        |
| Mothers, N (%)        | 205 (83.3)             | 78 (86.7)             |
| Fathers, N (%)        | 41 (16.7)              | 12 (13.3)             |
| Age, mean (SD) years  | 41.2 (6.2)             | 43.3 (6.6)            |
| Marital status, cohabitant/spouse N (%) | 217 (88.2)     | 80 (88.9)             |
| Education, N (%)      |                        |                        |
| Primary school        | 11 (4.5)               | 3 (3.3)               |
| Secondary school      | 89 (36.2)              | 37 (41.1)             |
| High school           | 119 (48.4)             | 39 (43.3)             |
| University            | 27 (10.9)              | 11 (11.2)             |
| Currently employed, N (% yes) | 133 (54.1)   | 55 (61.1)             |
| Daily hours in patient's caregiving, mean (SD)* | 6.3 (4.1) | 3.3 (2.7) |

DMD = Duchenne Muscular Dystrophy; BMD = Becker Muscular Dystrophy; differences between the two groups explored by X or ANOVA test, * $p < .05$ with Bonferroni correction
### Table 2. Psychological difficulties in DMD vs. BMD groups (N = 246 vs. N = 90).

<table>
<thead>
<tr>
<th>Items – section a of FPQ (MD Type)</th>
<th>Always N (%)</th>
<th>Often N (%)</th>
<th>Sometimes N (%)</th>
<th>Never N (%)</th>
<th>Mean (SD)</th>
<th>χ²</th>
<th>MissingN</th>
</tr>
</thead>
<tbody>
<tr>
<td>I felt that I would not be able to bear this situation much longer</td>
<td>DMD 8 (3.2)</td>
<td>35 (14.3)</td>
<td>103 (42.0)</td>
<td>99 (40.4)</td>
<td>1.8 (.0)</td>
<td>1.8</td>
<td>.0</td>
</tr>
<tr>
<td></td>
<td>BMD 2 (2.2)</td>
<td>4 (4.5)</td>
<td>21 (23.6)</td>
<td>62 (69.7)</td>
<td>1.4 (.7)</td>
<td>23.2</td>
<td>.0</td>
</tr>
<tr>
<td>I cried or felt depressed</td>
<td>DMD 7 (2.8)</td>
<td>59 (24.0)</td>
<td>130 (52.8)</td>
<td>50 (20.3)</td>
<td>2.1 (.7)</td>
<td>0.0</td>
<td>.0</td>
</tr>
<tr>
<td></td>
<td>BMD 4 (4.5)</td>
<td>8 (9.0)</td>
<td>49 (55.1)</td>
<td>28 (31.5)</td>
<td>1.9 (.7)</td>
<td>11.4</td>
<td>.0</td>
</tr>
<tr>
<td>I worry for the future of other family members</td>
<td>DMD 21 (8.6)</td>
<td>41 (16.8)</td>
<td>119 (48.8)</td>
<td>63 (25.8)</td>
<td>2.1 (.9)</td>
<td>2.0</td>
<td>.0</td>
</tr>
<tr>
<td></td>
<td>BMD 3 (3.3)</td>
<td>11 (12.2)</td>
<td>45 (50.0)</td>
<td>31 (34.4)</td>
<td>1.8 (.8)</td>
<td>5.2</td>
<td>.0</td>
</tr>
<tr>
<td>When I went to a public place with my ill relative, I felt that everyone was watching us</td>
<td>DMD 19 (7.9)</td>
<td>20 (8.3)</td>
<td>68 (28.1)</td>
<td>135 (55.8)</td>
<td>1.7 (.9)</td>
<td>4.0</td>
<td>.0</td>
</tr>
<tr>
<td></td>
<td>BMD 1 (1.1)</td>
<td>0 (0.0)</td>
<td>4 (4.4)</td>
<td>85 (44.4)</td>
<td>1.1 (.9)</td>
<td>44.1</td>
<td>.0</td>
</tr>
<tr>
<td>I feel guilty because I believe that I or my spouse may have passed on the illness to our relative</td>
<td>DMD 19 (7.8)</td>
<td>30 (12.2)</td>
<td>80 (32.7)</td>
<td>116 (47.3)</td>
<td>1.8 (.9)</td>
<td>1.0</td>
<td>.0</td>
</tr>
<tr>
<td></td>
<td>BMD 15 (17.0)</td>
<td>18 (20.5)</td>
<td>29 (33.0)</td>
<td>26 (29.5)</td>
<td>2.2 (1.1)</td>
<td>13.3</td>
<td>.0</td>
</tr>
<tr>
<td>I think that if our relative didn’t have this problem, everything would be all right in our family</td>
<td>DMD 39 (16.0)</td>
<td>32 (13.1)</td>
<td>64 (26.2)</td>
<td>109 (44.7)</td>
<td>2.0 (1.1)</td>
<td>2.0</td>
<td>.0</td>
</tr>
<tr>
<td></td>
<td>BMD 4 (4.5)</td>
<td>3 (5.6)</td>
<td>17 (19.1)</td>
<td>63 (70.8)</td>
<td>1.4 (.8)</td>
<td>19.9</td>
<td>.0</td>
</tr>
<tr>
<td>When I think of how our ill relative was beforehand and how he/she is now, I feel disappointed</td>
<td>DMD 75 (31.0)</td>
<td>50 (20.7)</td>
<td>79 (32.6)</td>
<td>38 (15.7)</td>
<td>2.7 (1.1)</td>
<td>3.0</td>
<td>.0</td>
</tr>
<tr>
<td></td>
<td>BMD 12 (13.8)</td>
<td>11 (12.6)</td>
<td>27 (31.0)</td>
<td>37 (42.5)</td>
<td>2.0 (1.0)</td>
<td>29.6</td>
<td>.0</td>
</tr>
</tbody>
</table>

DMD = Duchenne Muscular Dystrophy; BMD = Becker Muscular Dystrophy; * p < .05 with Bonferroni correction

### Table 3. Practical difficulties in DMD vs. BMD groups (N = 246 vs. N = 90).

<table>
<thead>
<tr>
<th>Items – section b of FPQ (MD Type)</th>
<th>Always N (%)</th>
<th>Often N (%)</th>
<th>Sometimes N (%)</th>
<th>Never N (%)</th>
<th>Mean (SD)</th>
<th>χ²</th>
<th>MissingN</th>
</tr>
</thead>
<tbody>
<tr>
<td>I have had to wake up during the night</td>
<td>DMD 29 (11.8)</td>
<td>10 (8.2)</td>
<td>67 (27.3)</td>
<td>129 (52.7)</td>
<td>1.8 (1.0)</td>
<td>1.0</td>
<td>.0</td>
</tr>
<tr>
<td></td>
<td>BMD 1 (1.1)</td>
<td>0 (0.0)</td>
<td>11 (12.2)</td>
<td>74 (82.2)</td>
<td>1.2 (1.6)</td>
<td>15.0</td>
<td>.0</td>
</tr>
<tr>
<td>I have had to neglect my hobbies and things I like doing in my free time</td>
<td>DMD 41 (16.8)</td>
<td>20 (8.2)</td>
<td>87 (35.7)</td>
<td>76 (31.0)</td>
<td>2.2 (1.0)</td>
<td>2.0</td>
<td>.0</td>
</tr>
<tr>
<td></td>
<td>BMD 2 (2.2)</td>
<td>2 (0.8)</td>
<td>19 (21.3)</td>
<td>60 (67.4)</td>
<td>1.5 (7.7)</td>
<td>38.4</td>
<td>.0</td>
</tr>
<tr>
<td>I have had difficulty in going on Sunday outings</td>
<td>DMD 16 (7.8)</td>
<td>21 (10.3)</td>
<td>43 (21.1)</td>
<td>124 (60.8)</td>
<td>1.6 (9.9)</td>
<td>1.0</td>
<td>.0</td>
</tr>
<tr>
<td></td>
<td>BMD 0 (0.0)</td>
<td>3 (3.7)</td>
<td>11 (13.6)</td>
<td>67 (82.7)</td>
<td>1.2 (5.5)</td>
<td>15.2</td>
<td>.0</td>
</tr>
<tr>
<td>I found it difficult to have friends at home</td>
<td>DMD 2 (0.8)</td>
<td>11 (4.5)</td>
<td>26 (10.7)</td>
<td>205 (84.0)</td>
<td>1.2 (5.5)</td>
<td>2.0</td>
<td>.0</td>
</tr>
<tr>
<td></td>
<td>BMD 1 (1.1)</td>
<td>0 (0.0)</td>
<td>2 (2.2)</td>
<td>87 (96.7)</td>
<td>1.1 (3.3)</td>
<td>10.9</td>
<td>.0</td>
</tr>
<tr>
<td>I found it difficult to meet friends and people I like to spend my leisure time</td>
<td>DMD 6 (2.5)</td>
<td>22 (9.0)</td>
<td>50 (20.5)</td>
<td>166 (68.0)</td>
<td>1.5 (8.1)</td>
<td>2.0</td>
<td>.0</td>
</tr>
<tr>
<td></td>
<td>BMD 3 (3.4)</td>
<td>0 (0.0)</td>
<td>9 (10.1)</td>
<td>77 (86.5)</td>
<td>1.2 (6.2)</td>
<td>15.2</td>
<td>.0</td>
</tr>
<tr>
<td>I found it difficult to carry out my usual work or household activities</td>
<td>DMD 11 (4.5)</td>
<td>31 (12.7)</td>
<td>94 (38.4)</td>
<td>109 (44.5)</td>
<td>1.8 (8.8)</td>
<td>1.0</td>
<td>.0</td>
</tr>
<tr>
<td></td>
<td>BMD 0 (0.0)</td>
<td>3 (3.3)</td>
<td>14 (15.6)</td>
<td>73 (81.1)</td>
<td>1.2 (5.5)</td>
<td>36.5</td>
<td>.0</td>
</tr>
<tr>
<td>I had to neglect other family members</td>
<td>DMD 1 (0.4)</td>
<td>27 (11.5)</td>
<td>75 (31.9)</td>
<td>132 (56.2)</td>
<td>1.6 (7.7)</td>
<td>1.0</td>
<td>.0</td>
</tr>
<tr>
<td></td>
<td>BMD 0 (0.0)</td>
<td>2 (2.2)</td>
<td>18 (20.2)</td>
<td>69 (77.8)</td>
<td>1.2 (5.5)</td>
<td>14.3</td>
<td>.0</td>
</tr>
<tr>
<td>I had difficulty in going on holiday</td>
<td>DMD 28 (13.8)</td>
<td>21 (10.3)</td>
<td>30 (14.8)</td>
<td>124 (61.1)</td>
<td>1.1 (1.1)</td>
<td>43.0</td>
<td>.0</td>
</tr>
<tr>
<td></td>
<td>BMD 1 (1.2)</td>
<td>2 (2.2)</td>
<td>7 (8.4)</td>
<td>73 (88.0)</td>
<td>0.5 (0.0)</td>
<td>21.8</td>
<td>.0</td>
</tr>
<tr>
<td>I had economic difficulties</td>
<td>DMD 12 (4.9)</td>
<td>20 (8.2)</td>
<td>71 (29.0)</td>
<td>142 (58.0)</td>
<td>1.6 (8.1)</td>
<td>1.0</td>
<td>.0</td>
</tr>
<tr>
<td></td>
<td>BMD 1 (1.1)</td>
<td>1 (1.1)</td>
<td>14 (15.6)</td>
<td>74 (82.2)</td>
<td>1.2 (5.5)</td>
<td>18.3</td>
<td>.0</td>
</tr>
</tbody>
</table>

DMD = Duchenne Muscular Dystrophy; BMD = Becker Muscular Dystrophy; * p < .05 with Bonferroni correction
friends, and considered two or more relatives as trustworthy friends (78.0% DMD and 78.9% BMD). Moreover, in case of patient’s emergencies, parents stated to have at least two friends/relatives (63.4% DMD and 58.8% BMD), on which rely, and to be confident to be helped by them always or often (72.3% DMD and 80.0% BMD). Furthermore, in the last two months, 50.0% of DMD relatives and 55.6% of BMD had been in contact with friends face to face or by phone, most days.

Burden was higher among parents of patients with lower functional autonomy (DMD burden total score: $r = -0.50$, BMD burden total score: $r = -0.38$, $p < 0.05$), in both groups. In the DMD group, burden correlated with duration of illness ($r = -0.32$, $p < 0.05$), patient’s age ($r = -0.36$, $p < 0.05$) and parents’ age ($r = -0.30$, $p < 0.05$). Furthermore, burden was higher among parents with fewer social contacts ($r = -0.28$, $p < 0.05$) and lower social support in emergencies ($r = -0.51$, $p < 0.05$). In the same group, difficulties in healthy siblings were higher among children whose parents were older ($r = -0.33$, $p < 0.05$) and with fewer social contacts ($r = -0.27$, $p < 0.05$).

Discussion

The results of this study confirm that parents of children with DMD experience higher difficulties than those of children with BMD, even when the patient’s functional ability is still relatively preserved. The main object of concern – significantly higher in DMD vs. BMD group – is the frequent feeling of loss and being inadequate to bear the situation and the conviction that whole family is influenced by the patient’s condition. Forty-four percent of DMD parents felt to be observed in public places when they are with the sick child, but this feeling is virtually absent in the BMD group. Perceived stigma, a phenomenon largely investigated in mental illness (23-25) and rarely considered in physical illness (26), may negatively influence parents’ and patient’s quality of life over time. In particular, the stigma may lead to family social withdrawal (23), and may be associated with feelings of depression and guilt among parents (23). Moreover, the parents’ perception of a stigma may contribute to a reduction of social contacts in patients, and negatively influence the adherence to treatment (25).

