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CONTENTS

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INVITED REVIEW

Muscle fatigue, nNOS and muscle fiber atrophy in limb girdle muscular dystrophy

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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 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.

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 provides a mechanical linkage between the extracellular matrix and the intracellular cytoskeleton. The structural and functional integrity of this connection is crucial to stabilize the sarcolemma during contractions. The SG complex is composed of 4 SG glycoproteins whose mutant genes cause a group of LGMD called sarcoglycanopathies (LGMD2C-2F), where the assembly of the SG complex is compromised, and the sarcolemma integrity and stability are lost. A similar pathogenetic mechanism leads to Duchenne muscular dystrophy (DMD), which is caused by mutations in the dystrophin gene. 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.

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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.

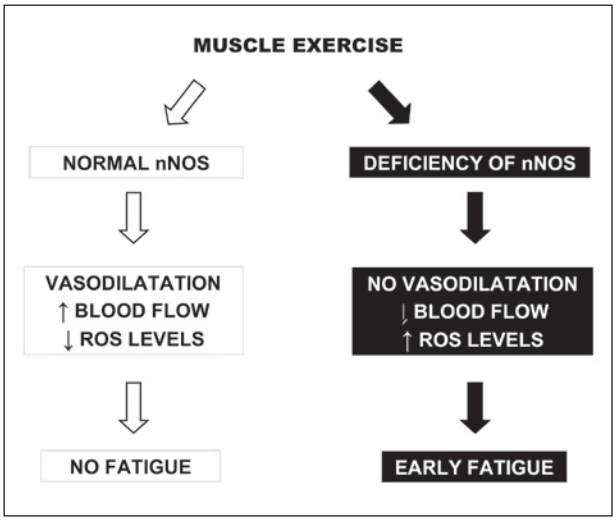

Figure 1. Cascade of events consequent to muscle exercise when normal (on the left) or defective (on the right) nNOS is present. Modified from Neuromuscular Disorders 2012;22:S214-220.

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

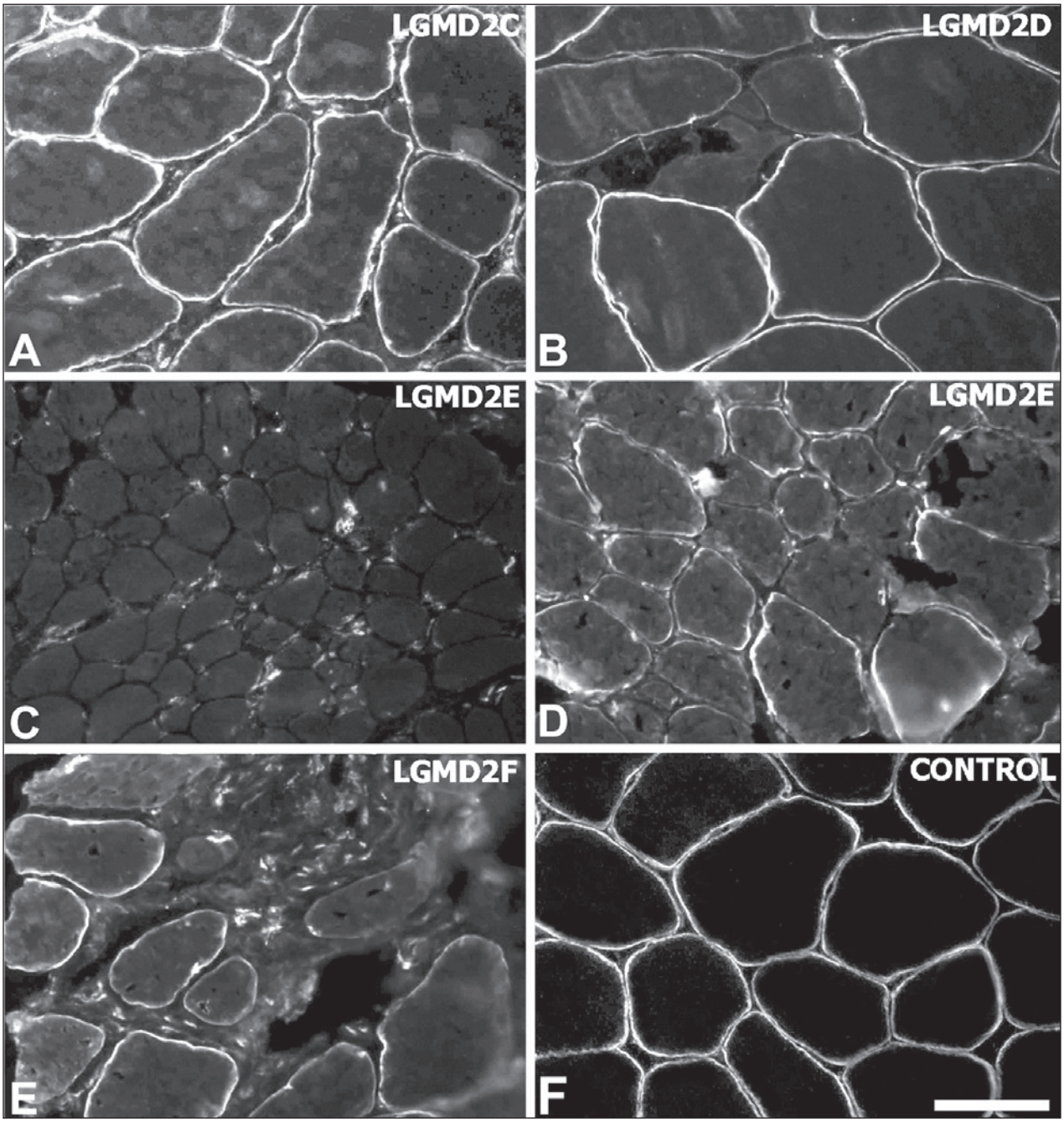

Figure 2. Sarcolemmal immunolabelling of nNOS in muscle biopsies from patients with sarcoglycanopathies (A-E) and control (F). The reaction was absent in LGMD2E patients with complete SG complex defect (C) and partial in LGMD2E patients with partial SG complex defect (D), indicating that the reduction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

(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).

nNOS and dilated cardiomyopathy in sarcoglycanopathies

In the study by Fanin et al. (14), the sarcolemmal nNOS expression correlated with the clinical severity, as

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

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

More detailed clinical and laboratory data from the same patients series are reported in Fanin et al. (14).

