ACTA MYOLOGICA

(Myopathies, Cardiomyopathies and Neuromyopathies)

Vol. XXXVI - December 2017

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

Founders: Giovanni Nigro and Lucia Ines Comi

Three-monthly

G. CONTE ACADEMY - NAPOLI -

PACINI EDITORE MEDICINA

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Acta Myologica publishes 4 issues per year in March, June, September, December. The Journal is available in OPEN ACCESS at: www.actamyologica.it

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E-mail: actamyologica@gmail.com, info@pacinieditore.it

Acta Myologica is cited in Index Medicus/MEDLINE, Medicine, Excerpta Medica Database (EMBASE), Index Copernicus and monitored for coverage in Chemical Abstracts Service. The Journal is available on PubMed Central (http://www.ncbi.nlm.nih.gov/pmc/journals/1221/).

Editor in Chief: Luisa Politano

Tribunal Authorization, Napoli N. 3827, January 10, 1989 - Journal registered at "Registro pubblico degli Operatori della Comunicazione" (Pacini Editore srl registration n. 6269 - 29/8/2001).

Publisher

Pacini
Editore

Via A. Gherardesca - 56121 Pisa, Italy

Published by Pacini Editore Srl - Pisa, Italy, December 2017

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Acta Myologica • 2017; XXXVI: p. 191-198

ORIGINAL ARTICLES

Differential diagnosis of vacuolar muscle biopsies: use of p62, LC3 and LAMP2 immunohistochemistry

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Intrafibral vacuoles are the morphological hallmark in a wide variety of human skeletal muscle disorders with different etiology. In most cases, differential diagnosis is feasible with a routine histochemical work up of muscle biopsy. Ultrastructural analysis is an important confirmatory tool, but it is not widely available. Immunohistochemical stainings for p62, LAMP2 and LC3 are commonly available as tissutal marker for autophagy. We compared the immunohistochemical patterns for autophagic markers p62, LC3 and LAMP2 with routine histochemical markers in 39 biopsies from patients with definite diagnoses of glycogen storage disease type 2 (LOPD or Pompe disease, PD), sporadic inclusion body myositis (sIBM), oculo-pharyngeal muscular dystrophy (OPMD) and necrotizing myopathy (NM). Moreover, we also analyzed muscles of 10 normal controls. In PD group, LC3 and LAMP2 showed an higher percentage of positive fibers, whereas p62 was limited to a minority of fibers, thus paralleling the results of histochemical stainings; in NM group, LAMP2 and LC-3 were diffusely and unspecifically expressed in necrotic fibers, with p62 significantly expressed only in two cases. OPMD biopsies did not reveal any significant positivity. The most interesting results were observed in sIBM group, where p62 was expressed in all cases, even in fibers without other markers positivity. There results, although limited to a small number of cases, suggest that the contemporary use of p62, LAMP2 and LC-3 staining may have an adjunctive role in characterizing muscle fiber vacuoles, revealing autophagic pathway activation and providing further clues for the understanding of pathogenetic mechanisms.

Key words: autophagy, Pompe disease, inclusion body myopathy, necrotizing myopathy, immunohistochemistry

Introduction

Autophagy is a highly conserved homeostatic process for lysosome mediated degradation of cytoplasmic components, including damaged organelles and toxic protein aggregates (1). The process of autophagy occurs through a multi-step mechanism, including the formation of a phagophore, which engulfs proteins and organelles destined for degradation, then the production of a membrane-bound vacuole (the autophagosome), which moves along microtubules and fuses with the lysosome to form the autolysosome. Microtubule-associated protein light chain 3 (LC3) is commonly used as a marker of autophagosome formation (2-3). Upon autophagy induction, its modified form LC3-II, associated with autophagic membranes, binds p62/SQSTM1, an adapter protein that targets ubiquitinated protein aggregates (4). The lysosomal-dependent turnover of LC3-II and p62 has emerged as a measure of autophagic proteolysis. Specifically, the accumulation of LC3-II-labeled autophagosomes and/or p62 aggregates is a robust marker of autophagic flux engulfment at any point beyond autophagosome formation (3).

Lysosome-associated membrane protein 2 (LAMP2) is a glycoprotein with a principal role in the adhesion of the lysosome, and therefore in their protection and maintenance studied in human tissues by immunohistochemical markers (5).

Autophagic vacuolar myopathies are a group of muscle disorders characterized by massive autophagic

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buildup. Pompe disease (PD) is due to a defect in lysosomal acid a-glucosidase (GAA), with intralysosomal glycogen accumulation (6); Danon disease is caused by lack of the lysosome associated membrane protein 2 (LAMP2) (7) with increase of dysfunctional lysosomes in skeletal and cardiac muscles; and the X-linked myopathy with excessive autophagy (XMEA) is due to mutations in V-ATPase (8). The exact role of autophagy dysfunction is still debated; however, in a recent work of Nascimbeni at al. (9) other autophagy regulators, like transcription factor EB (TFEB) and vacuolar protein sorting 15 (VPS15), seem to have an active role in the pathogenesis of both Danon disease and PD, showing an autophagy block correlated with the severity of the disease; therefore therapeutic approaches targeted to normalize these factors and restore the autophagic flux should be considered.

Other myopathies are characterized by the presence of intrafibral vacuoles, originated by different pathological processes; these include inclusion body myopathies (IBM), necrotizing myopathies (NM), and oculophayngeal muscular dystrophy (OPMD).

IBMs are classified as sporadic (s-IBM), a relatively common inflammatory myopathy classically presenting in older individuals (10-12), and hereditary (h-IBM), caused by gene mutations producing intrafibral protein storage, with disruption of cell architecture (i.e., UDP-Nacetylglucosamine-2 epimerase/N-acetylmannosamine-kinase or GNE gene).

Necrotizing myopathies (NM) have a multifactorial etiology; they may have an acute or subacute onset, can be severe, may have an autoimmune pathogenesis or be associated to cancer, and may be related to statin therapy. Diagnosis is based on the clinical picture and on muscle biopsy showing minimal or no inflammatory infiltrates and marked muscle necrosis with vacuolated fibers and macrophagic activation, unlike other inflammatory myopathies (10).

Oculopharyngeal muscular dystrophy (OPMD) is a late-onset muscle disease associated with progressive ptosis of the eyelids, dysphagia, and unique histological features, including intracytoplasmic rimmed vacuoles and tubule-filamentous intranuclear inclusions (INIs) in skeletal muscle. Polyalanine [poly(A)] expansion mutations in the polyadenine-binding protein 2 (PABN1) gene have been shown to cause OPMD (11). Since its impairment leads to accumulation of autophagosomes, autophagy can be detected by immunohistochemistry for autophagy proteins LC3 and p62/SQSTM1; immunostaining for either LC3 or p62 was proposed to replace electron microscopy in the diagnosis of autophagic vacuolar myopathies (12-15).

LC3 and p62 have also been evaluated as markers of IBM (16).

In routinary muscle biopsy evaluation, differential diagnosis of vacuolar myopathies can be challenging for the presence of only mild alterations, or unspecific/unrelated tissue changes; moreover, in some cases clinical data may lack or are only partially supportive.

Aim of this retrospective study is to verify the use of a simple immunohistochemical procedure to detect autophagic activation in a series of muscle biopsies with a defined diagnosis of vacuolar myopathy, in order to ameliorate the diagnostic accuracy.

Materials and methods

Ethics statement

All patients had undergone quadriceps muscle biopsy for diagnostic purposes, and signed full informed consent. No individually identifiable patient data are presented in this report.

Objectives

This is a retrospective study on muscle tissue samples stored in liquid nitrogen from patients affected with different types of vacuolar myopathies, namely late onset PD (LOPD), NM, s-IBM, OPMD. Immunohistochemistry for p62, LC3 and/or LAMP2 was compared with routine histological and histochemical staining, in order to evaluate their role as diagnostic tools for the differentiation of autophagic vacuolar myopathies.

Case selection

A search of the database of tissue bank at the Neuromuscular Unit was carried out, spanning the interval between 1988 and 2016. Only patients with complete clinical, genetic and follow up data were included.

Twenty patients with a confirmed diagnosis of LOPD (9 men, 11 women; mean age 44.4 ± 28.8); seven sIBM patients (4 men, 3 women; mean age 65 ± 14.9) and four OPMD patients (1 man, 3 women; mean age 55.2 ± 10.1) were included in the study. Moreover, eight patients with NM were also considered (5 men, 3 women; mean age 51.1 ± 21.4).

Normal controls (5 men, 5 women; mean age 60.5 ± 7.1) were selected from a larger pool of muscle biopsies characterized by lack of pathologic findings

Patients characteristics are reported in Table I; levels of plasma creatine kinase prior to biopsy were available in the clinical record in 47 out of 49 subjects. A review of the original muscle biopsy slides was also made.

Table I. Clinical characteristics in 49 cases.