Differences in the onset and clinical course of DMD vs. BMD can explain why a lower parental burden was observed in the BMD group. In DMD, the early onset of symptoms may influence the mother-child relationship, and may become an obstacle to the child’s social experiences (26, 27). Conversely, in BMD the later onset has a limited interference with patient’s emotional development in childhood and adolescence, and the slow progression allows a gradual adaptation of parents and patients to the disability itself (27).

Furthermore, a clear relationship between increased levels of burden and reduction in parents’ social ties was found only in the DMD group, though the social network did not differ between the two groups. It is likely that, as DMD progresses, parents feel overwhelmed by their caregiving role, and too exhausted to be involved in social activities. This situation may lead to a vicious cycle in which a progressive reduction of social network exposes the parents to greater levels of burden over time, with consequent further social withdrawal (10).

While parental burden is higher in the DMD, the difficulties observed by parents in their minor healthy children are similar in the two groups and relatively mild. It is likely that parents tend to protect unaffected children, not involving them in the care of the patient as longer as possible (19). However, when the disease progresses and burden increases (16), even healthy siblings are invited to take care of the patient, and this may lead to the onset of practical and psychological difficulties (20).

<table>
<thead>
<tr>
<th>Items – additional section of FPQ</th>
<th>MD Type</th>
<th>Always N (%)</th>
<th>Often N (%)</th>
<th>Sometimes N (%)</th>
<th>Never N (%)</th>
<th>Mean (SD)</th>
<th>$\chi^2$</th>
<th>Missing N</th>
</tr>
</thead>
<tbody>
<tr>
<td>I feel that the presence of S affects negatively the psychological well-being of my children (e.g., I see them crying, being fearful, aggressive, shy)</td>
<td>DMD</td>
<td>2 (1.5)</td>
<td>6 (4.5)</td>
<td>33 (25.0)</td>
<td>91 (68.9)</td>
<td>1.4 (.6)</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>BMD</td>
<td>1 (2.6)</td>
<td>0</td>
<td>4 (10.3)</td>
<td>34 (87.2)</td>
<td>1.2 (.5)</td>
<td>6.3</td>
<td>6</td>
</tr>
<tr>
<td>I feel that the presence of S affects negatively the social life of my children (school performance, leisure activities, etc.)</td>
<td>DMD</td>
<td>3 (2.3)</td>
<td>5 (3.8)</td>
<td>26 (19.7)</td>
<td>98 (74.2)</td>
<td>1.3 (.7)</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>BMD</td>
<td>0</td>
<td>0</td>
<td>7 (18.4)</td>
<td>31 (81.6)</td>
<td>1.2 (.4)</td>
<td>2.5</td>
<td>7</td>
</tr>
</tbody>
</table>

DMD = Duchenne Muscular Dystrophy; BMD = Becker Muscular Dystrophy
This study also showed that about two-thirds of parents, both DMD and BMD, acknowledged psychological benefits in their caregiving experience, especially "personal growth" and an increased sense of strength against adversity. It's likely that – as postulated by Lazarus and Folkman’s model (15) – when relatives feel they can manage the practical difficulties of care, they do not overcome the individual threshold of stress tolerance, and may also consider the positive aspects of caregiving.

This study presents some limitations: (a) the assessment of burden only in the key-parent – mainly the mother, as is customary in Italy – does not allow us to estimate the influence of the caregiving on the parental couple (5, 28); (b) the study did not assess psychological adjustment in healthy siblings themselves (19); (c) the lack of data from normal and healthy population collected by FPQ and SNQ; and (d) the cross-sectional design of the study that does not allow inferences regarding the evolution of burden or whether external resources may influence the burden or vice versa. These limitations will be addressed in future studies, using an online assessment to overcome the logistical difficulties found in the involvement of more relatives per patient.

On the other hand, the main strengths of this comparative study exist in the large sample size, the participation of centers located in different geographical areas of Italy, and the use of validated assessment tools (21, 22).

The results of this study underline the need to differentiate the type of parental support, taking into account the clinical features of MDs. In the case of BMD, education on the illness and its course could be sufficient to facilitate a parent’s adaptation and their active involvement in care (29). Conversely, in the more severe form of MD, targeted psychological support should be provided to parents in the different stages of the disorder, according to family need (29). Physicians, in collaboration with psychologists, should be trained in addressing parents’ psychological reactions to diagnosis (30) and disease course. Furthermore, these professionals should maintain a hope-oriented approach to provide parents with education on MD, helping them to see the child “beyond the illness” and to communicate with unaffected children about the diagnosis (14, 19, 20, 31). Finally, they should prompt parents to, as useful coping strategies, adopt a problem-solving approach to deal with difficulties, to carve out time for their social contacts and to joint associations (32).

In conclusion, our results highlight that parents’ and healthy siblings reactions to MDs vary in relation to type of the disease and parents’ social resources. Moreover, the study focuses on aspects that are usually neglected by physicians and that would require planned professional training and appropriate resource allocation (33).

Acknowledgments
This study was supported by a Telethon UILDM grant (Grant n. GUP10002). We are grateful to Angela Rinaldi, psychologist, who reviewed the literature on healthy siblings’ reactions in chronic illnesses.

List of abbreviations
BI: Barthel Index
BMD: Becker Muscular Dystrophy
DMD: Duchenne Muscular Dystrophy
FPQ: Family Problems Questionnaire
MD: Muscular Dystrophy
MD-CS: Muscular Dystrophy Care Schedule
MDs: Muscular Dystrophies
SNQ: Social Network Questionnaire

References
Psychological and practical difficulties among parents and healthy siblings of children with Duchenne vs. Becker muscular dystrophy


Introduction. CMT4B2 is a rare subtype of CMT caused by pathogenic mutations in the myotubularin-related protein-13/set binding factor 2 (MTMR13/SBF2) gene. Nerve conduction velocities are markedly reduced and focally folded myelin sheaths are present on nerve biopsies. We presented two patients from two related Portuguese families with peripheral neuropathy caused by a novel mutation in the MTMR13/SBF2 gene.

Case report. Family 1: Patient 1: A 30-year-old woman, with disease onset in early childhood presented pes cavus and hammertoes and walked with a steppage gait. Muscle weakness was present distally, myotactic reflexes were abolished and sensory examination revealed a stocking and glove pattern of hypoesthesia.

Family 2: Patient 2: A 43-year-old man, second degree cousin of patient 1, born of a consanguineous marriage. At the age of 9 months, he was diagnosed with congenital glaucoma on the left eye, with progressive visual loss up to total blindness. He presented bilateral claw hand deformity, pes cavus and hammertoes and walked with a steppage gait. Myotactic reflexes were abolished and muscle weakness was severe distally in the upper and lower limbs. Sensory examination revealed a stocking and glove pattern of hypoesthesia. In both patients electrodiagnostic studies evidenced an uniform and generalized sensorimotor demyelinating polyneuropathy and the molecular study found a frameshift/truncating homozygous novel mutation c.5073_5074del (p.Ser1692Tyrfs*42) in the MTMR13/SBF2 gene.

Conclusions. We report a novel mutation in the MTMR13/SBF2 gene associated with a classical CMT phenotype. Congenital glaucoma associated with a frameshift/truncating mutation in CMT4B2 is reported for the first time.

Key words: CMT4B2, MTMR13/SBF2 gene, autosomal recessive CMT, congenital glaucoma
We present the clinical, neurophysiologic and molecular findings of two patients from two related Portuguese families with CMT4B2, one with unilateral congenital glaucoma, caused by a novel frameshift/truncating homozygous mutation in the MTMR13/SBF2 gene.

A. Clinical findings

Family description

Family 1: Patient 1 – The patient is a 30-year-old woman, the single offspring of a non-consanguineous couple. The mother’s parents are first degree cousins (Fig. 1) and her parents were clinical and electrophysiological normal. The patient’s delivery was normal and she presented a normal motor and intellectual development in infancy. She attended University and graduated in Social Service.

At 4 years of age, the parents noticed a slight gait disturbance, which did not interfere significantly with her participation in the school physical activities. At the end of the first decade, running became increasingly more difficult and lastly impossible.

By the age of 9, the medical records from the local pediatric hospital described bilateral pes cavus, hammer toes, distal lower limb muscular atrophy, absent ankle jerks and a flexor plantar response.

The muscle weakness and atrophy were slowly progressive in the following years, with involvement of the intrinsic hand muscles at the end of the second decade. Several corrective feet orthopedic surgeries were performed at the ages 15, 18 and 19.

By the age of 30 she presented pes cavus and hammer toes (Fig. 2a), with inverse champagne bottle legs (Fig. 2b). The thenar muscles were severely atrophic (Fig. 2c). Walking was difficult on tiptoes and impossible on heels and she walked with a bilateral steppage gait without ataxia. In the upper limbs the finger flexors, extensors and abductor muscles were weak (4/5 MRC); the abductor pollicis brevis muscle was more severely affected (0/5 MRC), bilaterally. In the lower limbs, extensor (0/5 MRC) and flexor muscles (4-5 MRC) of the feet and toes were weak, bilaterally. Muscle stretch reflexes were absent throughout. There were no cranial nerve deficits (including II cranial nerve and the VII nerve innervated muscles). Sensory examination revealed diminished tactile and pain sensation in a stocking and glove pattern and vibratory sensation was reduced distally in the upper and lower limbs, 10 and 6 seconds, respectively.

Family 2: Patient 2 – The patient is a 43-year-old man, born of a first degree consanguineous marriage and second degree cousin of Patient 1 (Fig. 1). His mother is a first degree cousin of Patient’s 1 father and he has one brother with a suspected similar neuromuscular condition (not available to examination). His parents were clinical and electrophysiological normal (the father died recently). The patient’s delivery was normal and when he was 9 months old, he was diagnosed with congenital glaucoma on the left eye, with progressive visual loss up to total blindness. His right eye is not affected and does not present intra-ocular hypertension. He presented a normal motor and intellectual development in infancy.

By the age of 4, he walked on tiptoes. He underwent

![Figure 1. Pedigree of Families 1 and 2.](image-url)
corrective orthopedic surgeries, because of pes equino-varus, at 4 and 18 years of age.

At his first clinical appointment at the Neuromuscular Disease Unit in 2014, by the age of 43 years, neurological examination showed pes cavus and hammertoes (Fig. 2d) and inverse champagne bottle legs (Fig. 2e). The hand muscles were severely atrophic, with claw hand deformity (Fig. 2f). He walked with a bilateral steppage gait,

Table 1. Neurophysiologic investigations.

<table>
<thead>
<tr>
<th>Nerve Conduction Study</th>
<th>Median Nerve</th>
<th>Ulnar Nerve</th>
<th>Peroneal Nerve</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CMAP (mV)</td>
<td>DML (ms)</td>
<td>MNCV (m/sec)</td>
</tr>
<tr>
<td>Family 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient 1</td>
<td>0.5</td>
<td>9.1</td>
<td>14</td>
</tr>
<tr>
<td>Family 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient 1</td>
<td>Ø</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Nerve Conduction Study</th>
<th>Sural Nerve</th>
<th>Facial Nerve</th>
<th>Blink Reflex</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SAP (µV)</td>
<td>CMAP (mV)</td>
<td>DML (ms)</td>
</tr>
<tr>
<td>Family 1</td>
<td>Ø</td>
<td>0.4</td>
<td>6.5</td>
</tr>
<tr>
<td>Patient 1</td>
<td>Ø</td>
<td>0.4</td>
<td>6.5</td>
</tr>
<tr>
<td>Family 2</td>
<td>Ø</td>
<td>0.4</td>
<td>6.5</td>
</tr>
<tr>
<td>Patient 1</td>
<td>Ø</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

DML: distal motor latency; CMAP: compound muscle action potential; MNCV: motor nerve conduction velocity; SAP: sensory action potential; ms: milliseconds; mV: millivolt; m/sec: meters per second; µV: microvolt; Ø: absent
which was impossible on tiptoes and heels. Muscle stretch reflexes were absent throughout. In the upper limbs, the hand extensor muscles were weak (4/5 MRC), as well as the intrinsic hand muscles and the distal finger extensor muscles (0/5 MRC). In the lower limbs, the feet and toes extensor and flexor muscles were weak (0/5 MRC). Facial nerve muscles and right eye visual acuity were normal. Sensory examination revealed diminished tactile and pain sensation in a stocking and glove pattern and vibratory sensation was reduced distally in the upper and lower limbs, 8 and 5 seconds, respectively.

B. Neurophysiological Investigations (Table 1)

In both patients, the median, ulnar and sural sensory nerve responses and the peroneal motor response recorded in the EDB muscle were absent bilaterally. In the upper limbs, the distal motor latencies recorded were prolonged and the motor nerve conduction velocities were severely reduced. No temporal dispersion or motor conduction block were observed. The latency of the direct motor response of the facial nerve and the R1 and R2i components of the blink reflex study were significantly prolonged in both patients. All the recorded motor responses were of very low amplitude. It was diagnosed a generalized demyelinating sensorimotor peripheral neuropathy with hereditary features.