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) hydrolize 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,

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 studied 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-

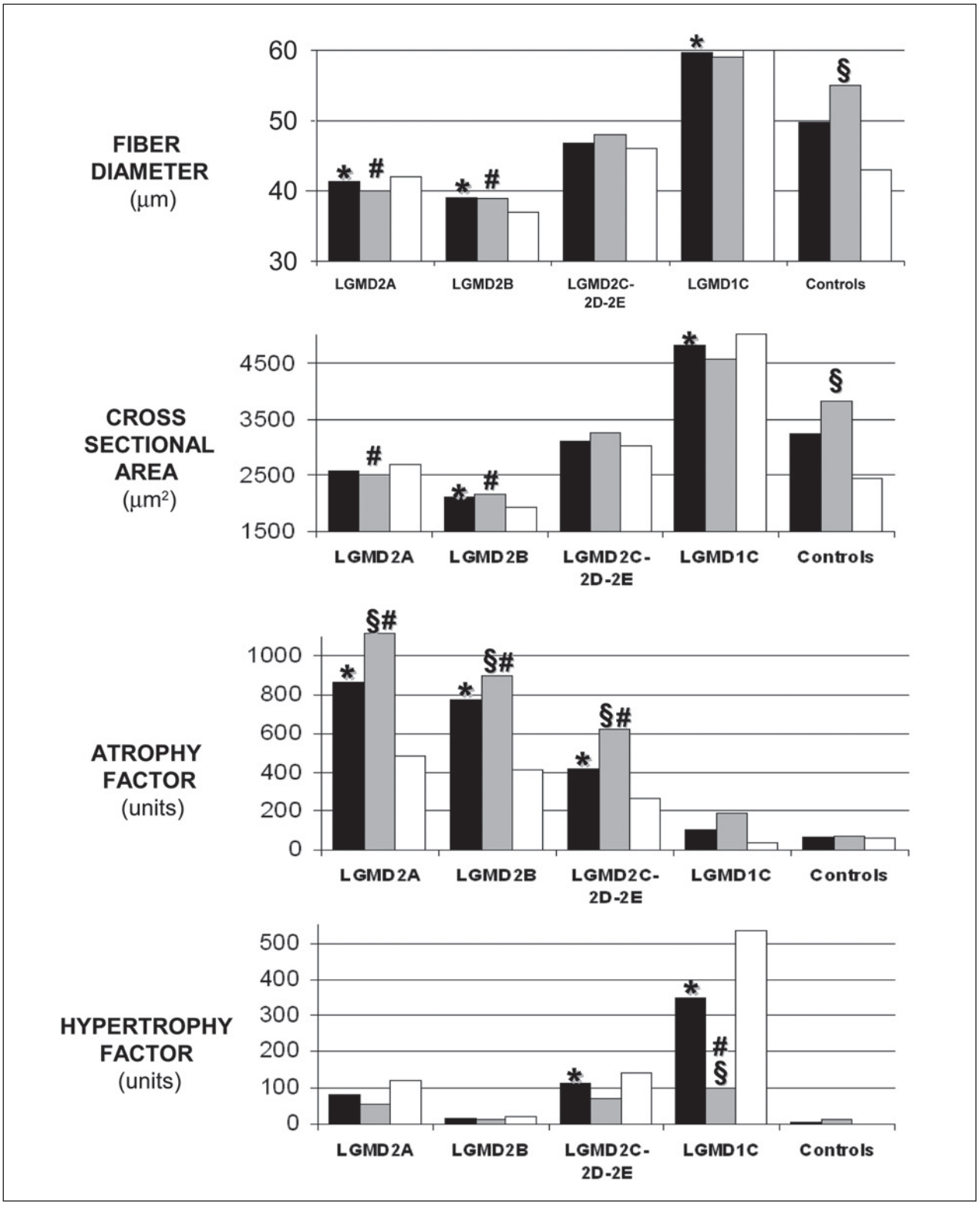

Figure 3. Histograms showing the comparison between the mean values of fiber diameter, cross sectional area, atrophy factor and hypertrophy factor in the different groups of LGMD patients and in the control group. Black bars = total cases of both genders, gray bars = male patients, white bars = female 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).

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ORIGINAL ARTICLES

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

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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 atrioventricula 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 atrioventricular 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

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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.

Table 1. Baseline characteristics of the study population. LVEDD (left ventricular end diastolic diameter), LVESD (left ventricular end systolic diameter), IVST (interventricular septum thickness), LVPWT (left ventricle posterior wall thickness).

Patients (n)	50
Age (years)	50,3 ± 7,3
Sex (M/F)	39/11
Atrioventricular block I grade	18
Atrioventricular block II grade type 1	12
Atrioventricular block II grade type 2	20
QRS duration (ms)	93 ± 13
LVEDD (mm)	42,7 ± 9
LVESD (mm)	27,24 ± 2,8
IVST (mm)	9,7 ± 1,3
LVPWT (mm)	9,9 ± 1,5
Ejection fraction (%)	60,39 ± 4
E wave (cm/s)	82,3 ± 15,5
A wave (cm/s)	57,9 ± 9,5
E/A ratio	1,5 ± 0,5

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).

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.

	APP ON Group	**APP OFF Group**	**p value**
AF episodes numbers(n)	117 ± 25	143 ± 37	0,03
AF episodes mean duration (min)	47 ± 17	43 ± 13	0,4
AF burden (min)	3059 ± 275	9010 ± 630	0,04
Atrial pacing percentage (%)	98	0	
Ventricular pacing percentage (%)	27	29	0,2
Atrial premature beats	13720 ± 7717	44903 ± 30689	0,005
Atrial pacing threshold (V)	0,7 ± 3	0,9 ± 2	0,6
Atrial sensing threshold (mV)	5 ± 3	7± 2	0,6
Atrial lead impedance (ohm)	582 ± 18	622 ± 12	0,7
Ventricular pacing threshold (V)	0,8 ± 3	0,6 ± 3	0,6
Ventricular sensing threshold (mV)	15 ± 5	17 ± 4	0,7
Ventricular lead impedance (ohm)	769 ± 45	889 ± 37	0,5