Subject ID	Group	CK level (x n.v.)	Sex	Age	Clinical involvement
1	LOPD	3X	F	44	Mild
2	LOPD	NA	F	34	Absent
3	LOPD	5X	M	52	Absent
4	LOPD	8X	M	66	Severe
5	LOPD	6X	M	34	Mild
6	LOPD	10X	F	30	Mild
7	LOPD	2X	F	57	Mild
8	LOPD	3X	F	74	Severe
9	LOPD	2X	M	42	Mild
10	LOPD	4X	F	49	Mild
11	LOPD	4X	M	1	Absent
12	LOPD	6X	F	52	Severe
13	LOPD	2X	M	61	Mild
14	LOPD	N	M	74	Severe
15	LOPD	5X	F	39	Absent
16	LOPD	5X	M	1	Severe
17	LOPD	4X	F	61	Absent
18	LOPD	2X	F	49	Absent
19	LOPD	2X	F	15	Absent
20	LOPD	N	M	53	Severe
21	NM	N	F	76	Mild
22	NM	6X	F	53	Mild
23	NM	4X	M	29	Severe
24	NM	N	M	34	Mild
25	NM	2X	M	82	Mild
26	NM	38X	M	25	Mild
27	NM	37X	M	64	Mild
28	NM	11X	M	46	Mild
29	IBMyositis	2X	F	33	Severe
30	IBMyositis	NA	F	61	Mild
31	IBMyositis	3X	F	71	Severe
32	IBMyositis	2X	M	68	Severe
33	IBMyositis	2X	M	77	Mild
34	IBMyositis	5X	M	73	Mild
35	IBMyositis	2X	M	72	Severe
36	OPMD	N	F	59	Mild
37	OPMD	N	F	66	Severe
38	OPMD	N	F	42	Mild
39	OPMD	N	M	54	Mild
40	CONTROL	N	M	47	Absent
41	CONTROL	N	M	54	Absent
42	CONTROL	N	M	58	Absent
43	CONTROL	N	M	65	Absent
44	CONTROL	N	M	71	Absent
45	CONTROL	N	F	62	Absent
46	CONTROL	N	F	58	Absent
47	CONTROL	N	F	61	Absent
48	CONTROL	N	F	59	Absent
49	CONTROL	N	F	70	Absent

Muscle biopsy sections

Serial 7-8 μm sections were cut from muscle samples stored in liquid nitrogen, and parallel processed for hematoxylin and eosin (H&E), modified trichrome Gomori, Periodic acid Schiff (PAS) stain and acid phosphatase stain according with routine procedures.

Immunohistochemistry

Immunoperoxidase staining for LC3 (mouse monoclonal antibody, clone 5F10, Nanotools; 1:100 dilution following antigen retrieval) and p62/SQSTM1 (guinea pig polyclonal antibody, Progen Biotechnik; 1:100 dilution following antigen retrieval) was performed on frozen tissue samples.

For LAMP2 we used purified rat anti-mouse CD107b monoclonal antibody, clone ABL-93, BD Biosciences, 1:100).

Quantification

Quantification was performed on muscle sections using a bright-field light microscope, with the investigator blind to group assignment of each subject. Prior to counting, each slide was viewed at low (2x-20x) and high power (40x) to determine whether positive fibers were present scarcely or in abundance. Muscle fibers containing the characteristic central inclusion, rimmed vacuoles, or punctate staining pattern were counted as positive, while fibers devoid of staining were counted as negative. The same criteria were used for morphological and histochemical stainings. A total of 200 fibers/slide were counted in specimens with abundant positivity, while a total of 600 fibers/slide was counted in specimens with scarce or patchy positivity (to reduce the sampling error). Tissue on the slide was divided into quadrants and randomly selected, non-overlapping fields were counted at high power in each quadrant until the total count was reached. The results were recorded as a percentage (the number of positive fibers divided by the total number of fibers counted).

Imaging

Images were taken with an AXIO digital camera on a BX41 bright-field light microscope using cellSens Entry 1.4 software (all by Olympus Corp) and were edited with Adobe Photoshop Version 12.0.2.

Statistical methods

Data were analyzed with SPSS statistical software (Version 18). For between-group comparison of the demographic data we used one-way ANOVA with post-hoc Bonferroni test (age and sex). To calculate sensitivity and specificity receiver operating characteristic (ROC) analysis was performed on all muscle biopsies. All tests were 2-tailed with $\alpha = 0.05$.

Results

Detailed results of the percentage of positive fibers for each staining are reported in Table II.

On light microscopy, we identified several histologic patterns suggestive of different cathegories of vacuolar myopathies.

In the LOPD patients group, characterized by variable clinical and muscle tissue involvement, LAMP2 and LC3 were positive in 65% of patients, whereas p62 positivity was seen only in 25% of subjects with a finely punctate staining pattern, paralleling morphological, PAS and acid phosphatase reactions (55%) (Figg. 1A, 2). No correlation with the clinical features was observed.

The NM group presented more extensive alterations with all methods, showing variable and heterogeneous expression of LAMP2 (7 out of 8 cases) and LC3 in 4 subject (Fig. 3), mainly in necrotic fibers, with less specificity; interestingly, p62 positivity was strongly observed only in 2 cases (22 and 27), both of them with a necrotizing myopathy of unknown origin and severe rabdomyolisis.

In IBM group, p62 and LC3 were diffusely expressed; in particular, p62 was positive in all eight subjects (Fig. 4B) respect to LC3 positivity in 6 cases (Fig. 4D). Differently from the NM group, LAMP2 showed a less significant expression in 6 cases (Fig. 4C).

In OPMD group, p62, LAMP2 and LC-3 were substantially negative in all cases, with LC3 mild unspecific staining only in a couple of fibers in 2 patients, LAMP2 in 1 case and no positivity for p62 antibody in all cases (data non shown).

In normal control sample, there was no intrafibral staining (Fig. 5B, C and D); a typical normal nuclear positivity was seen on LC3, LAMP2 and p62-stained sections.

There was no statistically significant difference in the mean age among the five groups (LOPD 44.4 ± 20.83 *vs* VM 51.1 ±21.49 *vs* IBM 65 ± 14.95 *vs* OPMD 55.2 ± 10.11 *vs* CONTROLS 60.5 ± 7.16 years; $p = 0.056$) or sex distribution (LOPD 55% *vs* VM 25% *vs* IBM 43% *vs* OPMD 75% *vs* CONTROLS 50% female, respectively; $p = 0.019$).

Figures 6 to 8 show the percentage of LC-3, LAMP2 and p62 positive fibers respectively in the different patients groups compared with the control group.

As expected, the higher percentage of positive fibers for autophagy markers is observed in LOPD patients, with a major occurrence of LAMP2 and LC3 staining;

Table II. Percentage of positive fibers for morphological, histochemical and immunohistochemial staining in 39 patients. All controls were completely negative.

Subject ID	Group	LC-3 (% positive fibers)	p62 (% positive fibers)	LAMP2 (% positive fibers)	HE (% vacuolated fibers)	TRIC (% vacuolated fibers)	PAS (% vacuolated positive fiber)	Acid phosphatase (% positive fibers)
1	LOPD	3,5	4	6.5	3	13.5	5.5	3
2	LOPD	0	0	0	0	0	2.5	0
3	LOPD	0	0	0	0	0	0	0
4	LOPD	37.5	2	25	25	45	35	20
5	LOPD	0	0	0	0	0	0	0
6	LOPD	0	0	0	0	0	0	0
7	LOPD	6	0	1.5	3	23.5	4	3
8	LOPD	7.5	4.5	14	7.5	13	5	2
9	LOPD	0	0	0	1.5	0	0	1.5
10	LOPD	0.5	0	3.5	2	1.5	1.5	3
11	LOPD	0	0	14	5	4.5	8.5	2.5
12	LOPD	12.5	4	26	12,5	10	20	7.5
13	LOPD	28	0	34	26.25	40	25	7.5
14	LOPD	8.5	0	10	6	3.5	3.5	3
15	LOPD	21.5	2	25.5	20	15	18.5	15
16	LOPD	6	0	0	75	75	75	75
17	LOPD	0	0	0	0	0	0	0
18	LOPD	16	0	0	0	0	0	0
19	LOPD	0	0	0.5	0	0	0	0
20	LOPD	0	0	0	0	0	0	0
21	NM	2.5	0	7	15	10	5	2.5
22	NM	0	21	1	0.5	1	1	2.5
23	NM	1	0	1.5	1.5	0	3	0
24	NM	0	0	1.5	10	7.5	10	3
25	NM	2	1	2,5	2,5	1	2	0
26	NM	0	0	9	0,5	0,5	0	12,5
27	NM	19	13	14	4,5	5	2	6,5
28	NM	0	0	0	1	0,5	0	0
29	sIBM	9	6	15	18,5	25	1,5	2
30	sIBM	9	5,5	6,5	6	6	0	1,5
31	sIBM	9,5	7	2,5	5	7,5	1,5	1,5
32	sIBM	5,5	15,5	3,5	2	2	2	0
33	sIBM	8	5,5	4,5	6	3,5	0	1,5
34	sIBM	0	14,5	2,5	1,5	0	0	0,5
35	sIBM	6	5,5	0	2,5	1,5	0	2
36	OPMD	0	0	1	0	1	0	0
37	OPMD	1,5	0	0	0	1	0	0
38	OPMD	1,5	0	0	0	0	0	0
39	OPMD	0	0	0	2	1,5	0	0,5

however, also in NM and in sIBM these three antibodies seem to have a different significance recognizing different patterns. In fact, whereas the percentage of LC3 and LAMP2 positive fibers is not statistically significant between different groups (Figs. 6, 7), the percentage of p62-positive fibers in muscle sections was significantly higher in sIBM group than in LOPD ($p < 0.001$ ANOVA with Bonferroni correction), OPMD ($p < 0.01$ ANOVA with Bonferroni correction) and in the control group ($p < 0.001$ ANOVA with Bonferroni correction) (Fig. 8); p62 positivity was also observed in muscle fibers showing normal histochemical features. ROC analysis of our data indicates a 100% specificity and 75% sensitivity of p62 staining for IBM.

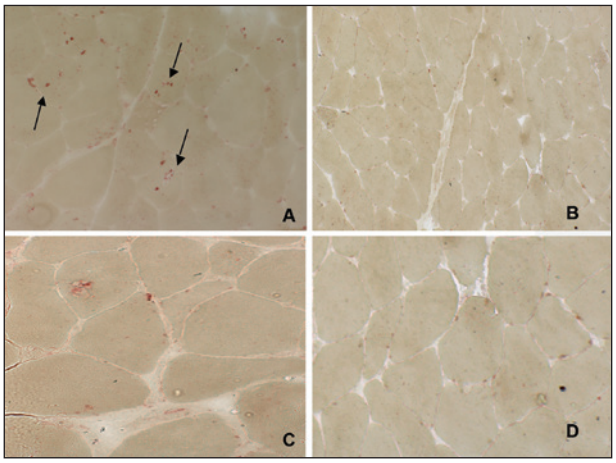

Figure 1. A representative case of acid phosphatase in LOPD (A), in NM (B), in sIBM (C),in OPMD (D) and in a control group (E). In the first three groups acid phosphate is present in focal regions in fibers (arrows).