C. Molecular studies (Fig. 3)

The patients, patient’s 1 parents and the mother of patient 2 underwent genetic testing. The molecular study included polymerase chain reaction and sequencing of the entire coding region, including the adjacent intronic regions, of the \textit{MTMR13/SBF2} gene (chromosome 11). Reference sequence: NM_030962.3 with the A of the initial ATG in position 1.

A frameshift/truncating homozygous novel mutation, variant c.5073_5074del (p.Ser1692Tyrfs*42), was found in a homozygous state in Patients 1 and 2 and in a heterozygous state in patient 1’s parents and the mother of Patient 2.

This mutation is predicted to be pathogenic as it introduces a premature stop codon, producing a truncated protein.

Discussion

These two Portuguese patients with CMT4B2 present a classical CMT phenotype: clinical onset of a sensorimotor peripheral neuropathy in the first decade of life, which was slowly progressive, predominantly motor and severe distally in the upper and lower limbs and associated feet deformities. There was no proximal muscle weakness, sensory symptoms have never been a major complaint.
and there was no sensory ataxia, cognitive impairment or psychiatric symptoms. The patients are still able to walk without support and are engaged on a productive life. A generalized demyelinating sensorimotor neuropathy was present in the neurophysiologic studies.

The characteristic histological feature of CMT4B2 is a markedly irregular contour and thickness of the myelin sheaths related to the presence of irregular myelin foldings, associated with marked reduction in the density of large myelinated fibers, segmental demyelination, and numerous onion bulb formations (6, 15). This pathologic feature is not unique to CMT4B2. The demyelinating nature of CMT4B can be easily confirmed by neurophysiologic techniques and nerve biopsies are exceptionally performed to confirm the demyelinating nature of the neuropathy.

Congenital glaucoma associated with a demyelinating CMT was reported in three members of a Brazilian family of Turkish ancestry (8). Early-onset glaucoma with a demyelinating CMT was later reported in families from Japan (10), Tunisia and Morocco (9). The affected members of these consanguineous families had nonsense mutations of the MTMR13/SBF2 gene, while CMT4B2 families without congenital or early-onset glaucoma had other types of mutations (in-frame deletions and mutations in the splicing site of the MTMR13/SBF2 gene) (6, 15). A genotype-phenotype correlation and a pathogenic mechanism were suggested, with nonsense mutations associated with complete absence of MTMR13/SBF2 protein and a partially functional protein in the other type mutations, capable of preventing the development of glaucoma, but not peripheral neuropathy (17). The mutation found in the Portuguese patients compromises the normal function of the MTMR13/SBF2 protein. It causes a premature stop codon, therefore it has the same consequence of the previously reported nonsense type of mutations related with the presence of congenital/early-onset glaucoma. Surprisingly, the glaucoma is not present in the female patient, which suggested that other genetic or epigenetic factors rather than this particular mutation could be responsible for the development of glaucoma.

References
CASE REPORT

Ventricular fibrillation induced by coagulating mode bipolar electrocautery during pacemaker implantation in Myotonic Dystrophy type 1 patient

Vincenzo Russo¹, Anna Rago¹, Federica Di Meo¹, Nadia Della Cioppa², Andrea Antonio Papa¹, Maria Giovanna Russo¹ and Gerardo Nigro¹

¹Department of Cardio-Thoracic and Respiratory Sciences, Chair of Cardiology, Second University of Napoli, Monaldi Hospital, Italy; ²Department of Anesthesiology, Monaldi Hospital, Napoli, Italy

Introduction

Electrocautery is often used to minimize bleeding during surgery. The probe used to perform electrocautery generates a high frequency electrical current that may be identified as noise, or mis-identified as an intrinsic heart activity by implanted pacemakers or defibrillators (1, 2).

Seven cases of electrocautery-induced ventricular tachycardia/fibrillation (VT/VF) during the implantation of pacemaker/defibrillators have been recently reported (3-6), four of them in patients with ischemic cardiomyopathy. In all cases the monopolar electrocautery system was used.

We report the first case of the occurrence of a ventricular fibrillation induced by bipolar electrocautery during elective dual chamber pacemaker implantation, in a patient affected by Myotonic Dystrophy type 1 with preserved left ventricular function.

Case report

A 46-years-old man with Myotonic Dystrophy type 1 (DM1) was referred to our division for pre-syncopal episodes. Electrocardiogram (ECG) revealed sinus rhythm, extreme left axis deviation, 2:1 atrioventricular block with a wide QRS complex. Trans-thoracic echocardiogram showed left ventricular concentric remodeling, a preserved left ventricular systolic function with an ejection fraction (EF) of 55%, calculated by the Simpson’s biplane method.

The patient was taking warfarin therapy for the occurrence of episodes of paroxysmal atrial fibrillation, and ACE-inhibitors. According to the current guidelines and personal experience, a dual chamber pacemaker implantation was performed. Because the high tromboembolic risk (CHA2DS2Vasc: 2) warfarin therapy was not discontinued before procedure and bipolar electrocautery system was used for cutting (setting: 40 watts) and coagulation (setting: 30 watts) during the pacemaker implantation. In the last step of the procedure, after the leads and generator placement, while applying short electrocautery pulses, in coagulating mode, near the subclavian vein lead access, the patient suddenly lost consciousness with ventricular fibrillation (VF) rhythm (rate 230 bpm) documented on the monitor (Fig. 1A). VF was immediately treated using an external biphasic defibrillator through a nonsynchronized 200 J shock, that restored sinus rhythm resulting in hemodynamic stability (Fig. 1B). Lead parameters remained
stable. The procedure of the pacemaker implantation was completed and the patient was discharged after two days, in which no further ventricular arrhythmias were reported. In the following 12 month follow-up, the patient did not experience episodes of pre-syncope nor episodes of ventricular arrhythmias were documented at the device interrogation.

**Discussion**

Myotonic dystrophy type 1, or Steinert Disease, is a severe autosomal-dominant hereditary disease with an estimated incidence of 1 in 8000 in adults. The phenotype is characterized by myotonia and muscle weakness, but a multisystemic involvement with highly variable clinical manifestation is very frequent. Cardiac involvement, often preceding the skeletal muscle involvement, occurs in 80% of MD1 patients and represents the second most common cause of death, after respiratory causes (7). The most frequent clinical event in DM1 patients is the development of a supraventricular arrhythmia (8-12), commonly observed on 12 lead ECG, 24 hour Holter monitoring or device interrogation, and often asymptomatic (12, 13). The supraventricular arrhythmias most commonly observed in up to 25% of patients – both

---

**Figure 1.** Rhythm strips during bipolar electrocautery application in coagulating mode. Ventricular fibrillation was seen after electrocautery was stopped (1A) and was treated (1B) using an 200 J external biphasic defibrillator non synchronized shock.
as not sustained and sustained forms (10, 14, 15) – are atrial fibrillation, atrial flutter and atrial tachycardia.

On the other hand, ventricular arrhythmias in MD1 patients may be related to the heterogeneity of ventricular repolarization, shown by an increase in QTc and JTc dispersion, as it has been reported in congenital (16-18) or acquired (19-21) heart diseases and in neuromuscular disorders (22-24).

The occurrence of VT/VF induced by electrocautery is uncommon; in the few cases so far reported (3-6), a monopolar electrocautery system was always used. The present report is the first case of VF induced by electrocautery during bipolar configuration. A possibly mechanism explaining the ventricular fibrillation induction may be attributed to the continuous transmission of the electrocautery radiofrequency pulses to the interface with the myocardium, through the ventricular electrode. The histopathological alterations observed in DM1 patient’s heart represent a highly sensitive arrhythmogenic substrate, with life-threatening consequence during the procedure of pacemaker/defibrillator implantation, also when bipolar electrocautery system is used in patients with preserved global systolic function. Therefore, the use of electrical scalpel should be only reserved to very high bleeding risk patients. In cases in which the electrocautery cannot be avoided, we strongly recommend to continuously monitor the patients and have advanced resuscitation equipment available during the procedure (25). Furthermore we suggest to perform short, intermittent, and irregular bursts at the lowest feasible energy levels in order to minimize the potential electromagnetic interference.

References


11. Russo V, Rago A, Papa AA, et al. Does a high percentage of right ven-


22. Negro G, Russo V, Rago A, et al. Regional and transmural disper-

23. Russo V, Rago A, Politano L, et al. Increased dispersion of ventricu-


25. Crossley GH, Poole JE, Rozner MA, et al. The Heart Rhythm Society (HRS)/American Society of Anesthesiologists (ASA) Expert Consensus Statement on the perioperative management of patients with implantable defibrillators, pacemakers and arryth-
In the present time there is the opinion that FSHD is a disease genetically heterogeneous, but homogeneous from a clinical point of view: “…clinical, genetic and epigenetic features of facioscapulohumeral muscular dystrophy (FSHD) allowed the identification of two forms of FSHD, the classical autosomal dominant FSHD type 1, and the FSHD type 2 characterized by an identical clinical phenotype but associated with a different (epi-) genetic defect” (1) and “Of the 33 patients with FSHD2 … the initial symptom was scapular weakness in 61%, foot dorsiflexor weakness in 27%, facial weakness in 10%, and hip girdle weakness in 3%”(2).

These authors, as many others, supposed that the facio-scapulo-peroneal topography of muscle weakness (early involvement of facial, shoulder girdle and tibialis anterior muscles) is the specific sign of both FSHD forms, at the beginning stage of the disease.

During years 1969-1971 the patterns of 67 bilateral muscles involvement were analyzed in 200 patients with FSHD, called FSLD, at different stages of the disease (3-5).

Of these, 145 cases were from the world literature while 55 were under Kazakov’s personal observation. Seventy-eight of them (31 personal cases and 47 from literature, 59 hereditary and 19 sporadic) had developed a descending Facio-Scapulo-Limb Dystrophy (FSLD2) with a jump type, 38 patients a FSP or FSP(H) phenotype and 40 patients a final FSPFGH [facio-scapulo-peroneo-femoro (posterior thigh muscles)-gluteo (gluteus maximus)-humeral (biceps brachial)] or FSPHFG phenotype. The “pure” FSP phenotype was clinically observed at an average age of 11-16 years. The diagnosis of FSHD (or FSLD2) in patients personally followed, was confirmed by molecular analysis in 1996 (6).

On the other hand, 60 out of 200 patients – 47 hereditary and 11 sporadic cases from the world literature and 2 Kazakov’s personal cases – had developed a FSLD1 gradually descending type. Among these, 31 presented with the FSHGF phenotype and 29 with the final FSHGFP phenotype. In the last group, pelvic girdle and thigh muscles were more severely affected when compared with peroneal muscles, except for some cases in which the peroneal muscles were similarly affected.

The existence of FSLD1 is also confirmed by the fact that in many Handbooks on Nervous Diseases and Handbooks on Muscle Diseases, FSHD is described as a “gradually descending muscular dystrophy (i.e. the FSLD1) with the affection of pelvic girdle and hip muscles as well as of peroneal muscles (in some patients) during 20-30 years after the involvement of the face, scapular, numeral and trunk muscles”.

In addition, we cannot ignore the publications of many authors who described the FSHD gradually descending type (i.e. the FSLD1). Furthermore, it’s necessary to remark that the famous discussion between Erb and Landouzy-Dejerine dealt with the priority of recognition (and description) of the FSHD as a descending type with a “jump” (i.e. the FSLD2); however both had to admit the priority of Duchenne in describing FSHD as a gradually descending type (i.e. the FSLD1) (7).

Therefore, because both FSLD1 and FSLD2 are diseases clinically and historically well documented, FSLD (or FSHD) must be considered a disease not only genetically but also clinically heterogeneous.

The FSLD2 descending type, with a “jump” and an initial FSP phenotype may develop as FSHD1 or FSHD2 clinical phenotypes. As both forms are linked to chromosome 4q35, what is the FSLD1 gradually descending type, with initial FSH phenotype?

In our opinion FSLD1 occurs very rarely and is limited to definite geographical areas. Two hypotheses can be advanced: 1. FSLD1 and FSLD2 recognize a same gene mutation but present a different phenotype, under the action of different modifier genes; 2. FSLD1 has a different gene defect, not linked to chromosome 4q35.