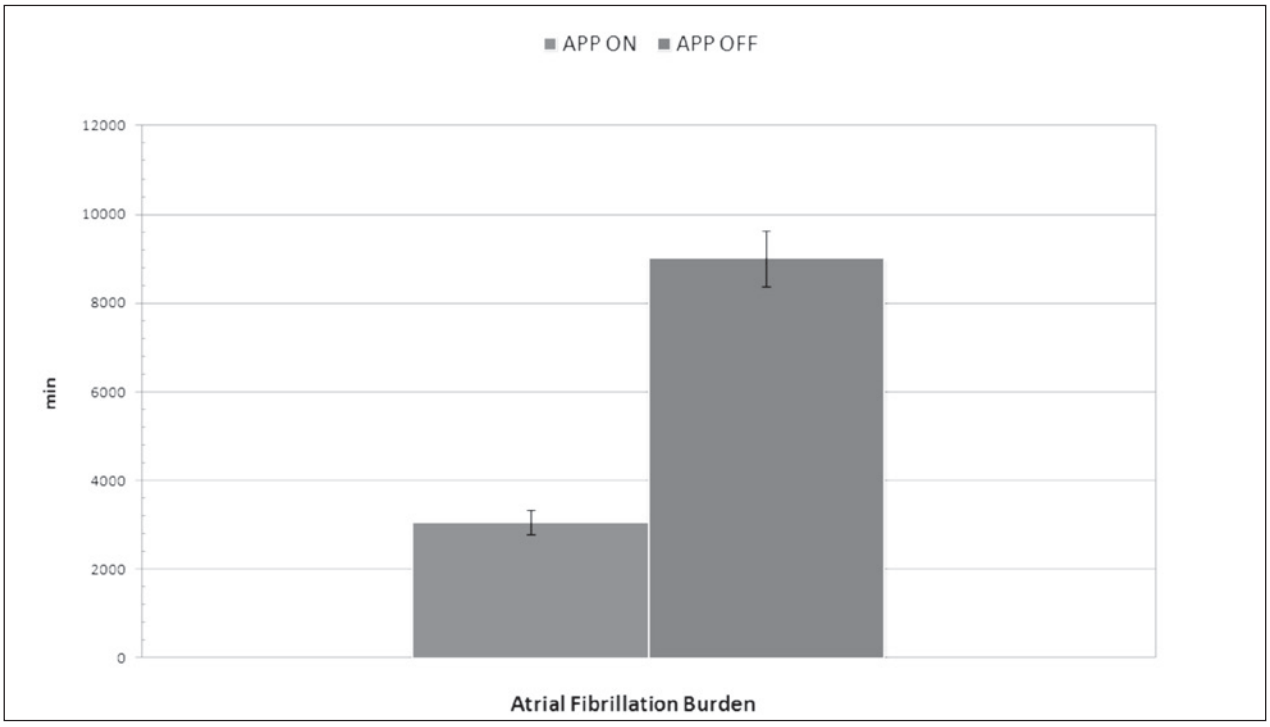

Figure 1. Difference in atrial fibrillation burden between APP ON Group and APP OFF Group (3059 ± 275 vs. 9010 ± 630 min; $p < 0.04$).

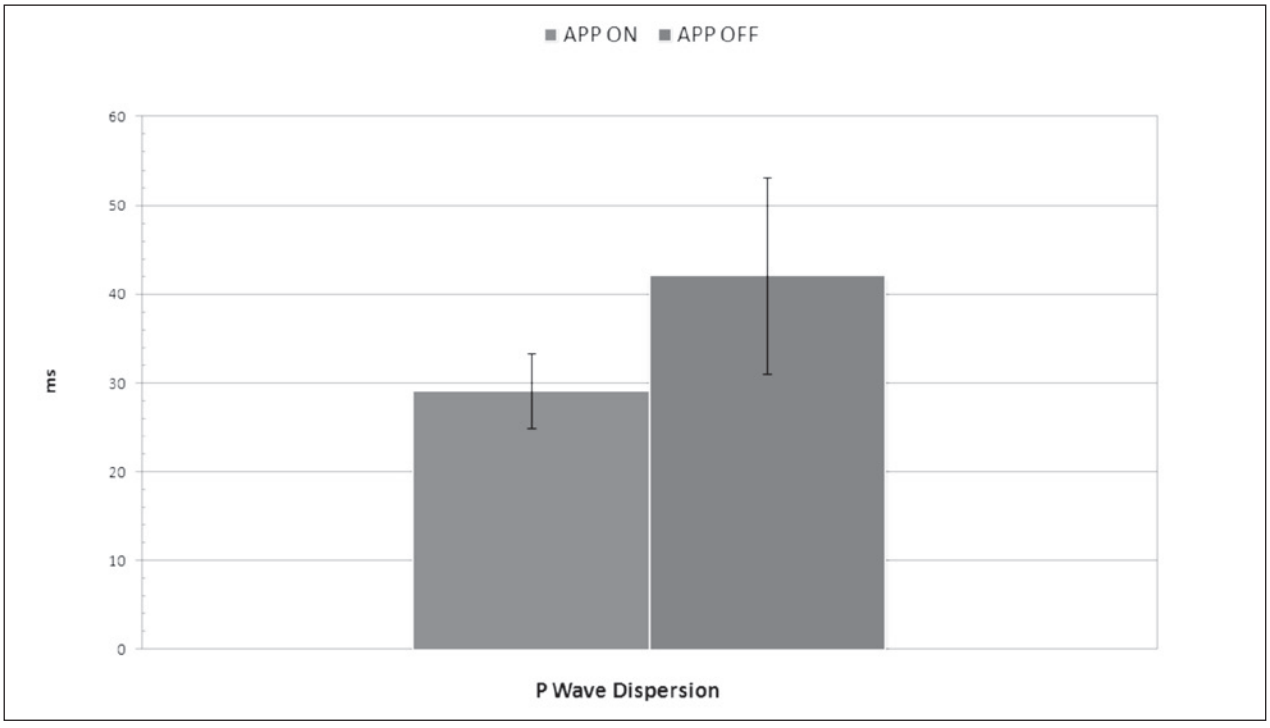

Figure 2. Difference in P-wave dispersion between APP ON Group and APP OFF Group (29,1± 4,2 ms vs. 42,1 ± 11 ms, $p = 0,003$).

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

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 asses 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-