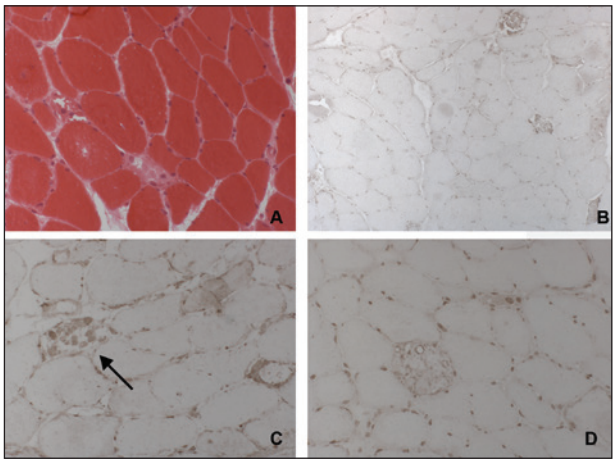

Figure 3. Necrotizing myopathy staining patterns. A representative case of necrotizing myopathy – (A-D) –; subject #25) shows vacuolated fibers on H&E 20X (A), mildly positive with p62 (B), LAMP2 (C) and LC3 (D).

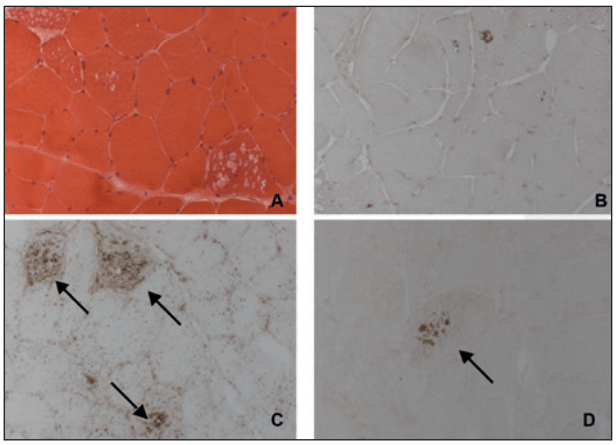

Figure 2. LOPD staining patterns. A representative case of LOPD (A-D; subject #15) shows several autophagic membrane-bound vacuoles seen on H&E 20X (A), LAMP2 (C) and LC3 (D), whereas only a few are p62 (B) positive.

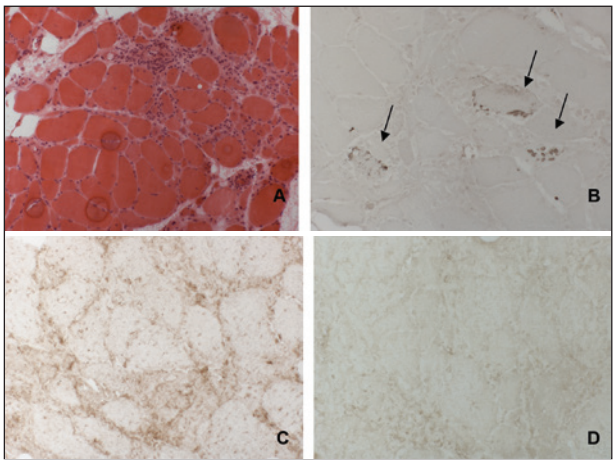

Figure 4. sIBM staining patterns. A representative case of sIBM – (A-D) –; subject #30) shows inflammatory cells and vacuolated muscle fibers seen on H&E 20X (A). There is a significant staining with p62 (B, arrows). Staining for LAMP2 and LC3 are negative.

Discussion

Differential diagnosis of vacuolar myopathies is usually achieved with the routine set of histological and histochemical staining on frozen muscle tissue; congruent clinical data are also necessary to distinguish among the great variety of myopathological entities. In some outlier cases, with only minor changes and with partial or incomplete clinical data, the ultrastructural exam may be necessary to reach a definitive diagnosis. However, this procedure is not diffusely available, is expensive and time-consuming.

In this study, we evaluated only by immunohistochemistry the potential adjunctive utility of p62, LC3 and LAMP2 in four groups of muscle disorders characterized by intrafibral vacuoles, and in a group of normal controls.

In LOPD, a lysosomal disease with defective autophagy, LC3, LAMP2 and p62 stainings were comparably positive with a punctate pattern, reflecting the association of LC3-II with the membranes of early autophagosomes, whereas p62 puncta correspond to the accumulation of protein aggregates within early autophagic (LC3-positive) vesicles; hence, the increased punctate staining seen with these markers corresponds to autophagosome

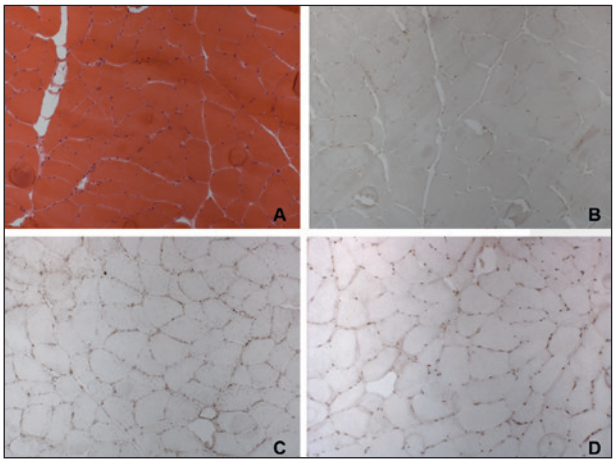

Figure 5. A representative case of normal control group – (A-D) –; subject #47). Lack of sarcoplasmic staining of p62, LAMP2 and LC3. There was background nuclear positivity wit all markers (B-D).

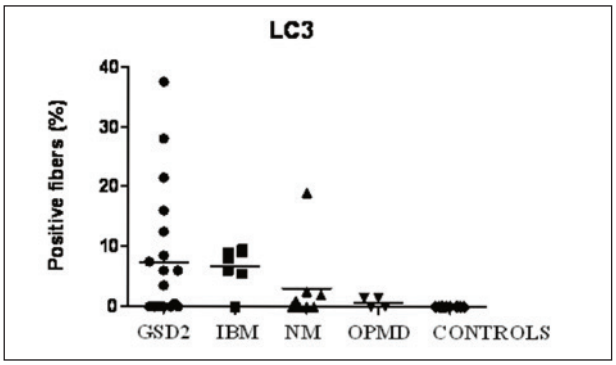

Figure 6. Quantification of LC3-positive fibers in frozen sections in different groups. The percentage of LC3-positive fibers was not statistically significant in LOPD, NM, sIBM, OPMD and in control group. Each study subject is represented with a symbol, lines indicate group means.

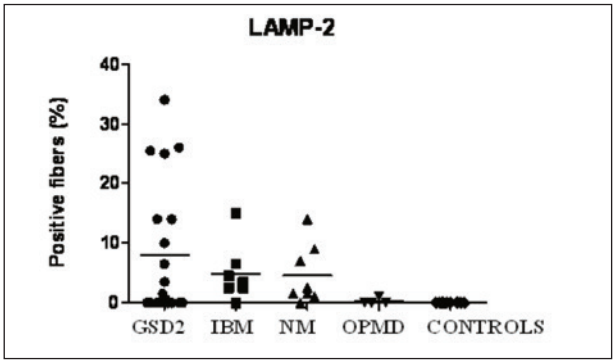

Figure 7. Quantification of LAMP2-positive fibers in frozen sections in different groups. The percentage of LAMP2-positive fibers was not statistically significant in LOPD, NM, sIBM, OPMD and in control group. Each study subject is represented with a symbol, lines indicate group means.

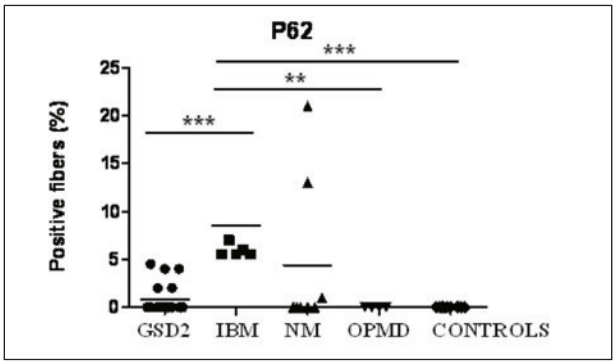

Figure 8. Quantification of P62-positive fibers in frozen sections in different groups. The percentage of p62-positive fibers was significantly higher in sIBM group than in LOPD ($p < 0.001$ ANOVA with Bonferroni correction), OPMD ($p < 0,01$ ANOVA with Bonferroni correction) and in the control group ($p < 0,001$ ANOVA with Bonferroni correction). p62 was marked positive only in two cases in NM group. Each study subject is represented with a symbol, lines indicate group means.

buildup (17). The observed minor incidence of p62 fibers in LOPD group may reflect the different stages of the autophagic process in these patients. In LOPD, LC3 seems to have the higher sensibility, in comparison to conventional stainings and the other markers. However, no LOPD case was detected by immunohistochemistry alone associated to a negative histochemical staining. In fact, in six out of 20 LOPD cases (30%), quadriceps muscle biopsy was totally normal, confirming the need to perform the biochemical test in all cases with a clinical suspect.