Valery Kazakov, Dmitry Rudenko, Vladislav Kolynin and Tima Stuchevskaya
Department of Neurology, Pavlov State Medical University St. Petersburg, Russia
References


PROCEEDINGS OF LGMD DAYS MEETING:
Prognosis and Treatment in LGMD

October 15-17, 2014

IRCCS “S. Camillo”,
Lido di Venezia (VE), Italy
LGMD DAYS

PROGNOSIS AND TREATMENT IN LGMD

15-17 OTTOBRE 2014

IRCCS «S. Camillo»
Via Alberoni 70, Lido di Venezia VE

www.beta-sarcoglicanopatie.it
WEDNESDAY OCTOBER 15th

9.15  Registration of participants
10.00  Annalena Venneri  
   Welcome greetings

Chairman: Francesco Piccione (Venice)

SESSION I - Introduction
10.15  Luisa Politano (Naples)  
   Genetics and cardiology of LGMD
10.40  Jordi Diaz Manera (Barcelona)  
   LGMD subtypes: MRI imaging
11.05  Corrado Angelini (Venice), Elisabetta Tasca (Venice), Marina Fanin (Padua)  
   Fatigue, gender and NOS in dominant and sporadic LGMD
11.30  Pascal Laforet (Paris)  
   Frequency and natural History of Sarcoglycanopathies: the experience of Paris-Est Neuromuscular Center
11.55  Discussion
12.20  Lunch

SESSION II - From diagnosis to patient care
14.00  M Savarese  
   Diagnosis by muscle chip
14.25  Francesca Bevilacqua (Venice), Rita Lorio (Venice)  
   Neuropsychological tests and QoL
14.50  Andrea Vianello (Padua)  
   Ventilation: home and hospital care
15.15  Paola Cudia (Venice), Antonio Merico (Venice)  
   Neuorehabilitation in LGMD
15.40  Tiziana Tosò (Padua)  
   Nutrition guidelines
16.05  Beatrice Vola (Sondrio)  
   The contribution of association
16.30  Discussion

THURSDAY OCTOBER 16th

9.15  Registration of participants
10.00  Paola Facchin (Padua)
10.10  Massimo Mirandola (Padua)
10.20  Luigi Querini (Padua)  
   Welcome greetings

Chairmen: Carlo Pietro Trevisan (Padova), Maurizio Moggio (Milano), Gabriele Siciliano (Pisa)

SESSION I - Therapy and natural History outcome in LGMD
10.30  Dorianna Sandonà (Padua)  
   Small molecule-based therapy for sarcoglycanopathy, a novel perspective.
10.55  Lucia Morandi (Milano)  
   Clinical features in LGMD
11.20  Elisabetta Gazzzerro (Genova), Claudio Bruno (Genova)  
   Improvement of molecular dystrophic process in alpha-sarcoglycan KO mice by blockade of extracellular ATP/P2P axis
11.45  Marija Meznaric (Ljubljana Slovenia)  
   Histopathology of cardiomyopathy in a patient with α-sarcoglycanopathy
12.10  Claudio Semplicini (Padua)  
   Follow-up and clinical study in Beta-sarcoglycans
12.35  Gabriele Siciliano (Pisa)  
   Muscle exercise evaluation in LGMD
13.00  Discussion
13.25  Lunch
15.00  Giacomo Comi (Milan)  
   Registry of LGMD
15.25  Paola Melacini (Padua)  
   Heart treatment in LGMD
15.40  C Borsato  
   LGMD: functional/electrophysiological outcomes
16.05  Corrado Angelini (Venice)  
   Discussion and closing remarks
16.30  Meeting GFB ONLUS and patient families

FRIDAY OCTOBER 17th

9.30  Vincenzo Nigro (Naples)  
   NGS and LGMD
10.00  Laura Drigo (Padua)  
   Discussion by groups of Horizon 2020 Projects
Thursday, 15 October
1st Session h. 10.15-11.55
Chairman: F. Piccione (Venice)

Genetics Aspects and Heart Involvement in LGMDs

L. Politano
Department of Experimental Medicine, Cardiomyology and Medical Genetics. Second Napoli University, Italy

Limb-girdle muscular dystrophies (LGMDs) represent a heterogeneous group of genetic rare disorders characterized by progressive deterioration and weakness of proximal limb muscles. A high variability in clinical course and phenotype has been observed, ranging from severe forms with rapid onset and progression, to very mild forms allowing affected people to have fairly normal life expectancy and daily activities.

The possibility to benefit of techniques of new generation, has dramatically improved the power of diagnosing these diseases, so that the number of disorders now included in the group of LGMD is almost doubled compared to just 10 years ago. So far, 31 LGMD loci have been identified, 8 autosomal dominant and 23 autosomal recessive. The dominant forms (LGMD 1A-1H) are generally milder and relatively rare, accounting for less than 10% of all Limb Girdle Muscular Dystrophies. The recessive forms (LGMD2A-2W) are more frequent, having a cumulative prevalence of 1:15,000, save some geographical differences. Among them, LGMD2A (calpainopathy) is the most frequent form observed in the Italian population, followed by LGMD2B (dysferlinopathy) and LGMD2C-2F (sarcoglycanopathies).

Differently from other forms of muscular dystrophy (dystrophinopathies, myotonic dystrophies, nuclear envelop cardiomyopathies etc.), in which cardiac involvement is a peculiar feature of the disease, myocardium is spared in the majority of LGMDs with the exception of particular subtypes. In these forms, cardiac abnormalities can range from conduction tissue defects (e.g., atrial fibrillations, flutters, atrio-ventricular blocks, supra-ventricular or ventricular ectopic beats, ventricular tachycardia) as is more frequently observed in LGMD1B, LGMD2M and LGMD2R to dilated cardiomyopathy characteristic of LGMD2C, LGMD2F and LGMD2I. Cardiac problems may precede, overlap with or follow skeletal muscle weakness. Therefore it is important to include cardiological evaluation in the regular follow-up of these patients, to adopt appropriate treatment when necessary.

Muscle MRI studies in patients with sarcoglycanopathies

J. Díaz-Manera1 2
1 Neuromuscular disorders Unit. Neurology department, Hospital de la Santa Creu I Sant Pau, Barcelona; 2 Centro de Investigación en Red en Enfermedades Raras (CIBERER), Valencia, Spain

Muscle MRI is becoming an important tool in the diagnosis and follow-up of patients with muscle dystrophies. Very good quality images from whole body can be obtained using 1.5 Teslas MRI devices that are available in many centers. There are at present several softwares that allow us to study different characteristics of the muscles such as fatty infiltration or edema. Muscle MRI can be used in daily clinics to select damages areas of the muscle increasing the performance of the muscle biopsy to obtaining a diagnosis. In the last 20 years many studies have been published analyzing the different patterns of muscle atrophy that are characteristic of every muscle disease. Although there are not pathognomonic patterns, muscle MRI is in general useful to suggest a diagnosis. Muscle MRI is useful also to follow-up patients over time because it does not irradiate patients and can be repeated safely every year. For this reason, MRI is becoming an interesting tool in natural history studies or in clinical trials.

In the case of sarcoglycanopathies there is not a clear pattern published yet but in my personal experience the patients have fatty infiltration involving deltoids, biceps and periscapular muscles in the upper limbs. Paraspinal and abdominal muscles are severely involved as well as pelvic muscles psoas and the glutei muscles. In the lower limbs there is a severe involvement of the quadriceps, semimembranosus, semitendinosus and biceps. In general, the muscles of the legs are not involved. This pattern is very characteristic and is not commonly found in other muscle dystrophies allowing to an easy detection when is found in patients with an undiagnosed muscle dystrophy. However a similar pattern can be found in patients with an adult onset Pompe disease, that have however a different clinical history and muscle biopsy. In conclusion, muscle MRI is a interesting tool that can be useful for the diagnosis and follow-up of patients with a muscle dystrophy produced by mutation in the sarcoglycan genes, but further studies are needed to know which is the pattern of muscle involvement of the patients.
Muscle fatigue, nNOS and muscle fiber atrophy in LIMB girdle muscular dystrophy

C. Angelini, E. Tasca, A.C. Nascimbeni, M. Fanin
1 Department of Neurosciences, University of Padova; 2 IRCCS Fondazione “San Camillo” Hospital, Lido di Venezia, Italy

Muscle fatigability and atrophy are frequent clinical signs in limb girdle muscular dystrophy (LGMD), but their pathogenetic mechanisms are still poorly understood.

We review a series of different factors that may be considered in causing fatigue and atrophy, particularly considering the role of neuronal nitric oxide synthase (nNOS) and additional factors such as gender in different forms of LGMD (both recessive and dominant) underlying different pathogenetic mechanisms.

In sarcoglycanopathies, the sarcolemmal nNOS reactivity varied from absent to reduced, depending on the residual level of sarcoglycan complex: in cases with complete sarcoglycan complex deficiency (mostly in beta-sarcoglycanopathy), the sarcolemmal nNOS reaction was absent and it was always associated with early severe clinical phenotype and cardiomyopathy.

Calpainopathy, dysferlinopathy, and cavelinopatia present gradual onset of fatigability and had normal sarcolemmal nNOS reactivity. Notably, as compared with caveolinopathy and sarcoglycanopathies, calpainopathy and dysferlinopathy showed a higher degree of muscle fiber atrophy.

Males with calpainopathy and dysferlinopathy showed significantly higher fiber atrophy than control males, whereas female patients have similar values than female controls, suggesting a gender difference in muscle fiber atrophy with a relative protection in females. In female patients, the smaller initial muscle fiber size associated to endocrine factors and less physical effort might attenuate gender-specific muscle loss and atrophy.

Frequency and natural history of sarcoglycanopathies: the experience of Paris-Est neuromuscular center

Centre de Référence de Pathologie Neuromusculaire Paris-Est, Institut de Myologie, GH Pitié-Salpêtrière, APHP, Paris; Laboratoire de Biochimie et Génétique Moléculaire, Groupe Hospitalier Cochin, APHP, Paris, France

Based on molecular and genetic criteria, sarcoglycanopathies are classified as LGMD2D (α-SG), LGMD2E (β-SG), LGMD2C (γ-SG), LGMD2F (δ-SG). Sarcoglycans are tightly bound to each other so that mutation in one normally results in partial or total deficiency of all of them. Alpha and gamma SGs are specific to skeletal muscle and heart, while beta-SG is expressed in multiple tissues, although expression is prominent in skeletal and cardiac muscle. Few epidemiological data is available concerning sarcoglycanopathies, prevalence based on biopsy and genetic analyses was estimated to be 5.6 x 10^-6 inhabitants.

We performed a retrospective study of all patients with sarcoglycanopathies followed in our center, in a cohort of 63 patients; 39 women, 24 men (32α-SG; 4 β-SG; 27 γ-SG; none δ-SG). Mean age of disease onset was 6.8 years old (6.9 y. for α-SG; 7.2 y. for β-SG; and 6.6 y. for γ-SG). Among all patients, 69.8% were wheelchair-bounded at last evaluation. Considering severity of each form, 50% of β-SG patients were wheelchairbound, while 68.75% of α-SG and 74.07% of γ-SG were in the same condition. Moreover, ambulation at 18 years old was possible in 100% of β-SG patients, but only 40.6% of α-SG and 48.14% of γ-SG were still ambulatory at same age.

Concerning systemic involvement, cardiomyopathy was observed in 24.07% of all patients (in 50% of β-SG cases, 39.13% of γ-SG cases, and in only 7.69% of α-SG cases). On the other hand, respiratory involvement was present in 37.7% of patients and a different distribution was observed among each subtype. None of β-SG patients was on non-invasive ventilation, but 26.08% of γ-SG and 52% of α-SG patients needed non-invasive ventilation (1 additional γ-SG had tracheotomy). Four patients with γ-SG died (2 men; 2 women, mean age 30 years old).

Genetic study showed that in α-SG, heterozygous R77C mutation was the most frequent mutation (9/32 cases). Regarding γ-SG we found the following mutations: homozygous del521T (13 patients), homozygous del525T (9 patients), heterozygous del521T (1 patient) and heterozygous del525T (2 patients). Each αβ-SG patient presented a distinct mutations being impossible to establish a more frequent mutation.

Our results point to a higher frequency of γ-SG, followed by α-SG and lastly by β-SG. Sarcoglycanopathy seems to be a severe form of muscular dystrophy with almost 70% of patients being wheelchair-bounded at long-term evaluation. Among these non ambulatory patients, 75% have lost ambulation before 18 years old and could be considered a “Duchenne-like” form. The remaining 25% of patients could then be considered a “Becker-like” form. In this large series of patients with sarcoglycanopathies, clinical and genetic data collected may enable search for correlations between mutations type, protein deficiency and clinical severity.
Large screening of a NMD cohort of patients by MotorPlex, an innovative strategy of targeted resequencing

M. Savarese1, G. Di Frusco1, A. Torella2, A. Garofalo1, T. Giugliano1, C. Fiorillo, G. Tasca1, C. Pisano2, F. Del Vecchio Blanco2, G. Piluso2, O. Musumeci2, M. Mora2, L. Morandi4, E. Ricci4, T. Mongini2, L. Santoro4, L. Politano9, C. Angelini10, G.P. Comi11,12, C. Bruno3,13, V. Nigro1,2

1 Telethon Institute of Genetics and Medicine (TIGEM), Napoli, Italy; 2 Dipartimento di Patologia Generale, Seconda Università di Napoli, Napoli, Italy; 3 UOC Neurologia Pediatrica e Malattie Muscolari, IRCCS Istituto “Giannina Gaslini”, Genova, Italy; 4 Istituto di Neurologia, Università Cattolica del Sacro Cuore, Roma, Italy; 5 Dipartimento di Neuroscienze, Università di Messina, Italy; 6 Dipartimento di Neuroscienze, Istituto Besta, Milano, Italy; 7 S.S. Malattie Neuromuscolari, Università di Torino, Italy; 8 Dipartimento di Neuroscienze e Scienze riproduttive ed odontostomatologiche, Università di Napoli “Federico II”, Napoli, Italy; 9 Servizio di Cardiologia e Genetica Medica, Seconda Università degli Studi di Napoli, Italy; 10 Dipartimento di Neuroscienze, Università di Padova, Italy; 11 Dipartimento di Fisiopatologia Medico-Chirurgica e dei Trapianti, Università Milano, Italy; 12 Italian Network for LGMD; 13 Italian Network for Congenital Myopathies

The identification of causative mutations in neuromuscular disorders is a crucial issue for the future possibility of a differentiated treatment on a genetic basis. However, the large genetic heterogeneity of neuromuscular disorders and the phenotypic overlap between the different forms hamper a rapid and cheap diagnosis, based on a gene-by-gene approach. In addition, interfamilial and intrafamilial phenotypic variability of patients sharing the same pathogenic variants suggests that additional modifier genes may be involved.