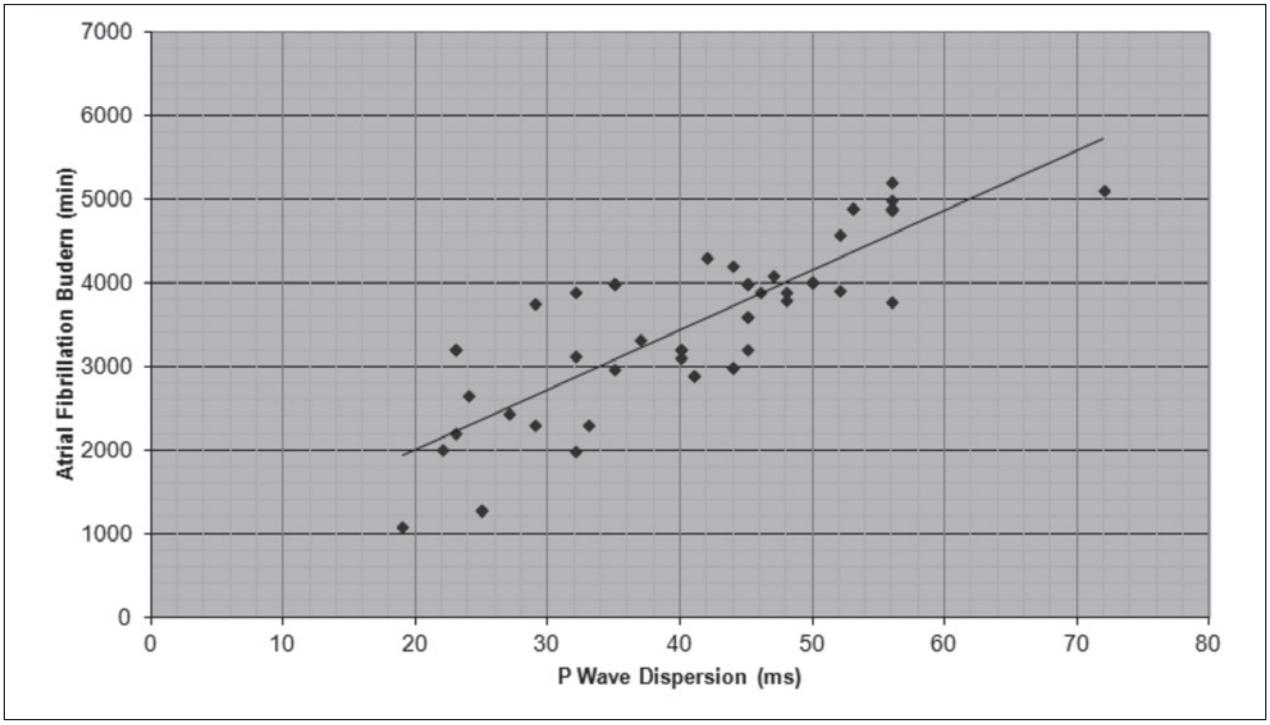

Figure 3. Correlation between P-wave dispersion and atrial fibrillation burden in DM1 patients (R = 0,8, p = 0.007).

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 Dilaveris 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.

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

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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 parents of 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).

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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; Crombach'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 GUP10002 study sample (7), Crombach'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

χ^2 and analysis of variance were used, as appropriate, to test differences in nominal and ordinal variables between DMD and BMD samples. χ^2 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 ($F = 13.9$, df 1, 334, $p < .05$), had lower levels of functional autonomy ($F = 95.7$, df 1, 334, $p < .05$) and more frequently received economic welfare benefits (177, 72.0% vs. 31, 34.4%, $\chi^2 = 39.3$, df 1, $p < .05$) than patients with BMD. Two-hundred-eight patients with DMD (84.6%) and 39 (43.3%) with BMD ($\chi^2 = 57.5$, 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%); cardiologic 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 ($\chi^2 = 87.3$, 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 ($F = 43.2$, 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), $F=44.7$, 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 Secondary school High school University	 11 (4.5) 89 (36.2) 119 (48.4) 27 (10.9)	 3 (3.3) 37 (41.1) 39 (43.3) 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).

Items – section a of FPQ	MD Type	Always N (%)	Often N (%)	SometimesN (%)	Never N (%)	Mean (SD)	χ2	MissingN
I felt that I would not be able to bear this situation much longer	DMD	8 (3.2)	35 (14.3)	103 (42.0)	99 (40.4)	1.8 (.0)		1
	BMD	2 (2.2)	4 (4.5)	21 (23.6)	62 (69.7)	1.4 (.7)	23.2*	1
I cried or felt depressed	DMD	7 (2.8)	59 (24.0)	130 (52.8)	50 (20.3)	2.1 (.7)		0
	BMD	4 (4.5)	8 (9.0)	49 (55.1)	28 (31.5)	1.9 (.7)	11.4	1
I worry for the future of other family members	DMD	21 (8.6)	41 (16.8)	119 (48.8)	63 (25.8)	2.1 (.9)		2
	BMD	3 (3.3)	11 (12.2)	45 (50.0)	31 (34.4)	1.8 (.8)	5.2	0
When I went to a public place with my ill relative, I felt that everyone was watching us	DMD	19 (7.9)	20 (8.3)	68 (28.1)	135 (55.8)	1.7 (.9)		4
	BMD	1 (1.1)	0	4 (4.4)	85 (94.4)	1.1 (.9)	44.1*	0
I feel guilty because I believe that I or my spouse may have passed on the illness to our relative	DMD	19 (7.8)	30 (12.2)	80 (32.7)	116 (47.3)	1.8 (.9)		1
	BMD	15 (17.0)	18 (20.5)	29 (33.0)	26 (29.5)	2.2 (1.1)	13.3	2
I think that if our relative didn't have this problem, everything would be all right in our family	DMD	39 (16.0)	32 (13.1)	64 (26.2)	109 (44.7)	2.0 (1.1)		2
	BMD	4 (4.5)	3 (5.6)	17 (19.1)	63 (70.8)	1.4 (.8)	19.9*	3
When I think of how our ill relative was beforehand and how he/she is now, I feel disappointed	DMD	75 (31.0)	50 (20.7)	79 (32.6)	38 (15.7)	2.7 (1.1)		3
	BMD	12 (13.8)	11 (12.6)	27 (31.0)	37 (42.5)	2.0 (1.0)	29.6*	3

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).