In most cases of clinically defined sIBM, intrafibral vacuoles show the typical 'red rim' staining with Gomori's trichrome and are easily recognized. However, non-rimmed vacuoles are also observed, and a possible activation of autophagic pathway is also hypothesized (18). Immunohistochemistry in sIBM biopsies is often characterized by aspecific and variable staining by a variety of antibodies utilized in the routinary muscle biopsy diagnostic study, making this procedure less significant in sIBM diagnosis. In all sIBM cases of our study, we found a significant positivity only for p62, with small positive puncta distributed throughout the sarcoplasm of a higher number of fibers in comparison to the other markers, supporting the hypothesis of a specific autophagic activation in these cases. (Fig. 4B, C and D). In LOPD cases, the puncta were larger and primarily (although not exclusively) located in the center of a reduced number of fibers. Several earlier studies have examined LC3, p62 or LAMP2 staining in the setting of IBM; however, no single work quantitatively compared all three markers on the same set of well-defined specimens (19). In our study p62, but not LC3 and LAMP2, effectively distinguished

the sIBM subject group from other vacuolar myopathy subject; moreover, p62 immunohistochemistry showed the best tradeoff between sensitivity and specificity for sIBM as a diagnostic test applied to an individual case. The p62 staining was qualitatively similar to LC3 staining, consistent with the idea that accumulation of either LC3-labeled autophagosomes or p62- positive aggregates are a marker of autophagic flux inhibition in sIBM.

In OPDM, also characterized by the presence of intrafibral vacuoles, the presence of autophagic activation was excluded in all cases; therefore immunohistochemistry may be useful in the differential diagnosis when clinical data are lacking or unsupportive, in particular in the presence of rimmed vacuoles.

Immunohistochemistry for autophagic markers did not add any additional information in necrotizing myopathies, since necrotic fibers showed a variable and unspecific staining with all antibodies. Interestingly, two patients presented a strong autophagic activation, thus challenging the diagnosis, and a specific follow up is ongoing.

Based on these findings, we can conclude that p62, LAMP2 and LC3 immunohistochemistry have a significant role in the routinary study of muscle when clinical data are not supportive, and could be included in the panel of antibodies when a vacuolar myopathy is observed with histochemical procedure. In particular, LC3 antibodies have a slightly higher specificity in LOPD biopsies, whereas a strong selective p62 positivity seem to be more indicative of sIBM. On the contrary, LAMP2 does not add important clues in differential diagnosis of these pathologies.

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Acta Myologica • 2017; XXXVI: p. 199-202

Study of anti-Müllerian hormone levels in patients with Myotonic Dystrophy Type 1. Preliminary results

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Myotonic dystrophy type 1 is a multisystemic disorder characterized by myotonia, muscle weakness and involvement of several organs and apparatus such as heart, lungs, eye, brain and endocrine system. Hypogonadism and reproductive abnormalities are frequently reported. A progressive testicular atrophy occurs in about 80% in the affected males leading to Leydig cell hyperproliferation and elevated basal follicle stimulating hormone (FSH) levels. Anti-Müllerian hormone (AMH) – a dimeric glycoprotein belonging to the super-family of transforming grow factor beta (TGF-beta) – is the earliest Sertoli cell hormone secreted in males and, together with inhibin B and FSH, is an important indicator of Sertoli cell function. AMH levels remain high during the whole prepubertal phase and are down-regulated in puberty by the increasing testosterone levels. Aims of the work were to assess the AMH levels in 50 patients with Myotonic Dystrophy type 1 aged less 50 years and to investigate whether it may contribute to the endocrine function impairment observed in these patients. The results confirmed a reduction of testosterone levels associated with an increase in Luteinizing Hormone (LH) and FSH compared to controls, suggesting a reduced function of the Sertoli cells. Conversely the average levels of AMH were significantly lower in patients compared with controls, and almost undetectable in about 60% of them. Further studies are necessary to better clarify these findings.

Key-words: myotonic dystrophy type 1, gonadal function, anti-Müllerian hormone

Introduction

Myotonic dystrophy type 1 (DM1) or Steinert disease is a multisystemic disorder characterized by myotonia, muscle and facial weakness, cataract, cognitive and gastrointestinal involvement, and cardiac conduction abnormalities (1). DM1 is the most common adult muscular dystrophy with a global incidence of 1:8000. Symptoms appear between 20 and 40 years of age and the localization of muscle weakness is predominantly distal (1-4). It is a RNA-mediated disease caused by a trinucleotide expansion, the CTG repeat in the DMPK gene (4) on the long arm of chromosome 19 (19q13-2). The endocrine system is also frequently involved as hypogonadism and reproductive abnormalities (1-4). Progressive testicular atrophy is a prominent feature and occurs with an incidence of 80% in the affected males (5). The observed histological abnormalities include tubular atrophy, hyalinization and fibrosis of seminiferous tubules as far as a reduced sperm number. Oligo/azoospermia is reported in 73% of DM1 patients while low serum testosterone levels are observed in most patients. The progression of the disease leads to Leydig cell hyperproliferation, elevated basal follicle stimulating hormone (FSH) levels and gonadal dysfunction (6-13). Therefore the evaluation of gonadal function, including interstitial Leydig cells and tubular Sertoli cell hormone production, is recommended in the workup of male hypogonadism.

Anti-Müllerian hormone (AMH) is a dimeric glycoprotein composed of two 72 KDa monomers belonging to the super-family of transforming grow factor beta (TGF-beta) (14). The coding gene for this hormone is localized in humans on the short arm of chromosome 19

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(19p13.3). The gene spans 275 bp and is subdivided into five exons. The expression of AMH in males is usually restricted to foetal and post-natal testosterone cells, and, in females, in post-natal granulosa cells.

The AMH molecule takes its name from the first function described in foetal sex differentiation, the regression of Müller ducts in the early phase of male differentiation. In the male foetus the Sertoli cells secrete AMH and androgens. Androgens in turn stimulate the evolution of Wolff's ducts into the male genital apparatus, while AMH causes the irreversible regression of the Müller's ducts, which is completed at the end of the ninth week of gestation. With the exception of a transient decrease in the perinatal period, the testicular secretion of AMH remains at high levels until puberty (Fig. 1) (14-16). For such behaviour, AMH dosage was proposed as a marker for the evaluation of Sertoli cell activity and an early identification of the pre-puberal male hypogonadism (17-23).

The study aimed at evaluating the gonadal function of patients with Myotonic Dystrophy type 1, and the possible involvement of the AMH in mechanisms underlying the hypogonadism and reproductive abnormalities frequently observed in these patients. The purpose was to highlight a possible association between testosterone, LH, FSH and estradiol levels with those of AMH, and to investigate a possible correlation between AMH levels and age of patients and/or degree of CTG triplet expansion.

Subjects and methods

Fifty male patients affected by Myotonic dystrophy type 1 aged between 18 and 50 years, regularly followed at the Cardiomyology and Medical Genetics of the "L.Vanvitelli" University, and 60 age-matched adult males were consecutively enrolled in the study.

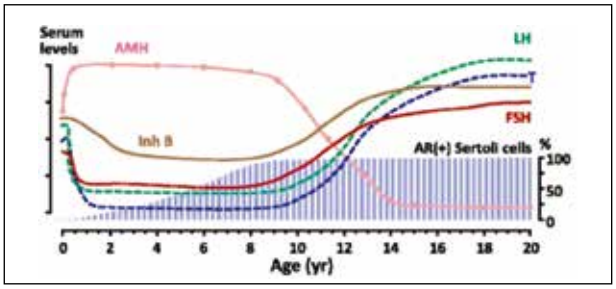

Figure 1. Schematic representation of serum levels of gonadotropins (FSH and LH), testosterone (T), inhibin B (Inh B) and AMH from birth through adulthood (left axis) and percentage of Sertoli cells expressing the androgen receptor (AR, right axis).

All patients had the clinical diagnosis confirmed by molecular analysis to define the magnitude of the triplet expansion. In practice, on the occasion of the routine follow up, a blood sample was taken for hormone (testosterone, 17ß-estradiol, luteinizing and stimulating follicle hormones) dosage, while an aliquot of the collected serum was used to dose the AMH. A written informed consent was obtained from all participants to the study, that was approved by the local ethical committee.

The hormone dosage was performed according to CLIA Method DiaSorin, while AMH was dosed by a II generation ELISA kit (Beckman Coulter, Brea, CA, USA).

Statistical analysis

The values are shown as mean ± SEM. Statistical differences were analysed by Student t test for non paired data; significance was put for p values < 0.05.

Results

The results are summarized in Table I and Figure 2.

Serum testosterone levels – although within the normal ranges – were on average significantly lower in DM1 than in controls (272.4 ± 55.8 ng/dL *vs* 563.3 ± 80.74 ng/dL, p value 0.0083; Fig. 2A).

The average values of LH and FSH were above the maximum limit of normality and statistically higher than in controls (11.1 ± 2.9 UI/L *vs* 4.4 ± 0.8 UI/L, p value < 0.0425, and 19.4 ± 4,7 UI/L *vs* 6.0 ± 1.4 UI/L, p value < 0.013, respectively; Fig. 2B-C).

Conversely, the average values of estradiol were not significantly different compared to controls (27.2 ± 3.7 pg/ml *vs* 30.5 ± 5.0 pg/ml, p value 0.6; Fig. 2D).

Focusing on AMH levels, a broad spectrum of values was observed in patients with DM1, ranging from 0.01 to 8.5 ng/ml. Furthermore 59% of them showed almost undetectable values (0.11 ± 0.07 ng/ml, p value 0.0064). The mean value of the whole group was 2.4 ± 0.97 ng/ml, significantly lower compared with controls (6.4 ± 1.4 ng/ml, p value 0.0318; Fig. 2E).

To be noted that patients who had almost undetectable values of AMH, presented also the highest values of LH and FSH (14.4 ± 4.3 UI/L *vs* 3,7 ± 0,95 ng/ml and 24.9 ± 5.6 *vs* 5,3 ± 1,3 UI/L), and the differences were statistically significant (p value 0.042, 0.006 and 0.006 respectively; Fig. 2F).

A trend to a negative correlation between AMH levels and age of patients and size of triplet expansion were observed (see Figures 3A and 3B, respectively).

Table I. Hormonal values in patients with Myotonic Dystrophy type 1.