In the last few years, the Next-generation sequencing (NGS) approaches have received unlimited consideration as universal test for almost all Mendelian conditions. In particular, NGS has demonstrated to be an excellent and cost-effective solution in highly heterogeneous diseases.

We have developed a NGS-based platform, named MotorPlex, to test 93 disease genes causing a muscular phenotype.

We have analyzed 391 patients with a clinical diagnosis of neuromuscular disorders. The patients were classified, according to their clinical phenotype, as being affected by Limb girdle muscular dystrophies (48.1%), by congenital myopathies (36.1%) or by other clinical conditions (15.8%), comprising, among others, distal myopathy (17 cases), isolated hyperCKemia (16 cases) and metabolic myopathy (7 cases). The molecular diagnosis has been found in 158 cases (40.4%), while 131 cases were judged incomplete, because these samples showed a single heterozygous variant in recessive genes fitting with the phenotype or with variations of likely pathogenicity in genes unrelated to the observed clinical condition.

In conclusion, MotorPlex is an ideal first tier test: it is cost-effective and thus applicable to a large number of patients and/or unaffected individuals; it is easily reproducible; it has high values of specificity and sensitivity; it includes all relevant NMD genes; and it is easily upgradable with novel discovered genes.

Respiratory assessment in children and adults with neuromuscular disorders (NMD)

F. Rao
Centro Clinico NeMO, Fondazione Serena onlus, Ospedale Niguarda Ca’ Granda, Milano

Neuromuscular diseases (NMD) in childhood are the most frequent causes of chronic respiratory failure (CRF), which requires the use of home mechanical ventilation (HMV). The respiratory care of the child and adults with NMD represents a major challenge for professionals involved and for the family. The key points in the treatment of the patient with NMD are the introduction and the monitoring of non invasive ventilation (NIV) and the management of airway secretions. A good care of these aspects allows a better control of the disease and the prevention of respiratory exacerbations which, if not recognized and promptly treated, weigh heavily on the clinical course of these illnesses. In this paper we examine every single aspect of the diagnostic respiratory functional assessment and its programs of periodic monitoring, the techniques of airway clearance required and their management, aiming to an integrated program involving all the professionals that work with the patient, starting from the physical therapist, in collaboration with the patient and family. First we considered the indications of NIV, its modalities and some criticisms such as the choice of masks, the management of leaks and the prevention of skin lesions associated to the use of interfaces. Another important feature of neuromuscular patients is represented by airway obstruction by secretions: we examined the standard of evaluation of ineffective cough and the ways of its improvement. Lastly, we stressed the importance of multidisciplinary approach to the neuromuscular patient during the evolution of the disease.
**Nutrition Guidelines**

T. Toso  
*Ambulatorio Nutrizionale, Padova*

The clinical heterogeneity of the various forms of LGMDs identified until today makes the outline of precise nutritional guidelines highly complex. This is especially true when the guidelines have to be associated with the variability of the clinical course, especially with regards to the level of physical activity and the presence/absence of respiratory and cardiac complications. Moreover, the different proteins involved in the various patterns of LGMDs are responsible for different pathogenetic mechanisms, to which can correspond specific deficiency and/or needs of micro and macronutrients.

It would be desirable, since the diagnosis, a constant monitoring of the weight, with the aim of avoiding overweight and obesity, which could negatively influence the motor autonomy of the subjects affected by the limb-girdle muscular dystrophy.

The acquisition of the sole body weight, which is a composit data, is not sufficient in order to identify the variances in the body composition, whose evaluation should be granted by impedenziometry and anthropometry techniques, with particular attention to the lean body mass and its preservation.

In the absence of specific guidelines for the LGMDs, general guidelines must be adopted. More specifically, the recommendations which incessingly come form the scientific research cannot be ignored, especially those which suggest to base the feeding mainly on food coming from vegetable sources (Healthy Eating Plate – Harvard Medical School 2011; World Cancer Research Found & American Institut for Cancer Research 2007).

In this context the abundance of antioxidants, which is a typical feature of the vegetable food, could be useful not only in the reduction of the oxidative stress, but also by playing a preventive role with respect to the progression of the disease.

Recent in vitro studies and of muscular biopsies, in fact, have showed an increase of the oxidative stress in the deficit of calpain 3 and dysferlinopathies, which can consequently be considered an important factor in the evolution of the muscular damage.

---

**Beta-sarcoglycanopathy: any longer an “orphan” disease?**

B. Vola, P. Bonetti  
*Family Group of Betasarcoglycanopathy ONlus (Gfb Onlus) and Italian Union Against Muscular Dystrophy (UILDM), Talamona, Italy; 1 Fondazione Istituto Italiano di Tecnologia IIT, Center for Genomic Science, Milan, Italy*

Limb girdle muscular dystrophy (LGMD) is a group of rare genetic diseases, including 31 different forms, inherited both in an autosomal dominant and recessive manner, and clinically characterized by a progressive involvement of limb musculature, proximal more than distal.

LGMD2E, also known as beta-sarcoglycanopathy, is an autosomal recessive dystrophy caused by mutations in the gene encoding beta-sarcoglycan, a cell plasma membrane that forms a tetrameric complex with other three types of sarcoglycans (alfa, gamma, delta).

Thanks to their genetic structure, formed by a few exons, sarcoglycanopathies in general may be suitable for the adenovirus-based gene therapy, and in fact a phase II clinical trial gene therapy for alfa -sarcoglycanopathy is on-going in the USA.

In 2013 the volunteer organization *Family Group of Beta-sarcoglycanopathy ONLUS (GFB ONLUS www.lgmd2e.org)* was founded aimed at: a) contacting the highest number of patients affected by LGMD and their families; b) collecting data and informations available on LGMD2E; c) stimulating both basic and clinical research. In the last two years the *GFB ONLUS* has involved 64 patients affected by all types of sarcoglycanopathies: 21 LGMD2E, 30 LGMD2D, 10 LGMD2C and 3 by a not well defined Sarcoglycanopathy.

Family Group of Beta-sarcoglycanopathy ONLUS is not a unique association; other six associations specifically dealing with other types of LGMDs are present: Coalition to care Calpain 3 (LGMD2A in the US), the Jain Foundation (LGMD2B in the US), Kurt + Peter Foundation (LDMD2C in the US), LGMD2D Foundation (in the US), Stichting Spierkracht (in the Netherlands) and LGMD2I Fund (in the US). In February 2014 five of these associations formed the “Consortium of LGMD Family Foundations”.

Family Group of Beta-sarcoglycanopathy ONLUS is not a unique association; other six associations specifically dealing with other types of LGMDs are present: Coalition to care Calpain 3 (LGMD2A in the US), the Jain Foundation (LGMD2B in the US), Kurt + Peter Foundation (LDMD2C in the US), LGMD2D Foundation (in the US), Stichting Spierkracht (in the Netherlands) and LGMD2I Fund (in the US). In February 2014 five of these associations formed the “Consortium of LGMD Family Foundations”.

The GFB ONLUS also promotes scientific research on LGMD2E, by creating collaborations with researchers to organize both informative and scientific meetings and, eventually, supporting those who are interested in the study of this disease. In this light, GFB ONLUS organised on April 19th 2013 October in Milan the first scientific Meeting and on 15th-16th-17th October 2014 in Venice the second scientific meeting, which will allow a fruitful confrontation between national and international researches.
Small molecule-based therapy for sarcoglycanopathy, a novel perspective

E. Bianchini, R. Sacchetto¹, P. Volpe², D. Sandonà
Department of Biomedical Sciences, University of Padova; ¹Department of Comparative Biomedicine and Food Science, ²Institute of Neuroscience, Padova, National Research Council of Italy

Sarcoglycanopathy, the collective name of four types of Limb Girdle Muscular Dystrophy (LGMD 2C-2F), is a rare genetic disorder mainly affecting the proximal musculature. It is well known that defects in any one of the genes coding for α-, β-, δ- or γ-sarcoglycan (SG), the components of a complex essential for the sarcolemma integrity of striated muscles, lead to the sever reduction or even the complete loss of SG-complex. Most of the mutations associated to sarcoglycanopathy are missense mutations. We have proven that the primary event in these cases is the premature degradation of a folding-defective mutant and the secondary loss of the wild-type partners, operated by the Endoplasmic Reticulum-Associated Degradation system. Interestingly, the entire complex can be rescued at the proper cellular site by reducing the degradation rate of the mutated-SG, opening a new perspective for the therapy of this neglected disease.

We have designed two small molecule-based strategies aimed at either “save” mutants from degradation or “assist” mutants in the folding process.

The pharmacological inhibition of the E3 ligase HRD1, key element of the sarcoglycan degradative route, leads to the quantitative and functional rescue of the mutant both in a cell model and in primary myotubes derived from a patient suffering of LGMD2D.

Regarding the “protein assisting” strategy, we are testing several small molecules, known as protein-folding correctors, both in a cell model and in patient-derived primary myotubes. By helping α-SG mutants to reach a native/native-like conformation, these treatments preserve the mutant from degradation allowing the assembly into a functional complex that properly localizes at the plasma membrane.

Altogether our results constitute the proof of concept for the development of novel pharmacological therapies for sarcoglycanopathy.

DNAJB6 myopathy

M.B. Pasanisi, L. Maggi, S. Zanotti, S. Saredi, F. Salerno, L. Morandi, M. Mora
Neuromuscular Diseases and Neuroimmunology Unit, Fondazione IRCCS Istituto Neurologico “C. Besta”, Milano, Italy

DNAJB6 gene mutations cause an autosomal dominant limb-girdle muscular dystrophy (LGMD1E). The disease usually manifests in adulthood and may be associated with distal muscle weakness. Muscle pathology is characterized by rimmed vacuoles and myofibrillar abnormalities. DNAJB6 belongs to the J-proteins (Hsp40) family, a class of co-chaperones characterized by a J-domain, and participates in autophagic and proteosomal turnover of proteins. We present clinical, pathological and molecular findings in four unrelated patients. Two females manifested early onset (mean 8.5 years) with distal lower limb muscle weakness, followed several years later by proximal upper and lower limb involvement. Both patients became wheelchair bound (mean age 38.5 years). One of them developed respiratory failure and dysphagia after 50 years of age, now (at 55 years) she is on non-invasive ventilation and has a percutaneous endoscopic gastrostomy for nutrition. The other patient manifested a moderate respiratory involvement at the age of 40. A third female patient presented difficulty climbing stairs at 16 years of age, then she developed slowly progressive proximal weakness, with loss of ambulation after about 20 years from onset. Now, at 40 years, she refers occasional dysphagia, and her neurological evaluation shows both proximal and distal limb weakness. The fourth patient is a 56-year old male who began to complain of mild difficulty climbing stairs at the age of 36. At present he refers difficulty getting up from a low chair and climbing stairs, he has predominantly proximal lower limbs weakness. In all patients morphological evaluation showed some rimmed vacuoles with acid phosphatase positivity around them. Electromyographic study demonstrated a myopathic pattern along with spontaneous activity (fibrillation and positive sharp waves); CK values were normal or only slightly increased. In all patients muscle imaging, by CT or MRI, showed fatty infiltration of biceps femuris and adductor magnus with a relative preservation of gracilis in thighs, and involvement of medial gastrocnemius with preservation of soleus in legs, in agreement with findings reported by Sandell et al. Molecular analysis detected two novel DNAJB6 mutations, and two known mutations, one of them recently reported by Jonson et al.
P2X antagonist oxidase-ATP (oATP) treatment in alpha-sarcoglycan null mice

Department of Neuroscience, Istituto “G. Gaslini”, Genova, Italy; 1 Novartis IRB, Basel, CH; 2 IRB, Bellinzona, CH

Limb-girdle muscular dystrophy 2D (LGMD2D), caused by mutations in the gene encoding alpha-sarcoglycan (a-SG), is a rare disorder characterized by progressive weakness and degeneration of skeletal muscle. Pathological features of muscle biopsies from these patients show myofiber degeneration and necrosis, endomyosial fibrosis, and reactive inflammatory response. In this scenario, extracellular ATP (eATP), a molecule released from the cytosol of dying cells, contributes to the initial phase of the immune response and later to the amplification of the inflammasome reaction. Excessive eATP causes protracted P2X receptors activation with alteration in muscle intracellular calcium homeostasis as well as recruitment of inflammatory cells.

Intriguingly, a-SG binds eATP and displays an ecto-ATPase activity, thus controlling eATP concentration at the surface of cells expressing P2X receptors and attenuating the magnitude and/or the duration of eATP-induced signals.

In order to evaluate the role of eATP in the in vivo inflammatory response and progression of the degenerative process associated to a-SG deficiency, we analyzed the consequences of P2X7 pharmacological inhibition in a-sarcoglycan (Sgcα-null mice) muscle function and morphology and on molecular markers of innate and adaptive immune response.

For this purpose we treated a-SK null mice with peridate-oxidized ATP (oATP), a compound that irreversibly antagonizes P2X receptors and which has been shown to ameliorate the phenotype of animal models with different inflammatory diseases. We determined that pharmacological inhibition of P2X purinergic receptors improved muscular function and morphology in a-SG knock-out mice. The beneficial effect exerted by purinergic blockade was associated with a reduction of the number and area of the inflammatory infiltrates and to a decrease of muscle transcript levels of II1 and II6.