Items – section b of FPQ	MD Type	Always N (%)	Often N (%)	SometimesN (%)	Never N (%)	Mean (SD)	χ2	MissingN
I have had to wake up during the night	DMD	29 (11.8)	20 (8.2)	67 (27.3)	129 (52.7)	1.8 (1.0)		1
	BMD	1 (1.1)	4 (4.4)	11 (12.2)	74 (82.2)	1.2 (.6)	25.7*	0
I have had to neglect my hobbies and things I like doing in my free time	DMD	41 (16.8)	40 (16.4)	87 (35.7)	76 (31.0)	2.2 (1.0)		2
	BMD	2 (2.2)	8 (9.0)	19 (21.3)	60 (67.4)	1.5 (.7)	38.4*	1
I have had difficulty in going on Sunday outings	DMD	16 (7.8)	21 (10.3)	43 (21.1)	124 (60.8)	1.6 (.9)		42
	BMD	0	3 (3.7)	11 (13.6)	67 (82.7)	1.2 (.5)	15.2	9
I found it difficult to have friends at home	DMD	2 (0.8)	11 (4.5)	26 (10.7)	205 (84.0)	1.2 (.5)		2
	BMD	1 (1.1)	0	2 (2.2)	87 (96.7)	1.1 (.3)	10.9	0
I found it difficult to meet friends and people I like to spend my leisure time	DMD	6 (2.5)	22 (9.0)	50 (20.5)	166 (68.0)	1.5 (.8)		2
	BMD	3 (3.4)	0	9 (10.1)	77 (86.5)	1.2 (.6)	15.2	1
I found it difficult to carry out my usual work or household activities	DMD	11 (4.5)	31 (12.7)	94 (38.4)	109 (44.5)	1.8 (.8)		1
	BMD	0	3 (3.3)	14 (15.6)	73 (81.1)	1.2 (.5)	36.5*	0
I had to neglect other family members	DMD	1 (0.4)	27 (11.5)	75 (31.9)	132 (56.2)	1.6 (.7)		11
	BMD	0	2 (2.2)	18 (20.2)	69 (77.5)	1.2 (.5)	14.3	1
I had difficulty in going on holiday	DMD	28 (13.8)	21 (10.3)	30 (14.8)	124 (61.1)	1.1 (.1)		43
	BMD	1 (1.2)	2 (2.4)	7 (8.4)	73 (88.0)	.5 (.0)	21.8*	7
I had economic difficulties	DMD	12 (4.9)	20 (8.2)	71 (29.0)	142 (58.0)	1.6 (.8)		1
	BMD	1 (1.1)	1 (1.1)	14 (15.6)	74 (82.2)	1.2 (.5)	18.3*	0

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

Table 4. Difficulties in healthy siblings as perceived by parents (N = 134 DMD vs. N = 45 BMD).

Items – additional section of FPQ	MD Type	Always N (%)	Often N (%)	Sometimes N (%)	Never N (%)	Mean (SD)	$\chi 2$	Missing N
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)	DMD	2 (1.5)	6 (4.5)	33 (25.0)	91 (68.9)	1.4 (.6)		2
	BMD	1 (2.6)	0	4 (10.3)	34 (87.2)	1.2 (.5)	6.3	6
I feel that the presence of S affects negatively the social life of my children (school performance, leisure activities, etc.)	DMD	3 (2.3)	5 (3.8)	26 (19.7)	98 (74.2)	1.3 (.7)		2
	BMD	0	0	7 (18.4)	31 (81.6)	1.2 (.4)	2.5	7

DMD = Duchenne Muscular Dystrophy; BMD = Becker Muscular Dystrophy

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: $r = -.50$, BMD burden total score: $r = -.38$, $p < .05$), in both groups. In the DMD group, burden correlated with duration of illness ($r = .32$, $p < .05$), patient's age ($r = .36$, $p < .05$) and parents' age ($r = .30$, $p < .05$). Furthermore, burden was higher among parents with fewer social contacts ($r = -.28$), and lower social support in emergencies ($r = -.51$, $p < .05$). In the same group, difficulties in healthy siblings were higher among children whose parents were older ($r = .33$, $p < .05$) and with fewer social contacts ($r = -.27$, $p < .05$).

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).

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

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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

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Acta Myologica • 2014; XXXIII: p. 144-148

Charcot-Marie-Tooth 4B2 caused by a novel mutation in the *MTMR13/SBF2* gene in two related Portuguese families

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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 to all sensory modalities.

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 to all modalities. 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

Introduction

Charcot-Marie-Tooth (CMT) disease, also called hereditary motor and sensory neuropathy (HMSN), is the most common inherited neuromuscular disorder with an estimated prevalence of 1/2500 (1).

According to mode of inheritance, it can be classified into autosomal dominant, recessive or X-linked forms; based on neurophysiologic findings it can be further sub classified into demyelinating or axonal, if nerve conduction velocity is below or above 38 m/s, respectively (2). The subtype CMT1A accounts for 60-70% of all cases of CMT. It is caused by a 1,4 Mb duplication of chromosome 17p11.2 that includes the *PMP22 g*ene (3) and nerve biopsy shows demyelination and remyelination with onion bulbs formations (4).

Autosomal recessive demyelinating CMT (CMT4) is a very rare form of CMT disease. It is clinically and genetically heterogeneous. Reported CMT4B2 families present similar clinical, electrodiagnostic and pathologic features (5-7). Congenital/early-onset glaucoma is a very characteristic, although not universal, clinical feature of the disease (8-10). The CMT4B2 locus was mapped in 1999 (6) and the myotubularin-related protein-13/set binding factor 2 (*MTMR13/SBF2*) gene was identified in 2003 (7, 9). MTMR13/SBF2 belong to the family of myotubularins and is a catalytic inactive phosphatase (11, 12). MTMR13/SBF2 interacts with MTMR2 to form tetramers and the interaction significantly increases the catalytic activity of MTMR2 (13), but a full understanding of its function it is still not known in detail (14).

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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, hammertoes, 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 hammertoes (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

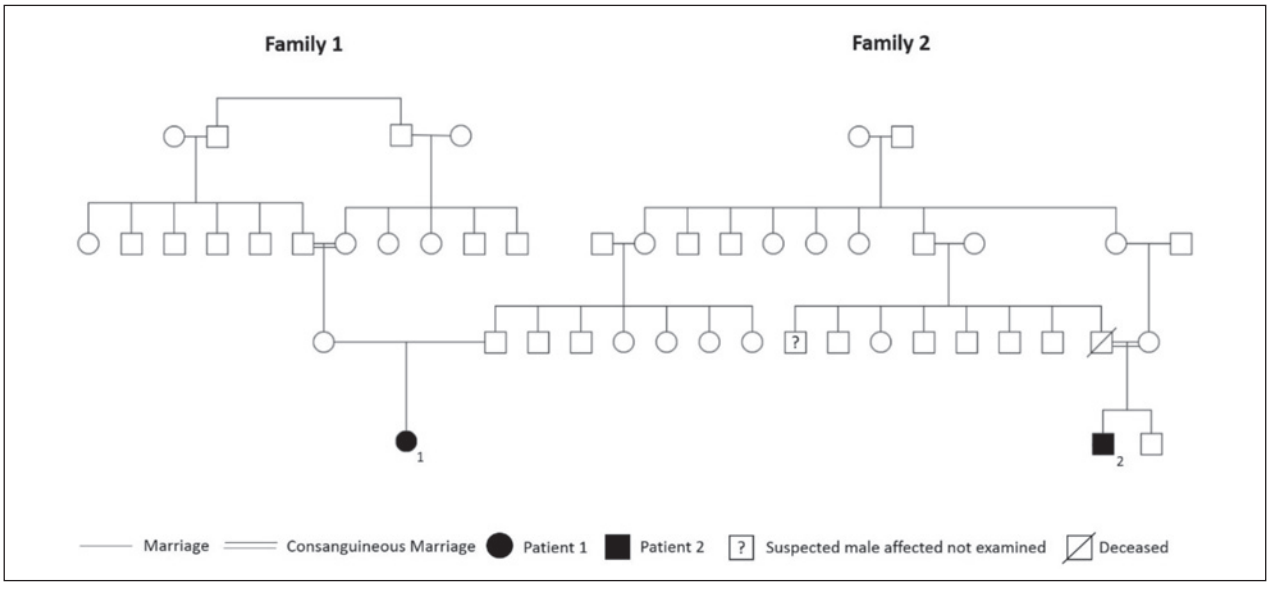

Figure 1. Pedigree of Families 1 and 2.