Serum hormones	DM1 patients	Controls	P value
Testosterone (ng/dL)	272,4 ± 55,8	563,3 ± 80,7	0,0083
LH (UI/L)	11,1 ± 2,9	4,4 ± 0,8	0,0425
FSH (UI/L)	19,4 ± 4,7	6,0 ± 1,4	0,013
Estradiol (pg/ml)	27,2 ± 3,7	30,5 ± 5,0	0,6
AMH (ng/ml)	2,4 ±1,0	6,4 ±1,4	0,0318

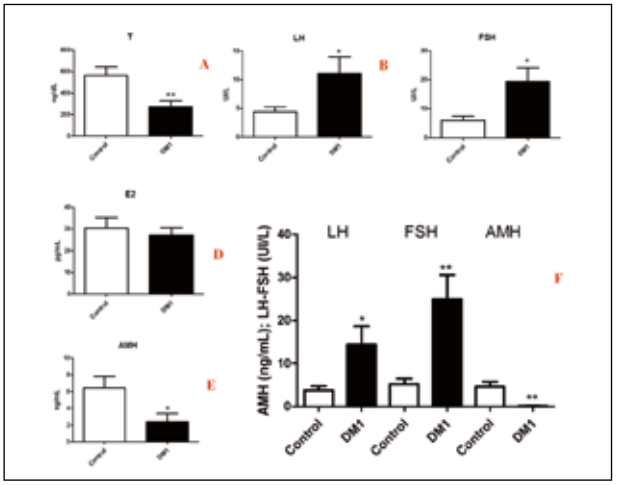

Figure 2. Hormone levels in DM1 patients and controls (A-F).
Testosterone levels (A); LH and FSH (B,C); Estradiol (D); AMH levels (E); Comparison of AMH and LH and FSH in the 2 groups (F).

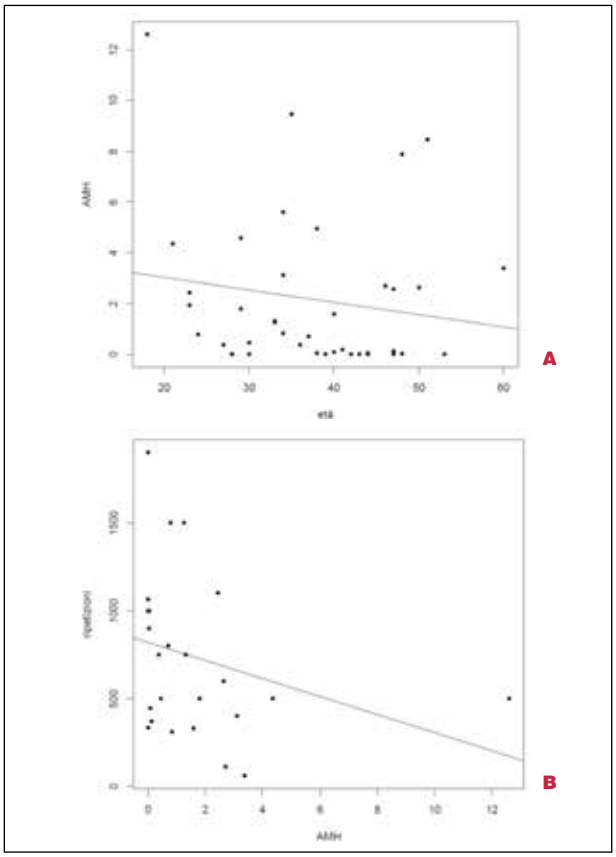

Figure 3. (A) AMH mean values according to the age of patients; (B). AMH mean values according to the number of triplets.

Discussion

Myotonic Dystrophy type 1 is a multisystem disease, with a wide pattern of clinical manifestations. Among these, the alterations of the endocrine system and in particular hypogonadism is one of the most frequently observed feature. The evaluation of the gonadal function, including interstitial Leydig cells and tubular Sertoli cells hormone production, is therefore recommended in these patients by the routine investigation of serum levels of testosterone, LH, FSH, and estradiol. As AMH has recently shown to play an important role in development of gonads and testicular function, and indicated as a possible marker of spermatogenesis, the dosage of serum AMH levels is also recommended. The evaluation of the gonadal function confirmed a condition of hypogonadism in our population, as serum testosterone levels were significantly lower compared to controls tough within the normal ranges. "However an impairment of the endocrine function and in particular of the Sertoli cells can be hypothesized by the observation that LH and FSH mean levels are statistically higher compared with controls.

Interestingly, it was observed that the average levels of AMH were significantly lower in patients compared with controls, and almost undetectable in about 60% of them. A trend to an inverse correlation between AMH and FSH levels was observed as the lower AMH levels were, the higher the levels of FSH. Further investigation are necessary to better define the contribution of the AMH in the impairment of the endocrine function in these patients, as far as that of proteins recently shown to be implicated in spermatogenesis (25-28).

Acknowledgements

The financial support of the Department of Experimental Medicine of University of Campania "Luigi Van-

vitelli" is gratefully acknowledged. We thank patients and their families for the cooperation.

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Acta Myologica • 2017; XXXVI: p. 203-206

The multifaceted clinical presentation of VCP-proteinopathy in a Greek family

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VCP-proteinopathy is a multisystem neurodegenerative disorder caused by mutations in valosin containing protein. Here, we report the first Greek case of VCP-proteinopathy in a 62 year old patient with a slowly progressing muscular weakness since his mid-40s and a severe deterioration during the last year. He also manifested dementia with prominent neuropsychiatric symptoms, including aggression, apathy, palilalia and obsessions. Brain MRI revealed frontal atrophy, while muscle MRI showed diffuse muscle atrophy. Family history was positive and several members of the family had been diagnosed with motor neuron disease, dementia or behavioral symptoms. Sequencing of the *VCP* gene revealed a pathogenic heterozygous missense mutation p.R159H. Conclusively, the present report highlights the intrafamilial variability and broadens the phenotypic spectrum of VCP-proteinopathy.

Key words: VCP, ALS, dementia

Introduction

Valosin Containing Protein (VCP) mutations have been well recognized as a cause of dominantly inherited Inclusion Body Myopathy, Paget's Disease and Frontotemporal Dementia (IBMPFD), although the term multisystemic proteinopathy has been proposed as a prevailing nomenclature in order to also include non-VCP IBMPFD and other rarer phenotypes, such as parkinsonism and peripheral neuropathy (1). IBMPFD (OMIM 167320) was firstly described in 2000 by Kimonis et al. (2) and the disease locus (*VCP* gene; 601023) was mapped to chromosome 9p13.3-p12 (3). VCP belongs to the cytosolic chaperone AAA class of ATPases of the endoplasmic reticulum-associated degradation (ERAD) system and is involved in many different cellular functions and signaling pathways, with its most important role facilitating proteasome-mediated degradation of misfolded polypeptides (4).

The clinical picture of the disease, as implied by its acronym, includes myopathy, Paget's disease and frontotemporal dementia with incomplete penetrance. Myopathy is the most common manifestation (~90%) and muscle weakness of variable pattern, either proximal or distal, may be usually the presenting symptom with onset in 3rd to 4th decade. Bone involvement is observed in almost half of the patients, while dementia of the frontotemporal type is a later onset symptom affecting approximately a third of the patients. However, the phenotypic spectrum of the disease has now broadened to include less frequent manifestations such as motor neuron disease, parkinsonism and Charcot-Marie-Tooth - like disease (1).

Herein, we describe the first Greek case of myopathy combined with an early onset progressive cognitive decline in a patient with a *VCP* mutation and a positive family history of neurodegenerative disorders, such as dementia and ALS.

Case report

The index patient is a 62 year old male with a personal history of arterial hypertension and an operation for herniated disc at the L4-L5 level at the age of 48 years. The symptoms began approximately in his mid-forties with steppage gait. His walking difficulties progressively worsened, especially during the last year and at the time of admission, he was no longer able to walk without aid. He also developed neuropsychiatric symptoms and in

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particular episodes of aggression and obsessions treated with atypical neuroleptics, such as quetiapine, with a satisfactory response, while he thereafter developed palilalia and apathy which still persist.

His mother had been diagnosed with "presenile dementia" and died at the age of 63 y (Fig. 1). His older brother had been diagnosed in his forties with classical ALS with a combination of upper and lower motor neuron involvement and subsequently died at 53 y. Another brother has been reported with behavioral symptoms since the age of 55 y.

On examination, the patient was unable to stand and walk without bilateral assistance. Although strength assessment of individual muscles was quite difficult due to poor cooperation, there was a severe symmetrical muscular weakness and a diffuse wasting in all muscles of upper and lower extremities, particularly prominent distally in lower limbs. Deep tendon reflexes were traced in upper and lower extremities, while no pathologic reflexes were elicited. Neuropsychological evaluation was abnormal with impaired conceptualization, mental flexibility and motor programming.

Laboratory studies, including serum creatine phosphokinase (CPK), transaminases (AST, ALT) and alkaline phosphates (ALP) were normal. Brain MRI revealed frontal lobe atrophy (Fig. 2). Bone radiographs did not reveal any abnormality suggestive of Paget's disease. Nerve conduction studies showed slightly low amplitudes of peroneal and tibial nerves obviously due to significant axonal loss, while EMG showed diffuse myopathic changes and mild spontaneous activity in the form of fibrillations and positive sharp waves in distal leg muscles. Muscle biopsy of the left vastus lateralis was not informative as it showed severe and non specific end stage changes with fibroadipose tissue replacement. Muscle MRI of lower limbs showed extended atrophy and fatty degeneration in almost all lower leg muscles with a relative sparing of the left biceps femoris at thigh level (Fig. 3), while muscle MRI of upper limbs revealed diffuse atrophy and fibroadipose tissue replacement especially of the posterior

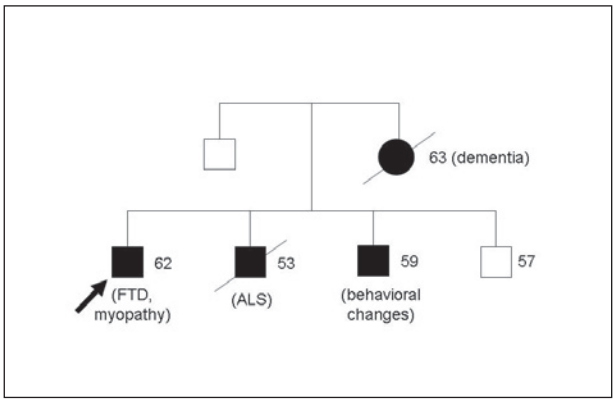

Figure 1. Pedigree of the patient's family (arrow indicates the index patient). Main symptoms and present age or age at death are indicated.