Histopathology of cardiomyopathy in a patient with α-sarcoglycanopathy

M. Meznaric, E. Kralj1, C. Angelini2, M. Fanin3
University of Ljubljana, Faculty of Medicine, Institute of Anatomy, Ljubljana, Slovenia; 1 University of Ljubljana, Faculty of Medicine, Institute of Forensic Medicine, Ljubljana, Slovenia; 2 Fondazione IRCCS “San Camillo” Hospital, Venezia, Italy; 3 University of Padova, Department of Neurosciences, Padova, Italy

The clinical phenotype of sarcoglycanopathies, caused by mutations in α-, β-, γ- or δ-sarcoglycan genes, is characterised by the limb-girdle distribution of muscle weakness of variable severity and may include also cardiomyopathy. Frequency of cardiomyopathy varies among individual subtypes and has rarely been reported in α-sarcoglycanopathy.

We report on autopsy heart examination of a 36-year old male patient, homozygous for α-sarcoglycanopathy gene mutation in exon 3 (c. 229C>T, p.Arg77Cys), who died suddenly. He had a Duchenne-like muscular dystrophy.

Characteristic was the outer – subepicardial localisation of focal lesions in the free left ventricle wall, most pronounced in the posterobasal segment of the left ventricle. Histopathological changes consisted of myocardial degeneration without inflammation, increased variability of cardiomyocytes diameters, fibrosis and fatty replacement of the myocardium. Dilated cardiomyopathy was not developed at the time of death which is in agreement with slow progression of cardiomyopathy in α-sarcoglycanopathy.

Follow-up and clinical study in Beta-sarcoglycans

C. Semplicini
University of Padova, Department of Neurosciences, Padova, Italy

Not arrived

Limb girdle muscle dystrophies and exercise

G. Siciliano, C. Simoncini, S. Giannotti1, G. Ricci
Department of Clinical and Experimental Medicine, Neurological Clinic; 1 Orthopaedic Clinic, University of Pisa, Italy

In limb-girdle muscular dystrophies (LGMD) different genetic mutations are responsible, by distinct pathogenic mechanisms, of muscle fibers degeneration and strength loss. Depending on that, exercise tolerance is affected in patients with LGMD, either as a direct consequence of the loss of muscle fibers or secondary to the sedentary lifestyle due to the motor impairment. It has been debated for many years whether or not muscle exercise is beneficial or harmful for patients with myopathic disorders. In fact, muscular exercise would be considered in helping to hamper the loss of muscle tissue and strength. On the other hand, muscle structural defects in LGMD can result in instability of the sarcolemma, making it more likely to induce muscle damage as a consequence of intense muscle contraction, such as that performed during eccentric training. Several reports have suggested that supervised aerobic exercise training is safe and may be considered effective in improving oxidative capacity and muscle function in patients with LGMD, such as LG-
MD2I, LGMD2L, LGMD2A. More or less comfortable investigation methods applied to assess muscle function and structure can be useful to detect the beneficial effects of supervised training in LGMD. However, it is important to note that the available trials assessing muscle exercise in patients with LGMD have often involved a small number of patients, with a wide clinical heterogeneity and a different experimental design. Based on these considerations, resistance training can be considered part of the rehabilitation program for patients with a limb-girdle type of muscular dystrophy, but it should be strictly supervised to assess its effects and prevent possible development of muscle damage.

4th Session h. 15.00-16.30

The National Registry of Limb Girdle Muscular Dystrophy: clinical and molecular characterization of a sample of 466 Italian patients

F. Magri, M. Moggio, N. Bresolin, M.G. D’Angelo¹, E. Pegoraro², C. Semplicini², C. Angelini², T. Mongini³, A. Toscano², O. Musumeci², G. Siciliano³, M. Mancuso³, G. Ricci³, G. Tomelleri⁴, M. Mora⁴, I. Moroni⁴,
L. Morandi⁵, V. Nigro⁶, G.P. Comi
IRCCS Ca’ Granda, University of Milano; ¹ IRCCS “E. Medea Bosio Parini”, LC; ² Dip. Neuroscienze, Università di Padova; ³ Dip. Neuroscienze, AOU “S. Giovanni Battista” di Torino; ⁴ Dip. Neuroscienze, Psichiatria e Anestesiologia, Messina; ⁵ Dip. Scienze Neurologiche, Università di Pisa; ⁶ Dip. Scienze Neurologiche, Verona; ⁷ IRCCS Istituto Neurologico “C. Besta”, Milano; ⁸ Telethon Institute of Genetics and Medicine (TIGEM), Napoli, Italy

Limb girdle muscular dystrophies (LGMD) are highly heterogeneous disorders characterized by predominant limb girdle weakness. Molecular analysis and clinical-genetic correlations are fundamental for genetic counselling, definition of natural history and insight into pathogenesis.

To verify the basic requirements for an national LGMD registry, we collected detailed clinical, biochemical, histological and molecular data of 466 Italian LGMD patients, belonging to 8 neuromuscular Italian centres, listed at the end of this summary.

Among them 309 patients are molecularly defined, 111 (24%) are still un-diagnosed and 46 (10%) carry heterogeneous mutations in genes determining autosomal recessive forms. Relative frequency was as follows: 5.5% LGMD1B, 11% LGMD1C, 25.2% LGMD2A, 27% LGMD2B, 9.2% LGMD2I, 9.1% LGMD2D, 6% LGMD2E, 4% LGMD2C, 2.1% LGMD2L, 0.3% LGMD2F, LGMD2R (0.3%) and LGMD2S (0.3%). Onset spans from the first decade to adulthood; LGMD2E being the most precious (6.2 ± 5.3 years) and LGMD2L the latest (36.6 ± 7.1 years). Creatine-kinase values were generally increased, especially in sarcoglycanopathies, LGMD2B, LGMD1C. Cardiomyopathy was more frequent in LGMD1B (100%), LGMD2E (47%) and LGMD2I (50%) and restrictive pulmonary involvement in LGMD2I (53%) and LGMD2E (47%). 30% of patients was wheelchair-bound.

Overall this study defined the relative frequency of Italian LGMD and improved the knowledge about clinical, morphological and molecular spectrum as far as their natural history. Furthermore the study of undiagnosed patients will potentially lead to identification of new LGMD causative genes.

LGMD: functional/electrophysiological outcomes

C. Borsato
Not arrived

Saturday, 17 October
4th Session h. 10.00-14.00
Chairman: C. Angelini (Padova)

NGS and LGMD

V. Nigro¹ ²
¹ Dipartimento di Patologia Generale, Seconda Università di Napoli, Italy; ² Telethon Institute of Genetics and Medicine (TIGEM), Napoli, Italy

See M. Savarese et al.

Discussion by groups of Horizon 2020 Projects

L. Drigo (Padua)

Discussion and closing remarks

C. Angelini (Padova)
AUTHOR INDEX

Angelini C., 159, 160, 163, 164
Assereto S., 163
Baldassari S., 163
Behin A., 159
Ben Yaou R., 159
Betto R., 162
Bianchini E., 162
Bonetti P., 161
Borsato C., 164
Bresolin N., 164
Bruno C., 160, 163
Comi G.P., 160, 164
D’Angelo M.G., 164
Del Vecchio Blanco F., 160
Di Fruscio G., 160
Díaz-Manera J., 158
Drigo L., 164
Eymard B., 159
Fanin M., 159, 163
Fiorillo C., 160, 163
Garofalo A., 160
Gazzotto E., 163
Giannotti S., 163
Giugliano T., 160
Grassi F., 163
Guimaraes-Costa R., 159
Kralj E., 163
Laforêt P., 159
Leturcq F., 159
Maggi L., 162
Magri F., 164
Mancuso M., 164
Meznaric M., 163
Minetti C., 163
Moggio M., 164
Mongini T., 160, 164

Mora M., 160, 162, 164
Morandi L., 160, 162, 164
Moroni L., 164
Musumeci O., 160, 164
Nascimbeni A.C., 159
Nigro V., 160, 164
Panicucci C., 163
Pasanisi M.B., 162
Pegoraro E., 164
Piluso G., 160
Pisano C., 160
Politano L., 158, 160
Rao F., 160
Ricci E., 160
Ricci G., 163, 164
Sacchetto R., 162
Salerno F., 162
Sandonà D., 162
Santoro L., 160
Saredi S., 162
Savarese M., 160
Semplicini C., 163, 164
Siciliano G., 163, 164
Simoncini C., 163
Stojkovic T., 159
Tasca E., 159
Tasca G., 160
Tomelleri G., 164
Torella A., 160
Toscano A., 164
Toso T., 161
Traggiai E., 163
Vola B., 161
Volpe P., 162
Zanotti S., 162
Erich Alfred Kuhn, former Director of the Policlinic for Internal Medicine in Heidelberg and erstwhile champion of German myology, passed peacefully away in his home on 8 October 2014. He was born on 23 November 1920 in the little Thuringian village of Oberrod close to the border to Bavaria and visited high school in the nearby town of Schleusingen. After passing his A-levels he was recruited to the then obligatory “imperial work duty”. After the start of World War II in 1939 he was drafted to the German Army, but at the same time was allowed to study medicine, first at Würzburg, then at Greifswald, where he passed his bachelor’s examination. Shortly after, he was promoted to the rank of a “junior medical officer” (Unterarzt) because doctors where needed on Germany’s Russian front. He was even able to complete his medical studies at Jena while still in the army. Nevertheless, after the war, he preferred to sit the official German state exam in medicine at Heidelberg.

In Heidelberg he was attracted to the Medical Policlinic because this hospital had by far the greatest number of outpatients. Here, he served on all the wards and his remarkable memory for the many and varied cases he saw made him an excellent medical all-rounder. In fact, he liked the atmosphere at this hospital so much that he stayed there to the end of his career, going through all stages from assistant, to consultant, and on to become its Medical Director. Scientifically, he focussed on his main interest in myotonic diseases which he had first encountered at Jena. Thus, in 1959 he devoted his “Habilitationsschrift” (the inaugural thesis required for entry to German-speaking academia), to myotonic dystrophy (published in updated form two years later, Ref. 1). In recruiting many families and recording all clinical details he felt inspired by the spirit of Heidelberg’s great myologist Wilhelm Erb who had published the first monograph on myotonia (2). “Wilhelm Erb (d. 29 Oct 1921 at the age of 81) could not close his eyes and rest until I was born.” Kuhn would banter.

One of Kuhn’s many achievements that benefitted muscle patients was the foundation in 1965 – together with six additional parents and physicians – of what is now the Deutsche Gesellschaft für Muskelkranke (German Muscular Disease Association, DGM). For several years he served on the board of governors and was president of the scientific council of this self-help organisation.

Kuhn was concerned not only about the muscle patients, but also their doctors. Even as late as 1965 Germany was still suffering from the bad reputation and the isolation that the atrocities of the Nazis had brought about. Kuhn found a remedy: Invite the muscle celebrities of the world to Germany! Actually an international conference on neuromuscular disorders had so far never been held. Kuhn was bold enough to organise such a conference – all by himself – at his home town of Heidelberg, and about 150 of the celebrities came. The usefulness of such a meeting was then so evident, that the influential myologists decided during the meeting that the World Federation of Neurologists (WFN) should henceforth organise similar conferences at regular time intervals. The
resulting series of International Conferences on Neuromuscular Disorders (ICNMD) numbers 13 to date. Kuhn was very proud to have given the impulse to this series and called his Heidelberg meeting jokingly: ICNMD-zero. The scientific proceedings of ICNMD-0 was published a year later by Springer (3).

Kuhn attended many of these international conferences, and when the younger generation of German myologists had acquired enough self-assuredness to apply for the organisation of ICNMD-VII in Munich, it was he who successfully defended the bid at ICNMD-VI in Los Angeles 1986. In the following years he attended the preparation of this conference with great enthusiasm.

After Kuhn had retired from his post at the Heidelberg Policlinic in 1986 he performed the Herculean task on behalf of his DGM by stipulating the foundation of Muscle Centres at the Medical Faculties of nearly all German Universities. Using his qualities as an elder statesman as well as a strong-headed Thuringian farm boy, he managed to get the existing specialist doctors in the various faculties to collaborate for the benefit of the neglected muscle patients. A yearly updated report contains the progress of the now 26 centres (4). It goes without saying that one of the centres, at Ulm, has its emphasis on ion channel diseases, the category that contains “his” myotonias and periodic paralyses (5). For that missionary achievement, the DGM bestowed on Kuhn an honorary lifetime membership.

As a doctor, Erich Kuhn was very much liked by his patients because he was open, honest and never patronising. He had many scholars and students who, amongst themselves, liked to call him “Vater Kuhn” because he cared for their progress like a real father. For more than 60 years Kuhn was married to his wife, Agnes, who bore him three daughters and a son.

Reinhardt Rüdel
Em. Professor für Allg. Physiologie,
Loschweg 16, D-89081 Ulm, Germany
E-mail: reinhardt.ruedel@uni-ulm.de

References
**NEWS FROM AROUND THE WORLD**

**MSM**

The 12th Congress of the Mediterranean Society of Myology will be held in Naples, Italy on May 2015, from 18th to 20th. The congress will be chaired by Prof. Giovanni Nigro, President of the Mediterranean Society of Myology. The Scientific Committee, is chaired by Prof. Giovanni Nigro, cooperated by the Board of the Society: Lefkos Middleton, London; George Serratrice (Marseille); Yeuda Shapira, Jerusalem; Luisa Politano, Naples; Ekram Abdel-Salam, Cairo; Marinos Dalakas, Athens; Fayçal Hentati, Tunis; Giovanni Meola, Milan; Gabriele Siciliano, Pisa; Eduardo Tizzano-Ferrari, Barcelona; Antonio Toscano, Messina; Janez Zidar, Ljubliana and by Vincenzo Nigro, Naples; Giuseppe Novelli, Rome; Reinhardt Rüdel, Ulm.