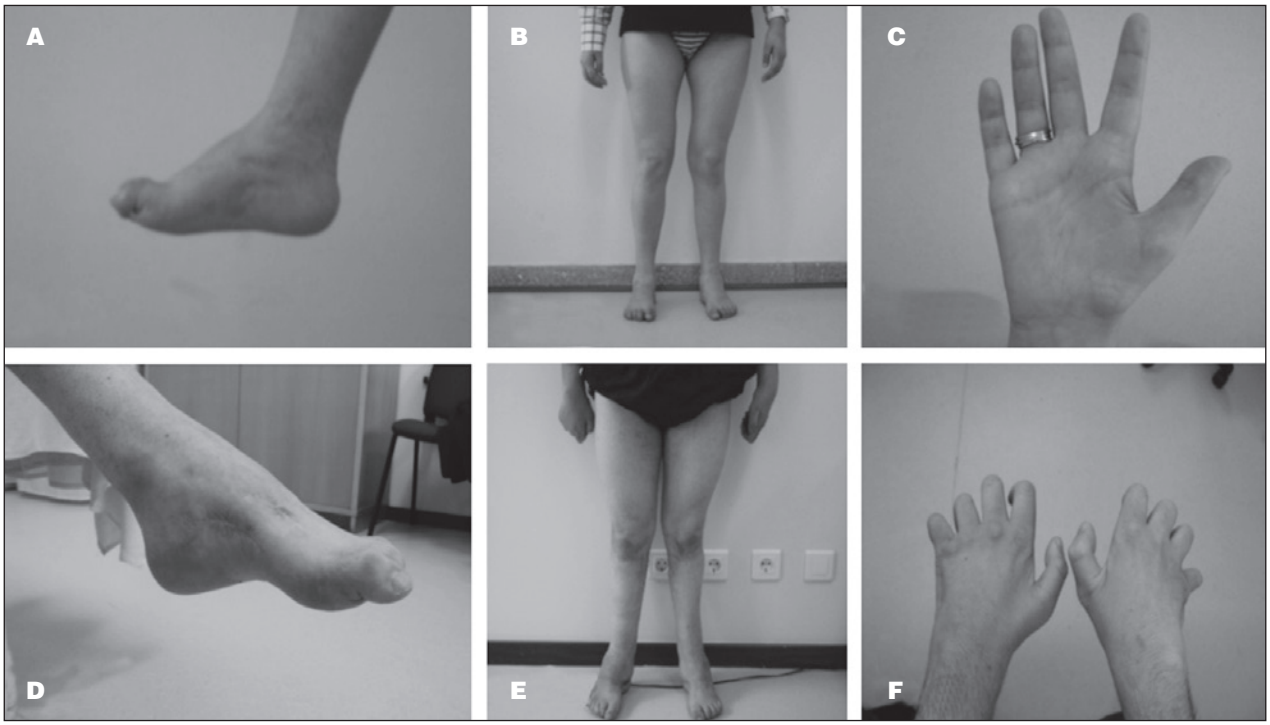

Figure 2. a, d: Pes cavus and hammertoes; b, e: inverse champagne bottle legs; c: atrophy of the thenar eminence; f: global intrinsic hand muscle atrophy with claw hand appearance.

corrective orthopedic surgeries, because of pes equinovarus, 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.

Nerve Conduction Study										
	Median Nerve				**Ulnar Nerve**				**Peroneal Nerve**	
	CMAP (mV)	**DML (ms)**	**MNCV (m/sec)**	**SAP (μV)**	**CMAP (mV)**	**DML (ms)**	**MNCV (m/sec)**	**SAP (μV)**	**CMAP (mV)**	**DML (ms)**
Family 1										
Patient 1	0.5	9.1	14	Ø	3.6	7.4	19	Ø	Ø	
Family 2										
Patient 1	Ø			Ø	0.5	7.3	14	Ø	Ø	

Nerve Conduction Study					
	Sural Nerve	**Facial Nerve**		**Blink Reflex**	
	SAP (μV)	**CMAP (mV)**	**DML (ms)**	**R1 (ms)**	**R2i (ms)**
Family 1					
Patient 1	Ø	0.4	6.5	22	57.6
Family 2					
Patient 1	Ø	0.4	6.5	18	54

DML: distal motor latency; CMAP: compound muscle action potential; MNVC: motor nerve conduction velocity; SAP: sensory action potential; ms: miliseconds; mV: milivolt; 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 *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.