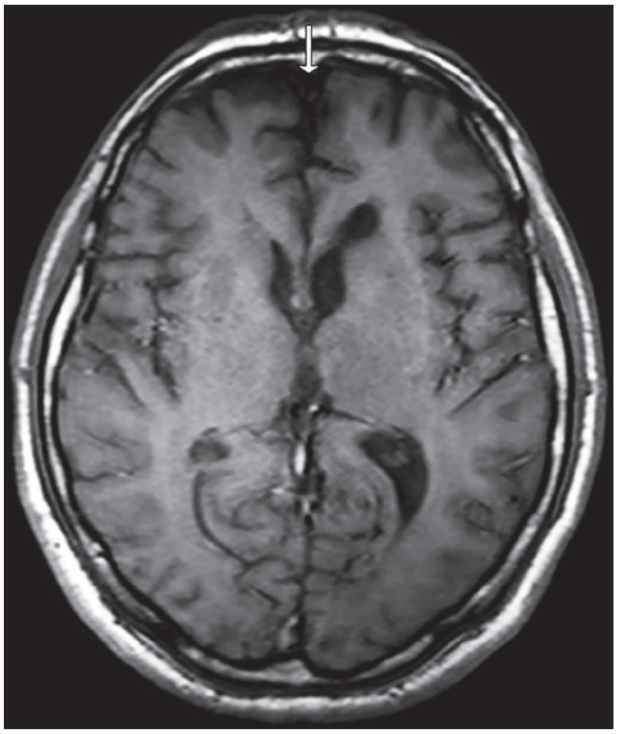

Figure 2. T1 weighted brain magnetic resonance image (MRI) transverse image showing frontal lobe atrophy (arrow).

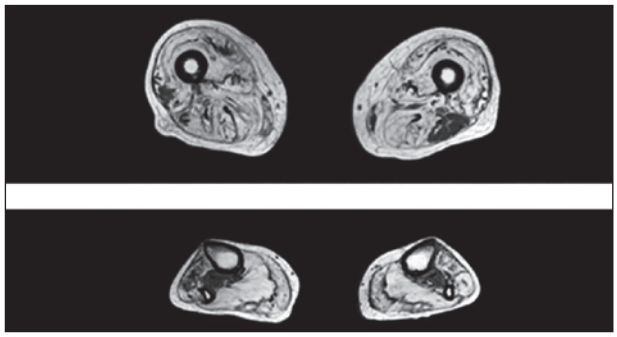

Figure 3. Muscle MRI of thigh and lower legs showing extensive T1w hyperintensity in most muscle groups suggesting severe fatty and/or fibrous degeneration. At thigh level there is an asymmetric relative sparing of the left biceps femoris and to a lesser extent of the left gracilis with a severe involvement of the other muscles and a characteristic patchy appearance particularly in vastus lateralis. At lower leg level there is also a severe involvement of both anterior and posterior compartment with a patchy appearance of anterior tibialis and peroneal muscles and a relative sparing of tibialis posterior muscle.

and anterior compartment of the arm (more pronounced in biceps and triceps) and to a lesser extent of the forearm muscles. Diagnosis of IBMPFD was confirmed by direct Sanger sequencing of coding regions and flanking intronic regions in the *VCP* gene, which revealed a heterozygous missense mutation p.R159H (c.476G>A).

Discussion

IBMPFD is a rare, clinically heterogeneous disorder transmitted in an autosomal dominant manner (1). Myopathy is by far considered as the prevailing presenting symptom in most cohorts, though with variable pattern of muscle involvement. Although proximal shoulder and pelvic muscle weakness is frequently observed, a scapuloperoneal or a predominant distal phenotype has also been reported, even at the onset of the disease (5-7). A selective pattern of muscle involvement mainly affecting glutei, hamstrings and calf muscles, may be observed on muscle MRI, usually with an inhomogeneous distribution of fatty replacement (8). Early-onset dementia of frontotemporal type may occur in approximately one third of patients, diagnosed at a mean age of 55 years (1). Quite interestingly, cognitive decline and behavioral changes are observed in most affected members of the present family and indeed the mother of the index patient has suffered from a pure dementing syndrome, although some degree of a concomitant myopathy cannot be definitely excluded.

In the present case, the family history with the constellation of symptoms, such as myopathy, dementia and an ALS-like syndrome in members of consecutive generations, which are indicative of an apparently autosomal dominant inheritance pattern, raised the suspicion for a possible VCP-associated syndrome. Sequencing analysis of the *VCP* gene in the index patient revealed the already known p.R159H pathogenic mutation. This mutation has been previously described in few families with a heterogeneous presentation and severity of disease. More specifically some affected members have been diagnosed with familial ALS (fALS) and/or FTD without signs of myopathy, whereas another family had affected members with a milder phenotype consisting of myopathy and PD without signs of dementia despite their age being far above the mean age at onset of FTD in *VCP* carriers (9, 10). Since an increasing body of evidence suggests that ALS may be part of a wide clinical spectrum, targeted genetic panel testing may be considered in familial cases of co-occurrence of ALS with dementia syndrome and/or myopathy including *VCP*, *C9orf72*, *TARDBP*, *SQSTM1*, *MATR3*, *HNRNPA2B1*, and *CHCHD10* (11).

Currently, more than 20 genes were identified to cause ALS and FTD. On the genetic basis, the complex network that underlies the pathogenesis of ALS and FTD was elucidated. Interestingly, disease-related mutant proteins form aberrant aggregates in two essential cellular machineries: The RNA quality and protein quality control machineries. Aggregations are hallmarks of many neurodegenerative disorders, and inclusions of the ubiquitous, highly conserved RNA binding protein TDP-43 represent the most important unifying marker throughout ALS molecular pathology. VCP/p97 is an important TDP-43 interaction partner and VCP proteinopathy contributes to TDP-43 dysregulation. Furthermore, TDP-43 proteinopathy is also predominantly associated with a certain frontotemporal lobar dementia (FTLD) subtype Ub+(FTLD-U)/TDP+ (FTLD-TDP). Therefore, ALS and the FTLD-TDP subtype are thought to represent different clinical manifestations of a common pathological pathway (12). Notably, aggregated, cytoplasmic TDP-43 inclusions have been detected in both hereditary and sporadic ALS with or without *TARDBP* mutations. Toxic gain of function and loss of function mechanisms for TDP-43 are discussed. Furthermore, at least three ALS-related genes, including *VCP*, *MATR3* and *SQSTM1/p62*, have been implicated in distal myopathies (11). The evidence of combined ALS/distal myopathy phenotypes in some individuals and the presence of TDP-43 inclusions on muscle biopsy further support the hypothesis of an ALS-FTLD/myopathy continuum.

Conclusively, the present case adds to the phenotypic heterogeneity in VCP proteinopathy and highlights an even striking intrafamilial variation. Myopathy followed by rapidly cognitive decline was the clinical presentation of the index case, while the other siblings presented with different phenotypes, such as dementia and ALS. Overall, the poor genotype-phenotype correlation possibly implies that other modifying factors may contribute to the clinical heterogeneity of *VCP* mutations. The elucidation of the underlying pathomechanisms may explain the clinical diversity and will be essential in providing relatively accurate prognostic information and genetic counseling.

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Acta Myologica • 2017; XXXVI: p. 207-212

Three new cases of dilated cardiomyopathy caused by mutations in LMNA gene

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Three cases of delated cardiomyopathy (DCM) with conduction defects (OMIM 115200), limb girdle muscular dystrophy 1B (OMIM 159001) and autosomal dominant Emery-Dreifuss muscular dystrophy 2 (OMIM 181350), all associated with different LMNA mutations are presented. Three heterozygous missense mutations were identified in unrelated patients – p. W520R (c.1558T > C), p.T528R (c.1583C > G) and p.R190P (c.569G > C). We consider these variants as pathogenic, leading to isolated DCM with conduction defects or syndromic DCM forms with limb-girdle muscular dystrophy and Emery-Dreifuss muscular dystrophy. The mutations were not detected in the ethnically matched control group and publicly available population databases. Their de novo occurrence led to the development of the disease that was not previously detected in the extended families. Mutations at the same codons associated with laminopathies have been already reported. Differences in the clinical phenotype for p.R190P and p.T528R carrier patients are shown and compared to previous reports.

Key words: dilated cardiomyopathy, limb-girdle muscular dystrophy, Emery-Dreifuss muscular dystrophy

Background

The LMNA gene (1q21-22, MIM 150330) encodes two proteins of the nuclear envelope – lamin A and C. They are intermediate filament proteins necessary for functioning and structural integrity of the nucleus. Lamins consist of an amino-terminal head domain, a coiled-coil central rod domain and a carboxy-terminal tail domain (Fig. 1A). They form dimers by rod domains and then associate in head-to-tail polymers creating complex network conjunction with other proteins located underneath the inner membrane of the nucleus. Mutations in LMNA affect lamins' dimerization and assembly (1, 2). It apparently leads to nuclear stability loss and inability to perform functions in its entirety. The mutations in LMNA lead to at least 10 clinically distinct phenotypes, termed laminopathies, affecting different tissues including cardiac and skeletal muscle, cutaneous, nervous and adipose tissue. There is no explicit relation between syndrome development and mutation domain localization. A number of hot spots were described in LMNA, but the mutations common for laminopathies were not found. Matching definite laminopathy symptoms with LMNA mutations brings us closer to understanding the genetic basis of the disease. Linkage analyses in affected families allow for prognosis and medication steps for mutation carriers.