The symposium will be in the traditional two-days MSM format with 6 selected topics:

- Spinal Muscular Atrophies
- Nuclear Envelop Diseases
- Heart involvement in NeuroMuscular Disorders
- Inflammatory Myopathies
- Next Generation Sequence and NeuroMuscular Disorders
- New therapeutic approach in NeuroMuscular Disorders

Further information is available in the website of the Organizing Secretariat www.fclassevents.com

**GCA**

During the Gala dinner of the 12th Congress of the Mediterranean Society of Myology to be held in Naples, Italy on May 2015, the 2015 Gaetano Conte Prizes will be assigned for both basic research and clinical research.

**AIM**

The 15th Congress of the Italian Association of Myology will be held 8 in Naples, Italy on May 2015, from 18th to 20th, closely linked to the Congress of the Mediterranean Society of Myology. The Scientific Committee is chaired by Prof. Giovanni Nigro, cooperated by the Board of the Society: Maurizio Moggio, Milan; Antonio Toscano, Messina; Claudio Bruno, Genua; Paola Tonin, Verona; Angela Berardinelli, Pavia; Massimiliano Filosto, Brescia; Giovanni Marrosu, Cagliari; Lucia Ovidia Morandi, Milano; Elena Pegoraro, Padua; Gabriele Siciliano, Pisa and by Luisa Politano and Vincenzo Nigro, Naples.

The local Organizing Committee is chaired by Luisa Politano and Vincenzo Nigro with the cooperation of Liberato Berrino, Gerardo Nigro, Orlando Paciello, Alberto Palladino, Luigia Passamano, Raffaele Russo, Cira Solimene, Paola D’Ambrosio, Roberta Petillo, Esther Picillo, Antonella Taglia and Emanuela Viggiano.

The symposium will be in the traditional three-days AIM format with the following selected topics:

- Laminopathies: a clinical-molecular update
- Spinal Muscular Atrophies
- LGMD: Update on the new phenotypes
- Rehabilitative aspects in MD
- Advances in the treatment of MD

Further information will be available in the website of the Society www.miologia.org and of the Organizing Secretariat www.fclassevents.com

**LGMD-EuroNet**

During the conference LGMD DAYS held at the IRCCS “S. Camillo” in Lido di Venezia (VE). From 15th to 17th October 2014, which was attended by a number of researchers from France, Italy, Slovenia and Spain, was established LGMD EuroNET having the following purposes: 1) To develop the aspects of scientific and clinical research on the Limb-girdle muscular dystrophies, especially on Sarcoglycanopathies and 2) to participate in calls for Horizon 2020 with a project of LGMD EuroNET.

Prof. Corrado Angelini was appointed as Coordinator of the network. The association GFB ONLUS will act as the organizing secretary. Next meeting of the LGMD EuroNET is planned to be in Naples, May 22nd, during the Congress of the Mediterranean Society of Myology and the Italian Association of Myology. Further information will be available on the website www.lgmd2e.org

**WMS**

The 20th International WMS Congress will be held in Brighton, UK from 30th September to 4th October, 2015. The Congress will be held in the traditional WMS format with three selected topics. One day of the symposium will be dedicated to each of the selected topics addressing emerging discoveries in the field of:

- Muscle metabolism in health and disease
- Immune mediated Peripheral Nerve, Neuromuscular Junction, and Muscle Disorders
- Advances in the treatment of Neuromuscular Disorders

Abstracts will also be welcome on advances across the neuromuscular field. Further information is available in the website of the Society www.wms2015.com
FORTHCOMING MEETINGS

2015

January 15-16
The 1st French-Italian Meeting on Laminopathies. Marseille, France.

March 26-28

April 16-18

May 5-9

May 18-20
12th Mediterranean Society of Myology Congress. Naples, Italy. Information: giovanni.nigro@unina2.it; luisa.politano@unina2.it; vincenzo.nigro@unina2.it

May 21-23
15th Congress of the Italian Society of Myology. Naples, Italy. Information: giovanni.nigro@unina2.it; luisa.politano@unina2.it; vincenzo.nigro@unina2.it

June 6-9
The European Human Genetics Conference. Glasgow, United Kingdom. Information: website: www.esgh.org

June 8-12
IDMC10 – International Myotonic Dystrophy Consortium Meeting. Paris, France. Information: website: www.idmc10.org; E-mail: contact@idmc10.org

September 30 – October 4

October 6-10

2016

March 17-20
The 10th World Congress on CONTROVERSIES IN NEUROLOGY. Lisbon, Portugal. Information: website: www.comtecmed.com/cony

April 3-7

September 4-9

October 20-24

October (to be announced)

2017

October 17-21

2018

October 16-20
ASHG Annual Meeting. San Diego, CA, USA. Information: website: www.ashg.org

2019

October 22-26

2020

October 27-31
ASHG Annual Meeting. San Diego, CA, USA. Information: website: www.ashg.org
VOLUME XXXIII - CONTENTS

Issue N. 1 • May 2014

INVITED REVIEW
Genetic basis of limb-girdle muscular dystrophies: the 2014 update
Vincenzo Nigro and Marco Savarese

ORIGINAL ARTICLE
Evaluation of neural damage in Duchenne muscular dystrophy patients
Ekram Abdel Salam, Iman Ehsan Abdel-Meguid, Rania Shatla and Soheir Korraa

CASE REPORT
Limb girdle muscular dystrophy type 2L presenting as necrotizing myopathy
Ilka Schneider, Gisela Stoltenburg, Marcus Deschauer, Martin Winterholler and Frank Hanisch

2013 GAETANO CONTE PRIZE LECTURE
A gating model for wildtype and R1448H Nav1.4 channels in paramyotonia
Boris Holzherr, Frank Lehmann-Horn, Elza Kuzmenkina, Chunxiang Fan and Karin Jurkat-Rott

MEMORIES BY A MYOLOGIST
Vladimir Karlovich Roth (1848-1916): the founder of neuromuscular diseases studies in Russia
Valery M. Kazakov, Dmitry I. Rudenko and Tima R. Stuchevskaya

PROCEEDINGS OF THE XIV MEETING OF THE ITALIAN ASSOCIATION OF MYOLOGY SIRMIONE (BS), ITALY – MAY 8-10, 2014
Program
Abstracts (in alphabetical order of the 1st Author)
Author index

NEWS FROM AROUND THE WORLD
MSM
GCA
AIM
WFN
WMS

FORTHCOMING MEETINGS

Issue N. 2 • October 2014

EDITORIAL
Myology: the passion of a lifetime
Giovanni Nigro

ORIGINAL ARTICLES
Mitochondrial disease heterogeneity: a prognostic challenge
Maurizio Moggio, Irene Colombo, Lorenz Peverelli, Luisa Villa, Rubjona Xhani, Silvia Testolin, Salvatore Di Mauro and Monica Sciaccio

Far field R-wave sensing in Myotonic Dystrophy type 1: right atrial appendage versus Bachmann’s bundle region lead placement
Vincenzo Russo, Gerardo Nigro, Andrea Antonio Papa, Anna Rago, Federica Di Meo, Anna Cristianio, Antonio Molino, Raffaele Calabrò, Maria Giovanna Russo and Luisa Politano

Sleep breathing disorders and nocturnal respiratory pattern in patients with Glycogenosis type II
Giuseppe Fiorentino, Anna Annunziata and Luisa Politano

CASE REPORT
Adenosine-induced sinus tachycardia in a patient with Myotonic Dystrophy type 1
Vincenzo Russo, Gerardo Nigro, Andrea Antonio Papa, Anna Rago, Nadia Della Cioppa, Anna Cristianio and Maria Giovanna Russo

2013 GAETANO CONTE PRIZE LECTURE
GNE myopathy: a personal trip from bedside observation to therapeutic trials
Zohar Argov

MEMORIES BY A MYOLOGIST
How we developed, at the Centre/Institute for Neuromuscular Diseases, differential diagnostics of Spinal Muscle Atrophies / Amyotrophic Lateral Sclerosis (SMA/ALS) and tried to influence the development of the disease
Anica Jušić
## NEWS FROM AROUND THE WORLD
- MSM 115
- GCA 115
- AIM 115
- WMS 115

## FORTHCOMING MEETINGS
116

Retraction statement *(for the article published in the May 2014 issue - Acta Myologica - 2014; XXXIII: 22-33)*

Instructions for Authors 118

### Issue N. 3 • December 2014

### INVITED REVIEW
Muscle fatigue, nNOS and muscle fiber atrophy in limb girdle muscular dystrophy
Corrado Angelini, Elisabetta Tasca, Anna Chiara Nascimbeni and Marina Fanin 119

### ORIGINAL ARTICLES
The effect of atrial preference pacing on atrial fibrillation electrophysiological substrate in Myotonic Dystrophy type 1 population
Vincenzo Russo, Gerardo Nigro, Federica Di Meo, Andrea Antonio Papa, Nadia Della Cioppa, Riccardo Proietti, Maria Giovanna Russo, Raffaele Calabrò and Luisa Politano 127

Psychological and practical difficulties among parents and healthy siblings of children with Duchenne vs. Becker muscular dystrophy: an Italian comparative study
Lorenza Magliano, Maria Grazia D’Angelo, Giuseppe Vita, Marika Pane, Adele D’Amico, Umberto Balottin, Corrado Angelini, Roberta Battini and Luisa Politano 136

Charcot-Marie-Tooth 4B2 caused by a novel mutation in the MTMR13/SBF2 gene in two related Portuguese families
Luís Negrão, Luciano Almendра, Joana Ribeiro, Anabela Matos, Argemiro Geraldo and Jorge Pinto-Basto 144

### CASE REPORT
Ventricular fibrillation induced by coagulating mode bipolar electrocautery during pacemaker implantation in Myotonic Dystrophy type 1 patient
Vincenzo Russo, Anna Rago, Federica Di Meo, Nadia Della Cioppa, Andrea Antonio Papa, Maria Giovanna Russo and Gerardo Nigro 149

### LETTER TO THE EDITOR
Facio-scapulo-humeral muscular dystrophy and its connection with facio-scapulo-peroneal muscular dystrophy 4q35-linked: some historical remarks
Valery Kazakov, Dmitry Rudenko, Vladislav Kolynin and Tima Stuchevskaya 152

### PROCEEDINGS OF LGMD DAYS MEETING: PROGNOSIS AND TREATMENT IN LGMD
Lido di Venezia, Italy – October 15-17, 2014
Program 157
Abstracts 158
Author index 165

### OBITUARY
Erich Kuhn
Reinhardt Rüdel 166

### NEWS FROM AROUND THE WORLD
- MSM 168
- GCA 168
- AIM 168
- LGMD-Euro-Net 168
- WMS 168

### FORTHCOMING MEETINGS
169

Volume XXXIII - CONTENTS 170
Volume XXXIII - AUTHOR INDEX 172
Volume XXXIII - SUBJECT INDEX 175
Volume XXXIII - LIST OF REFEREES CONSULTED IN 2014 176