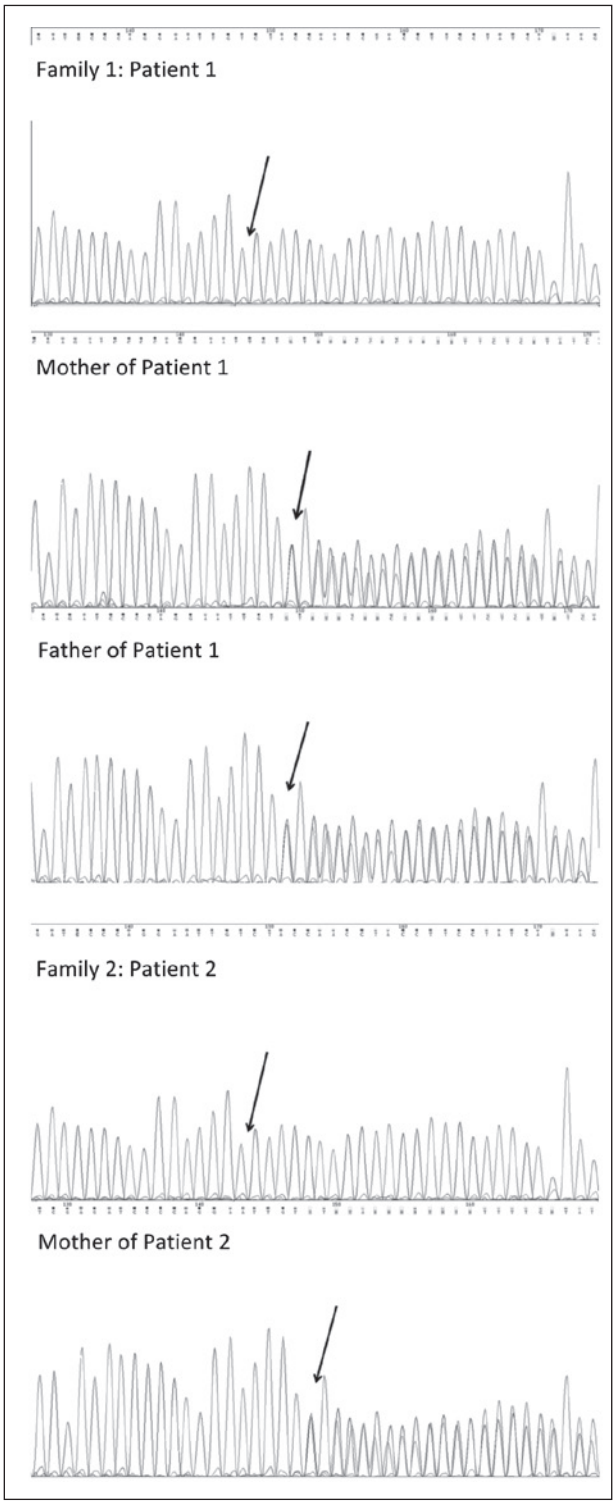

Figure 3. Chromatograms of the mutation site sequence (*arrow*).

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, 16). 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.

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Acta Myologica • 2014; XXXIII: p. 149-151

CASE REPORT

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

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The occurrence of ventricular fibrillation, induced by bipolar electrocautery during elective dual chamber pacemaker implantation, is reported in a patient affected by Myotonic Distrophy type 1 with normal left ventricular ejection fraction

Key words: ventricular fibrillation, bipolar electrocautery, pacemaker implantation, Myotonic Dystrophy type 1

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 Distrophy 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 atrioventricolar 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 (CHA2DS2Vasc2: 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

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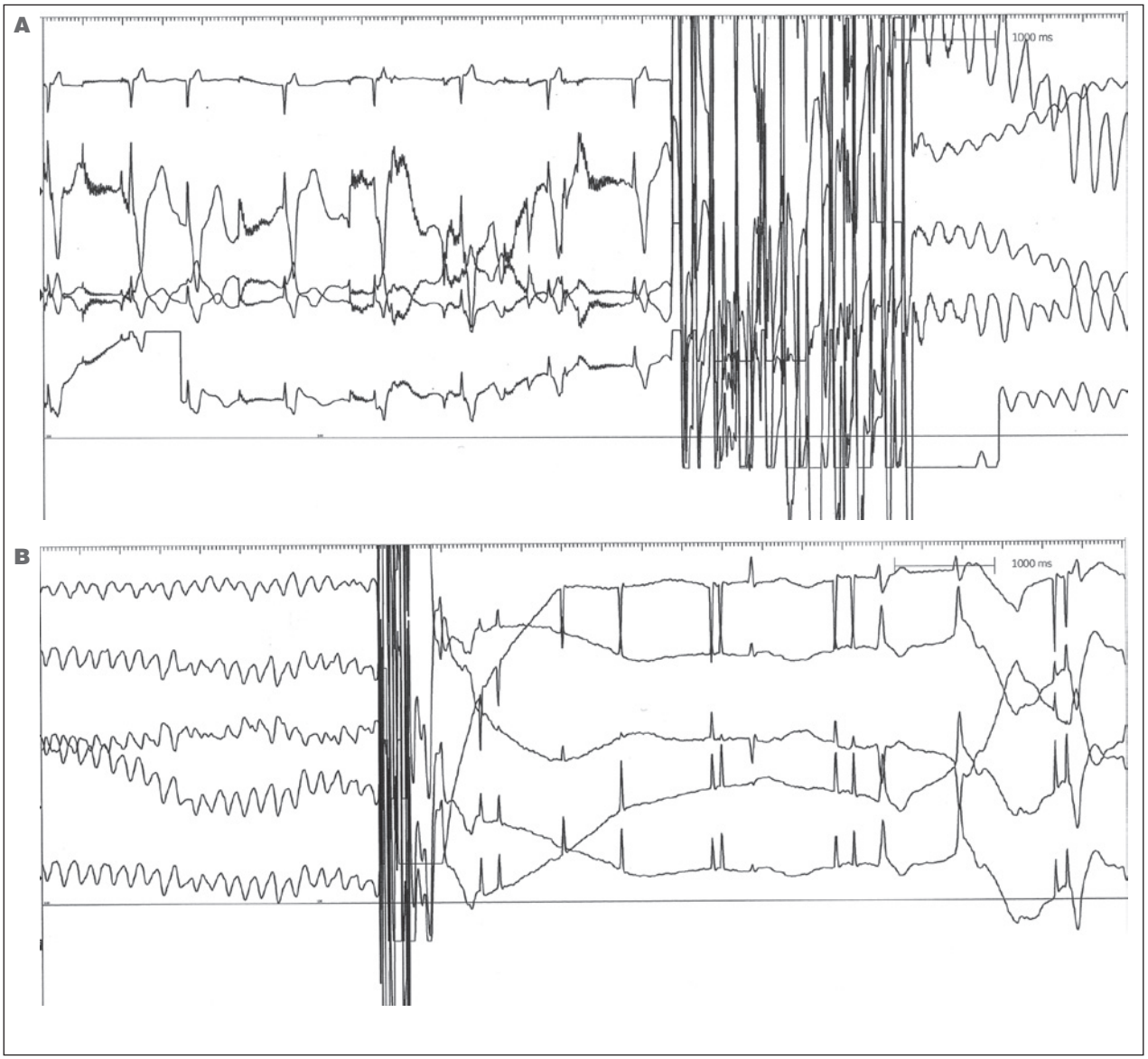

Figure 1. Rhythm strips during bipolar electrocautery application in coagulating mode. Ventricular fibrillation was seen after electrocautetry was stopped (1A) and was treated (1B) using an 200 J external biphasic defibrillator non synchronized shock.

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 experienced 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

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 possibily 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.

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Acta Myologica • 2014; XXXIII: p. 152-153

LETTER TO THE EDITOR

Facio-scapulo-humeral muscular dystrophy and its connection with facio-scapulo-peroneal muscular dystrophy 4q35-linked: some historical remarks

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, humeral 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.