This report presents three cases of laminopathy: dilatation cardiomyopathy with conduction defects (DCM, OMIM 115200), limb girdle muscular dystrophy 1B (LGMD, OMIM 159001) and autosomal-dominant Emery-Dreifuss muscular dystrophy 2 (EDMD, OMIM 181350) associated with the different LMNA mutations.

Methods

Patients and controls

Patients with DCM and conduction defects from the Scientific and Practical Center of Cardiology (Minsk, Belarus) were referred to Institute of Genetics and Cytology (Minsk, Belarus) for mutation analysis of the LMNA gene. The clinical diagnoses of patients included isolated DCM with conduction defects and syndromic DCM forms with limb-girdle muscular dystrophy and Emery-Dreifuss

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muscular dystrophy. Clinical syndromes were diagnosed according to the currently established criteria (3-5).

The control group consisted of 315 ethnically matched individuals without cardiovascular diseases, physical and neurological abnormalities, a family history of DCM or sudden cardiac death. They were selected from a previously studied population cohort (6).

Informed consent was obtained from all participants. Clinical surveillance and genetic investigation were performed in accordance with the recommendations of the local ethics committee of the Belarusian State Medical University and the Scientific Board of the Institute of Genetics and Cytology of the National Academy of Sciences, Belarus.

Genetic analysis

Genomic DNA was obtained from blood with phenol/chloroform extraction. Each LMNA exon and exon-intron boundaries were sequenced using the BigDye© Terminator v3.1 Cycle Sequencing Kit on a 3500 Genetic Analyzer (Applied Biosystems, Thermo Fisher Scientific, Waltham, MA). The primer sequences are available upon request.

All mutations were verified by restriction fragment length polymorphism (RFLP) in affected individuals and family members and the control group. Exons were PCR amplified and digested with endonucleases (p.R190P removes the FauI site in exon 3, p.W520R introduces the MspI site and p.T528R removes the RsaI site in exon 9).

Bioinformatics tools

Sequence variants were described according to the NCBI Reference Sequences NM_170708.3 and checked for population frequencies in Exome Sequencing Project, 1000 Genomes and Genome Aggregation Database. Multiple alignment of various orthologous sequences was built with Clustal Omega (http://www.ebi.ac.uk/Tools/msa/clustalo/). To predict the effects of amino acid substitutions on motif assembly, the wild-type (GenBank: AHL67294.1) and mutated sequences of lamin A/C were analysed in the Protein secondary structure prediction server JPred4 (7).

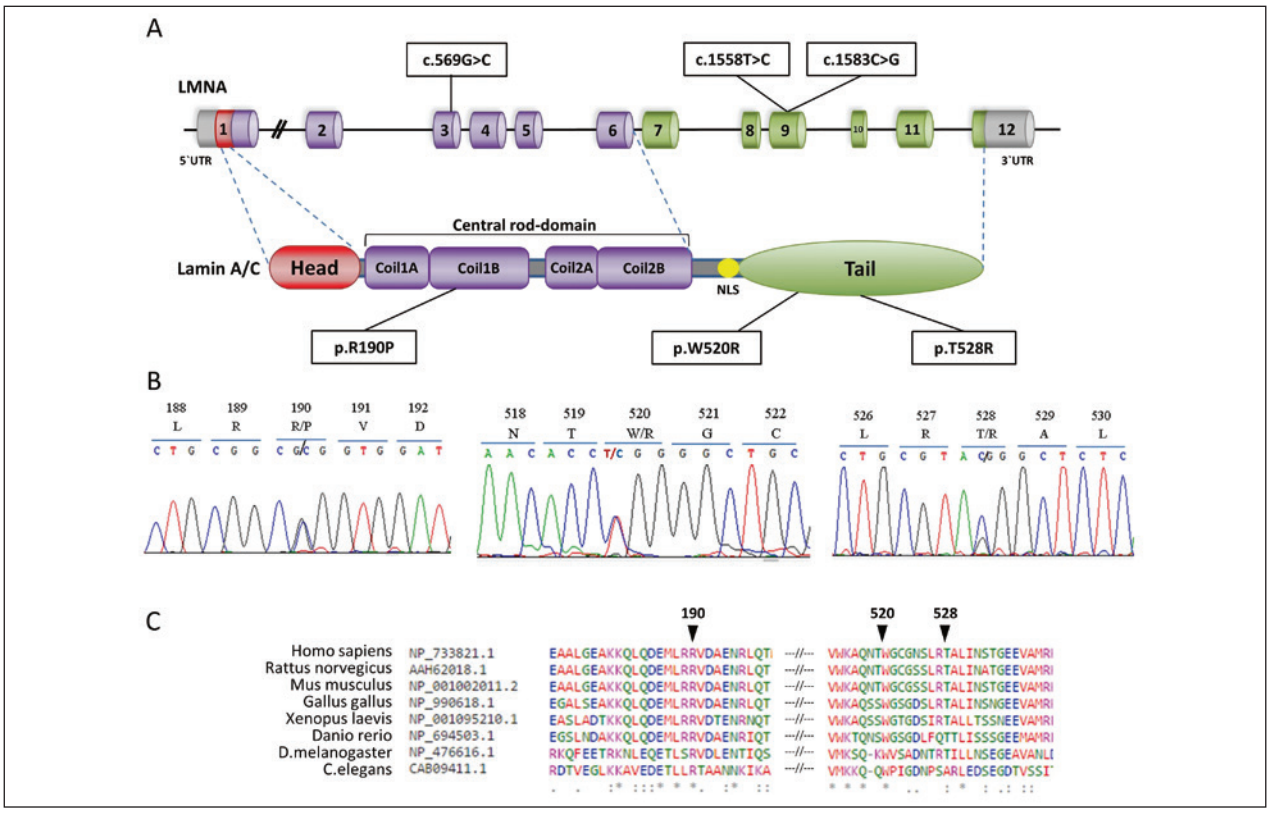

Figure 1. LMNA mutations detected in patients. (A) Representation of mutations localization in gene and protein domains; (B) Mutation detection by DNA sequencing. Heterozygosity is revealed as two overlapping peaks; (C) Illustration of the evolutionary conservation of residues associated with identified mutations located in the coding region of LMNA by multiple alignment of various orthologous sequences.
Symbol (*) indicates identical residues in all aligned sequences, (:) - conserved substitutions, (.) - semi-conserved substitution.

Table I. Clinical characteristic of patients.

Individual	Cardiac phenotype, (age at onset)	Systolic function, LVEF*	ECG characteristics in series	Neurological phenotype (age at onset)	Dominant muscle defeat	sCPK level, u/l (age)	PM/ICD implantation (age)	HTx (age)
Patient 1	DCM (23)	27%	SB, AVB, SSS, AF, RBBB, VES, CHB, nsVT	Subclinical (21)	Quadriceps minimal hypotrophy	293 (23)	ICD (23)	24
Patient 2	DCM (27)	25%	AVB, AF, LBBB, CHB, nsVT	LGMD1B (27)	The limb-girdle pattern of weakness	784 (30)	PM (27) ICD (30)	32
Patient 3	DCM (40)	44%	AF, AVB, CHB, sVT	AD-EDMD (5)	Proximal muscles, scapula-humeroperoneal pattern, early contractures	471 (40)	PM (40) ICD (46)	-

LVEF refers to the first clinical admission. AD-EDMD: autosomal dominant Emery-Dreifuss muscular dystrophy; AF: atrial fibrillation; AVB: atrioventricular block; CHB: complete heart block; DCM: dilated cardiomyopathy; ECG: electrocardiogram; HTx: heart transplantation; ICD: implantable cardioverter defibrillator; LBBB: left bundle branch block; LGMD1B: limb-girdle muscular dystrophy type 1B; LVEF: left ventricular ejection fraction; nsVT: nonsustained ventricular tachycardia; PM: pacemaker; RBBB: right bundle branch block; SB: sinus bradycardia; sCPK: serum creatine phosphokinase, normal level 24-190 U/L; SSS: sick sinus syndrome; sVT: sustained ventricular tachycardia; VES: ventricular extrasystoles.

Results

LMNA exon sequencing led to the identification of different heterozygous mutations in three unrelated patients: p.R190P (c.569G > C, rs267607571), p.W520R (c.1558T > C, rs267607557) and p.T528R (c.1583C > G, rs57629361) (Fig. 1A,B). Detailed clinical characteristics of the patients are presented in Table I. The evolutionary conservation of residues was confirmed by multiple alignment of the region surrounding these variants against various orthologous sequences (Fig. 1C).

Patient 1. The proband is a 24-year-old woman. The initial symptoms of DCM appeared when she was 23 (Tab. I). There was no family history of DCM or sudden cardiac death. Clinical presentation manifested in atrial fibrillation and atrial ventricular block (AVB). Complete heart block developed suddenly and quickly within three months after her first symptoms (dyspnea and weakness). No visible pathological changes of the coronary arteries were found by coronary angiography. Cardiac magnetic resonance imaging showed dilatation of the heart chambers and systolic biventricular dysfunction. The cardioverter-defibrillator implantation and pharmacological therapy did not stop heart failure from progressing, so orthotopic heart transplantation was performed. Neurological examination showed hyperlordosis, mild quadriceps hypotrophy, hypertrophy of calf muscles without reduced limb strength. Reflexes and nerve conduction were normal. Serum creatine phosphokinase level elevated up to 293 U/L (normal range 24-190 U/L) (Tab. I).

The missense p.R190P at exon 3 of the LMNA gene was identified in patient 1. First-degree relatives did not have cardiovascular disease or skeletal muscle involvement. Family genotyping showed that p.R190P occurred de novo (Fig. 2). To estimate the pathogenicity of the missense variant, population screening was performed. No mutation in LMNA gene was detected in the control group consisting of 315 adult subjects.