Instructions for Authors 178
VOLUME XXXIII - AUTHOR INDEX

A
Abdel Salam E., 13
Accorsi A., 75
Agennouz M., 49, 60, 68
Aguglia U., 71
Alfonsi E., 69
Ali G., 75
Almendra L., 144
Altamura C., 47, 59
Altavista M.C., 58
Amato A., 73
Ambrosi A., 57
Angelini C., 48, 51, 55, 61, 62, 63, 68, 69, 70, 74, 75, 78, 119, 136, 159, 160, 163, 164
Annunziata A., 100
Antonini G., 47, 55, 56, 63, 69, 70
Arca M., 75
Ardissone A., 47, 61
Argov Z., 107
Armoric A., 75, 76
Arnoldi M.T., 49, 66
Assereto S., 56, 57, 163
Astrea G., 47, 49, 68, 138
Attico E., 48, 70
Azzolin F., 50
B
Badiali De Giorgi L., 65
Baldacci J., 54
Baldanzi S., 48
Baldassari C., 50
Baldassarri S., 56, 57, 163
Balestri P., 49
Ballottin U., 50, 64, 68, 71, 136
Baranello G., 49, 66
Barbero M., 66
Barca E., 71
Barcellona C., 49, 53, 60, 78
Barison A., 51
Baron A., 73
Baroncelli C., 72
Barp A., 50
Battini R., 47, 49, 68, 136
Beatriz Romero N., 56
Behin A., 159
Bell L., 50, 55, 74, 76
Ben Yau R., 159
Berardinelli A., 48, 50, 61, 64, 66, 68, 69, 70, 74, 78, 136
Bernardi C., 58
Bernardo D., 50, 68, 73
Bernasconi P., 47, 51, 59, 61, 69
Berti E., 51, 62, 78
Bertolin C., 69
Berton L., 50
Beshiri F., 59
Betto R., 162
Beuvin M., 56
Bevilacqua F., 48
Bianchi M., 67
Bianchi M.L., 66
Bianchini E., 162
Binda A., 76
Bitto A., 49, 60
Blasevich F., 47, 60
Bonannini L., 53
Bonetti P., 161
Bonuccelli U., 74, 75
Bordoni A., 52, 71, 78
Borgione E., 74
Borsato C., 164
Boschetti E., 73
Botta A., 52, 76
Bottitta M., 74
Bragato C., 47
Brenna G., 66
Bresolin N., 52, 55, 61, 64, 67, 164
Brighina E., 136
Brighina E., 55, 67, 68
Briska G., 76
Brochier G., 56
Brondi M., 74
Brugnoni R., 47, 59, 61
Bruno C., 54, 55, 56, 57, 62, 160, 163
Brusa R., 61, 62
Bucchia M., 52
Bucci E., 47, 56, 69, 70
Bugiardini E., 52, 76
Buria J., 73
Bushby K., 59, 77
Bussolino C., 66
C
Calabrò R., 94, 127
Caldarazzo lenco E., 74, 75
Caliendo C., 57
Camia M., 68
Canioni E., 51, 59
Cannon S.C., 73
Cao M., 51, 69, 70
Capasso M., 53
Capelletti C., 51
Carbonara R., 53
Carboni N., 51
Cardioli E., 62, 72
Cardini R., 52, 76
Carelli V., 62, 73
Carnevale A., 58
Cassandrini D., 47
Castello F., 74
Castellotti B., 70
Catalano A., 65
Catteruccia M., 62, 68, 136
Cavallaro F., 53
Cavallini A., 67
Cecchi P., 48
Cello N., 59
Cencacchi G., 65, 70, 73
Cereda C., 67
Cescon C., 66
Chambers D., 52
Chiapparini L., 49
Chico L., 70, 74
Clafalon E., 73
Ciceri F., 57, 58
Cipullo F., 54
Giranni A., 49, 60, 63, 65
Cirillo F., 76
Ciscato P., 62, 77
Civitini F., 68, 136
Codemo V., 55
Coi A., 50
Colia G., 68, 136
Colleoni L., 59, 61
Colombo D., 55
Colombo L., 50, 52, 62, 67, 86
Comanducci G., 66
Comi G., 52, 64
Comi G.P., 50, 55, 60, 61, 62, 63, 64, 67, 71, 77, 78, 160, 164
Consolo C., 53
Conte Camerino D., 47, 53
Conte D., 59
Cortese A., 52
Corti S., 52, 60, 61, 64, 71
Cosottini M., 48
Cossia S., 51
Cossu G., 57, 58
Costanzi-Porrini S., 66
Cotelli M., 77
Cristiano A., 94, 104
Crow Y., 64
Crumb B., 73
Cuccagna C., 50, 68, 73
Cuomo G., 52
D
D’Adda E., 52
D’Ambrosio P., 66
D’Amico A., 53, 60, 68, 136
D’Amico M.C., 53, 69, 70
D’Angelo G., 48, 55, 69, 70
D’Angelo M., 78
D’Angelo M.G., 55, 61, 62, 67, 136, 164
D’Antona G., 61, 66
D’Apice M., 54
D’Arrigo S., 60
D’Elia A., 69
Da Pozzo P., 72
DalMasi A., 64
Darnetti S., 64
Danesino C., 63
Danielli M.G., 60
Daiolo J., 48, 70
De Angelis M.V., 53
De Filippis P., 63
De Giorgio R., 73
Deconinck N., 63
Del Bo R., 61, 62
Del Vecchio Blanco F., 160
Delbarba E., 79
Dell’Ossio G., 70
Della Cioppa N., 104, 127, 149
Demontis A., 59
Desaphy J.F., 47, 53, 69
Deschauer M., 19
Di Battista G., 58
Di Bella P., 60
Di Bella V., 53
Di Blasi F., 74
Di Fruscio G., 160
Di Iorio G., 54, 63
Di Mauro S., 86
Di Meo F., 94, 127, 149
Di Muzio A., 48, 53, 63, 69, 70
Di Pasquale A., 56
Díaz-Manera J., 158
DiMauro S., 71
Dinardo M.M., 47
Dictfano M.G., 49, 53, 60, 78
Docci S., 54
Donati A., 62
Donato C., 53
Drigo L., 164
E
Eagle M., 59
Ehsan Abdel-Meguid I., 13
Ellis M., 52
Entwistle K., 63
Esposito T., 54
Eymard B., 56, 159
F
Fabbri S., 48
Fagiolari G., 67, 71
Faclier E., 58
Falin A., 57
Fan C., 22
Fanin M., 119, 159, 163
Fardeau M., 56
Farina L., 52
Farina O., 54
Federico A., 62, 72
Fertazz E., 71
Ferlini A., 76
**Volume XXXIII - Author index**

<table>
<thead>
<tr>
<th>A</th>
<th>Abate T., 51, 55, 60, 61, 72, 81, 84, 163</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>Baccarani M., 55, 59, 70, 78, 80, 81, 84, 164</td>
</tr>
<tr>
<td>C</td>
<td>Capoferri A., 54, 55, 63, 64, 71, 78, 79, 80, 81, 84, 164</td>
</tr>
<tr>
<td>D</td>
<td>Della Corte E., 50, 51, 55, 56, 57, 58, 59, 72, 78, 80, 81, 84, 164</td>
</tr>
<tr>
<td>E</td>
<td>Ercolani L., 50, 51, 55, 56, 57, 58, 59, 72, 78, 80, 81, 84, 164</td>
</tr>
<tr>
<td>F</td>
<td>Fabbian F., 50, 51, 55, 56, 57, 58, 59, 72, 78, 80, 81, 84, 164</td>
</tr>
<tr>
<td>G</td>
<td>Gattagalloni M., 50, 51, 55, 56, 57, 58, 59, 72, 78, 80, 81, 84, 164</td>
</tr>
<tr>
<td>H</td>
<td>Hanisch F., 19, 79, 80, 81, 84, 164</td>
</tr>
<tr>
<td>I</td>
<td>Istrumenti M., 50, 51, 55, 56, 57, 58, 59, 72, 78, 80, 81, 84, 164</td>
</tr>
<tr>
<td>J</td>
<td>Juretta-Kott K., 22, 79, 80, 81, 84, 164</td>
</tr>
<tr>
<td>K</td>
<td>Kapetis D., 47, 51, 59, 61, 72, 78, 79, 80, 81, 84, 164</td>
</tr>
<tr>
<td>L</td>
<td>La Foresta S., 63, 79, 80, 81, 84, 164</td>
</tr>
<tr>
<td>M</td>
<td>Maggi L., 47, 51, 57, 59, 61, 66, 69, 70, 72, 78, 79, 80, 81, 84, 164</td>
</tr>
<tr>
<td>N</td>
<td>Napoli L., 52, 79, 80, 81, 84, 164</td>
</tr>
<tr>
<td>O</td>
<td>Olivieri I., 64, 79, 80, 81, 84, 164</td>
</tr>
<tr>
<td>P</td>
<td>Padovani A., 70, 72, 75, 77, 79, 80, 81, 84, 164</td>
</tr>
<tr>
<td>Q</td>
<td>Quarteroni A., 50, 51, 55, 56, 57, 58, 59, 72, 78, 80, 81, 84, 164</td>
</tr>
<tr>
<td>R</td>
<td>Rezza G., 50, 51, 55, 56, 57, 58, 59, 72, 78, 80, 81, 84, 164</td>
</tr>
<tr>
<td>S</td>
<td>Sansone L., 50, 51, 55, 56, 57, 58, 59, 72, 78, 80, 81, 84, 164</td>
</tr>
<tr>
<td>T</td>
<td>Tedeschi G., 50, 51, 55, 56, 57, 58, 59, 72, 78, 80, 81, 84, 164</td>
</tr>
<tr>
<td>U</td>
<td>Ugo V., 50, 51, 55, 56, 57, 58, 59, 72, 78, 80, 81, 84, 164</td>
</tr>
<tr>
<td>V</td>
<td>Vecchione M., 50, 51, 55, 56, 57, 58, 59, 72, 78, 80, 81, 84, 164</td>
</tr>
<tr>
<td>W</td>
<td>Wieland M., 50, 51, 55, 56, 57, 58, 59, 72, 78, 80, 81, 84, 164</td>
</tr>
<tr>
<td>X</td>
<td>Xerri L., 50, 51, 55, 56, 57, 58, 59, 72, 78, 80, 81, 84, 164</td>
</tr>
<tr>
<td>Y</td>
<td>Yeh L., 50, 51, 55, 56, 57, 58, 59, 72, 78, 80, 81, 84, 164</td>
</tr>
<tr>
<td>Z</td>
<td>Zanetti F., 50, 51, 55, 56, 57, 58, 59, 72, 78, 80, 81, 84, 164</td>
</tr>
</tbody>
</table>
VOLUME XXXIII - SUBJECT INDEX

Adenosine, 104
Amyotrophic syndromes, 111
Anoctamin 5, 19
Apnoea, 100
Atrial fibrillation, 127
Atrial lead, 94
Atrial preference pacing, 127
Autosomal recessive CMT, 144
Bachmann's bundle, 94
Becker muscular dystrophy, 136
Bipolar electrocautery, 149
Burden, 136
Channelopathy, 22
Clinical heterogeneity, 86
Closed-state inactivation, 22
CMT4B2, 144
Cognitive function, 13
Congenital glaucoma, 144
D-penicillamine, 111
Distal myopathy, 34
Dual genetic control, 86
Duchenne muscular dystrophy, 13, 136
Far field, 94
Far field R-wave sensing, 94
Glycogenosis, 100
Healthy siblings, 136
Hospice Movement in Croatia, 111
Hypopnea, 100
Hypoventilation, 100
Intrafamilial variability, 86
LGMD, 1, 119
Limb girdle muscular dystrophy 2L, 19
Limb-girdle muscular dystrophies, 1
Markov model, 22
MTMR13/SBF2 gene, 144
Multisystem disorders, 86
Muscular tabes, 34
Myotonic dystrophy, 104, 127
Myotonic dystrophy type 1, 94, 149
Necrotizing myopathy, 19
Neural damage, 13
NGS, 1
nNOS, 119
Non invasive ventilation, 100
Oversensing, 94
Pacemaker implantation, 149
Paramyotonia, 22
Parents, 136
Peripheral neuromuscular involvement, 34
Proarrhythmic effect, 104
Sarcoglycan, 119
Sinus tachycardia, 104
Skeletal muscle, 22
Social network, 136
Sodium channel, 22
Ventricular fibrillation, 149
VOLUME XXXIII - LIST OF REFEREES CONSULTED IN 2014

Angelini, Corrado
Barresi, Rita
Hausmanowa-Petrusewicz, Irena
Jurkat-Rott, Karin
Kochański, Andrzej
Mantegazza, Renato
Monsurrò, Maria Rosaria
Mora, Marina
Nigro, Gerardo
Nigro, Vincenzo
Piluso, Giulio
Politano, Luisa
Rizzo, Vincenzo
Rüdel, Reinhardt
Santorelli, Filippo
Siciliano, Gabriele
Toscano, Antonio
Trevisan, Carlo Pietro
For application or renewal to MSM

MEDITERRANEAN SOCIETY OF MYOLOGY* (MSM)

G. Nigro, President
L. T. Middleton, G. Serratrice, Vice-Presidents
Y. Shapira, Secretary
L. Politano, Treasurer

APPLICATION/RENEWAL FORM

Application/Renewal for 1yr 2 yrs

☐ ☐

Prof. Luisa Politano, MSM Treasurer, Viale dei Pini 101, 80131, Napoli, Italy
Fax: 39 081 5665100 E-mail: actamyologica@gmail.com
Fax or Mail to the above address. Type or print.

Name __________________________________________ Degree(s) __________

Last

First

Department ____________________________________________________________

Institution ____________________________________________________________

Street Address _______________________________________________________

City, State, zip, country _______________________________________________

Tel (__________) __________________ Fax (__________) __________________

Area code

Area code

* Amount payable: 1 year Euro 100
2 years Euro 150

☐ I enclose copy of the bank transfer to:

Bank name: Banco di Napoli – Filiale 00660
Bank address: Via Riviera di Chiaia, 131 – 80122 Napoli, Italy
Account holder: MSM – Mediterranean Society of Myology
IBAN code: IT48T0101003488100000100680
BIC code (for foreign countries): IBSPITNA

* The membership fee is inclusive of free subscription to Acta Myologica.
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, 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.

E-mail submission

Manuscripts will be sent to the Editorial Office (actamyologica@gmail.com • luisa.politano@unina2.it) by email only, with a covering note, subject to prior agreement from the Editorial Office, 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 4 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. Check that it represents the content of the paper and is not misleading. Also suggest a short running title.

Key words. Supply up to three 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. Colour 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. Reference numbers in the text must be in brackets. References in the list must be numbered as they appear in the text.


Please check each item of the following checklist before mailing:

- Three 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 telephone and fax numbers and 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.

The editor remains at the complete disposal of those with rights whom it was impossible to contact, and for any omissions.

Photocopies, for personal use, are permitted within the limits of 15% of each publication by following payment to SIAE of the charge due, article 68, paragraphs 4 and 5 of the Law April 22, 1941, No 633.

Reproductions for professional or commercial use or for any other other purpose other than personal use can be made following a written request and specific authorization in writing from AIDRO, Corso di Porta Romana, 108, 20122 Milan, Italy, E-mail: segreteria@aidro.org and web site: www.aidro.org.