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Acta Myologica • 2014; XXXIII: p. 155-165

PROCEEDINGS OF LGMD DAYS MEETING: Prognosis and Treatment in LGMD

October 15-17, 2014

IRCCS "S. Camillo", Lido di Venezia (VE), Italy

GRUPPO FAMILIARI
BETA-SARCOGLICANOPATIE
ONLUS

IRCCS
FONDAZIONE OSPEDALE
SAN CAMILLO

UILDM
Unione Italiana Lotta alla
Distrofia Muscolare ONLUS

LGMD DAYS

PROGNOSIS AND TREATMENT IN LGMD

15-17 OTTOBRE 2014

IRCCS «S. Camillo»
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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)
Neurorehabilitation in LGMD
15.40 Tiziana Toso (Padua)
Nutrition guidelines
16.05 Beatrice Vola (Sondrio)
The contribuition 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 Doriana Sandonà (Padua)
Small molecule-based therapy for sarcoglycanopathy, a novel perspective.
10.55 Lucia Morandi (Milano)
Clinical features in LGMD
11.20 Elisabetta Gazzerro (Genova), Claudio Bruno (Genova)
Improvement of molecular dystrophic process in alpha-sarcoglycan KO mice by blocade 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

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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, supraventricular 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

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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, semimebranosus, 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

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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 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

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Limb girdle muscular dystrophies (LGMDs) are a heterogeneous group of inherited progressive muscle disorders affecting predominantly the pelvic and scapular girdle muscles. Sarcoglycanopathies are a group of recessive LGMDs caused by mutations in the genes that encode for the components of the sarcoglycan (SG) complex. 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 β-SG is expressed in multiple tissues, although expression is prominent in skeletal and cardiac muscle. Few epidemiological data is available concerning sarcoglycanopathies, prevalence based in biopsy and genetic analyses was estimated to be 5.6 χ 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.

2nd Session h. 14.00-16.30

Large screening of a NMD cohort of patients by MotorPlex, an innovative strategy of targeted resequencing

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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)

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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 choise 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 incresingly 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 progresison of the disease.

Recent in vitro studies and of muscolar 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?

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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-sarcoglyconopathy, 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, sarcoglyconopathies 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".

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.

Friday, 16 October
3rd Session h. 10.30-11.55

Chairmen: C.P. Trevisan (Padova), M. Moggio (Milano), G. Siciliano (Pisa)

Small molecule-based therapy for sarcoglycanopathy, a novel perspective

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Department of Biomedical Sciences, University of Padova; [1] Department of Comparative Biomedicine and Food Science, [2] Institute 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 proteasomal 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 muscles 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

E. Gazzerro, S. Baldassari, S. Assereto, C. Panicucci, C. Fiorillo, C. Minetti, E. Traggiai[1], F. Grassi[2], C. Bruno
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, endomysial 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 (Sgca-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 periodate-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 Il1 and Il6.

Histopathology of cardiomyopathy in a patient with α-sarcoglycanopathy

M. Meznaric, E. Kralj[1], C. Angelini[2], M. Fanin[3]
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 ventricular 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. Giannotti[1], 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'Angelo[1], E. Pegoraro[2], C. Semplicini[2], C. Angelini[2], T. Mongini[3], A. Toscano[4], O. Musumeci[4], G. Siciliano[5], M. Mancuso[5], G. Ricci[5], G. Tomelleri[6], M. Mora[7], I. Moroni[7], L. Morandi[7], V. Nigro[8], G.P. Comi

IRCCS Ca'Granda, University of Milano; [1] IRCCS "E. Medea Bosisio 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 heterozygous 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 precocious (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. Nigro[1 2]

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See M. Savarese et al.

Discussion by groups of Horizon 2020 Projects

L. Drigo (Padua)

Discussion and closing remarks

C. Angelini (Padova)

AUTHOR INDEX

Acta Myologica • 2014; XXXIII: p. 166-167

OBITUARY

Erich Kuhn (1920-2014)

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.

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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, Barçelona; 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
- Infiammatory Myopathies
- Next Generation Sequency and NeuroMuscular Disorders
- New therapeutic approach in NeuroMuscular Disorders

During the General Assembly of the Society, the new Board of the Society will be elected.

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 18th -20th, 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

The 9th World Congress of controversies in Neurology (CONy). Budapest, Hungary. Information: website: *www.comtecmed.com/cony*

April 16-18

MDA NZ & ANN 2015 Conference: 'Life Without Limits' Neuromuscular Conference. Auckland, New Zealand. Information: *http://www.mda2015.org.nz*

May 5-9

2015 ISBER Annual Meeting. Phoenix, Arizona. Information: website: *www.isber.org*

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. 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

20th World Muscle Society Congress. London/Brighton, UK. Information: website: *http://www.worldmusclesociety.org*

October 6-10

American Society of Human Genetics ASHG Annual Meeting. Baltimore, MD, USA. Information: website: *www. www.ashg.org*

2016

March 17-20

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

April 3-7

The European Human Genetics Conference. Kyoto, Japan. Information: *website: www.esgh.org*

September 4-9

International Congress of Human Genetics 2016. Yokohama, Japan. Information: website: *www.esgh.org*

October 20-24

ASHG Annual Meeting. Vancouver,Canada. Information: website: *www.ashg.org*

October (to be announced)

21st World Muscle Society Congress. Granada, Spain. Information: website: *http://www.worldmusclesociety.org*

2017

October 17-21

ASHG Annual Meeting. Orlando, Florida, USA. Information: website: *www.ashg.org*

2018

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

2019

October 22-26
ASHG Annual Meeting. Toronto, Canada. Information: website: *www.ashg.org*

2020

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

VOLUME XXXIII - CONTENTS

Issue N. 1 • May 2014

Issue N. 2 • October 2014

Issue N. 3 • December 2014

VOLUME XXXIII - AUTHOR INDEX

G

H

I

J

K

L

M

N

O

P

Q

R

S

T

U

V

W

X

Z

VOLUME XXXIII - SUBJECT INDEX

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

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INSTRUCTIONS FOR AUTHORS

Acta Myologica publishes articles related to research in and the practice of primary myopathies, cardiomyopathies and neuromyopathies, including observational studies, clinical trials, epidemiology, health services and outcomes studies, 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.

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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:
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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.
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Standard journal article: Figarella-Branger D, Bartoli C, Civatte M, et al. Cytokines, chemokines and cell adhesion molecules in idiopathic inflammatory myopathies. Acta Myol 2000;19:207-8.
Books and other monographs: Dubowitz V. Muscle disorders in childhood. London: WB Saunders Company Ltd; 1978.
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