Mutation p.R190P is located in the α-helical rod domain that forms a dimeric coiled coil (CC) necessary for creating lamin network. The JPred4 server was chosen for evaluating CC formation. A low probability of regular

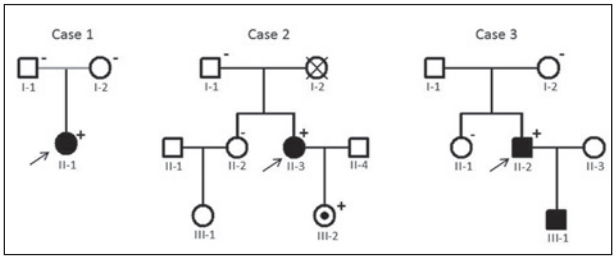

Figure 2. Pedigrees of patient's families. Squares indicate males, circles – females, open symbols – unaffected members, solid symbol – clinically affected subjects, slanted bars – deceased individuals, the black filled dot within symbol – mutation carrier. The arrow denotes the proband. Symbols (+) and (-) indicate LMNA mutation carriers and non-carriers, respectively. The absence of such symbols denotes that no DNA was available for analysis.

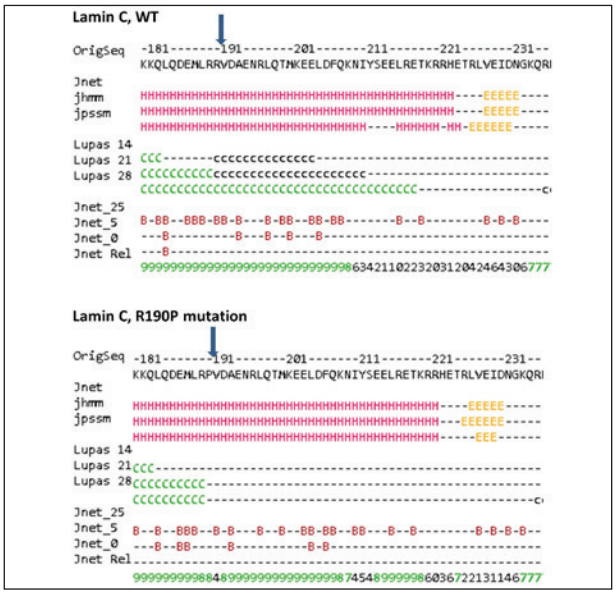

Figure 3. Coiled coil disruption by mutation p.R190P predicted by Jpred4.
Numerous 'H' denote extended alpha-helical secondary structure, 'C'- coiled coil formation with different probability for capital and small letter. Arrow indicates the position 190 and interruption of coiled coil structure due to p.R190P.
Number shows amino acid position in protein;
OrigSeq – protein sequence (GenBank: AHL67294.1);
Jnet – final secondary structure prediction for query: extended (E), helical (H) and other (-) types of secondary structure;
jhmm – Jnet HMM (hidden Markov models) profile prediction;
jpssm – Jnet PSIBLAST PSSM (Position-Specific Scoring Matrix) profile prediction.
Lupas – coiled-coil prediction for the sequence (binary predictions for each location): C = greater than 90% probability, c = 50-90% probability, (-) = less than 50% probability.
Jnet_25 – Jnet prediction of burial, less than 25% solvent accessibility;
Jnet_5 – Jnet prediction of burial, less than 5% exposure;
Jnet_0 – Jnet prediction of burial, 0% exposure, B = buried, (-) = exposed. JnetRel – Jnet reliability of prediction accuracy, ranges from 0 to 9 (bigger is better).

coiled-coil assembly for P190 was shown: less than 50%. This is particularly low in comparison to R190, which has a probability of more than 90% (Fig. 3).

Patient 2. The proband is a 34-year-old woman. The first symptoms of AVB appeared when she was 27. At this period, no signs of heart dilatation were observed. During the next 3 years, heart failure symptoms progressed rapidly. Negative myocardial remodeling and progressive heart failure were observed despite the biventricular resynchronization therapy with optimal medical management and prescribed heart transplantation. The proband suffered from slight limb muscle weakness since childhood. Progressive skeletal muscle pain and weak, lower-limb muscle hypotrophy developed simultaneously with heart failure symptoms. LGMD was diagnosed when limb-girdle wasting with symmetric weakness predominantly affecting the proximal legs and arms distinctly manifested.

Patient 2 is a carrier of the p.W520R mutation. Family genotyping did not detect p.W520R in the patient's father or sister. The mother's death was not associated with cardiac diseases. There was no family history of DCM, skeletal muscle involvement or sudden cardiac death. The mutation was inherited by a daughter (Fig. 2), still asymptomatic except for a high serum CPK level.

Patient 3. This case is characterised by atypical clinical presentations of EDMD. Very severe muscular dystrophy and mild DCM were observed in the proband, a 47-year-old man. Wasting and weakness of the upper- and lower-limb muscles were slowly progressing from the age of 5. During adolescence, EDMD manifested through contractures of the elbows and the Achilles' tendons; muscular dystrophy affected the arms, legs, spine, face, and neck. On the third decade of his life, a progressive proximal muscular atrophy with multiple contractures has developed. No heart disorder presented in the patient before the age of 40. The first cardiac sign was syncope caused by a complete heart block. Echocardiography showed DCM: mild left ventricular dilatation with a decrease of ejection fraction. A permanent pacemaker was implanted.

The mutation p.T528R at exon 9 of the LMNA gene was identified in patient 3. Genetic testing of his first-degree relatives confirmed a de novo origin of the mutation. The proband has an affected child, a son diagnosed with a severe form of EDMD. Informed consent was not obtained from the son, so LMNA analysis was not performed.

Discussion

The p.R190P carrier is unique. Its detailed clinical manifestation was reported before (8). In this article, we presented data supporting its pathogenicity. We consider the variant p.R190P as pathogenic, leading to delated cardiomyopathy and conduction defects. Its de novo occurrence led to the disease development that was not previously detected in the proband family. The mutation alters an amino acid residue in the highly conserved position where another missense changes have been determined as 'pathogenic' before. The mutation was not detected in the control group consisting of 315 adult subjects and was absent in publicly available population databases.

Codon 190 is one of the most prevalent LMNA mutation hot spots that provoke DCM in Europe. Other amino acid replacements, p.R190Q and p.R190W, were identified in patients from several European countries, as well as South Korea and China (9-11). All of the missense mutation carriers showed nearly the same cardiac involvement, namely conduction abnormalities and/or arrhythmias, and thus the necessity of heart transplantation. We note that this case is characterised by relatively early DCM manifestation, in comparison with carriers of other amino acid substitutions in codon 190. The p.R190Q carriers were asymptomatic under 40 years of age, according to case reports described (9, 10). The p.R190W mutation manifested in a broad age range (30-58 years) or was hidden during the entire life (12, 13).

Codon 190 is located in the protein α-helical rod domain that forms a simple dimeric left-handed coiled coil (CC), which is the building block of higher-order lamin structures. The studies of LMNA mutations in the rod domain confirmed their impact on protein dimerization and assembly both *in vitro* and *in vivo* (14). In general, proline is not typical for CC and is known as the 'helix breaker.' We suppose its intercalation in position 190 of lamins could critically destabilize the CC structure and abolish the assembly of the normal nuclear lamina (Fig. 4).

The genetic testing allowed specifying the diagnosis of patient 2 to LGMD type 1B. This pathology form is accompanied by severe cardiomyopathy and potentially life-threatening cardiac arrhythmias presented from the first to the fourth decade of life. Our patient with LGMD predominantly suffered from cardiomyopathy with slight skeletal muscle involvement. These features became the basis for the search for LMNA mutation. We suppose the substitution of aromatic tryptophan to arginine residue, p.W520R, was crucial in LGMD development in patient 2. Codon 520 can be rightfully considered as the hot spot of LMNA gene. Several case reports associated with EDMD or LGMD have described other substitutions in this position: p.W520S (15) and p.W520G (16).

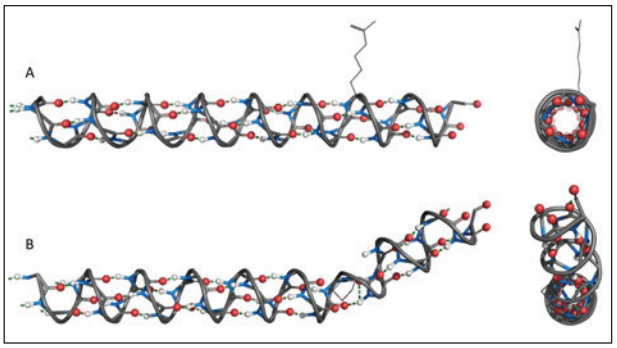

Figure 4. Representation of lamin α-helix model and simulation of amino acid substitution. The model was built with PyMol software: O atoms in red, N atoms in blue, H atoms in white and hydrogen bonds as green dotted lines. The residue side chains are not shown, except R190 and P190. (A) The α-helix of 168-199 amino acid residues: QVAKLEAALGEAKKQLQDEMLRRVDAENRLQT and side view. (B) The same α-helix with p.R190P mutation and side view. Proline curves α-helix away from the coiled coil axis: it disrupts the helical H-bond network and its side chain interferes sterically with the backbone of preceding turn.

The mutation p.T528R caused severe muscular dystrophy without heart disease manifestation for an extended period in our patient 3. The laminopathy was suspected only at age of 40 when the specific cardiac pathology manifested. The genetic investigation verified the diagnosis of autosomal-dominant EDMD type 2. We note that cardiomyopathy in other described cases of EDMD associated with p.T528R was manifested before the age of 30. The early symptoms of heart failure were shortness of breath, atrial fibrillation and sinus tachycardia at the age of 12-23 years (17-20). Zhang et al. (2015) observed this variant in two patients with EDMD and showed positive segregation in their families, supporting pathogenicity for p.T528R (20).

Residue W520 and T528 are composed of the lamin C-terminal domain with an immunoglobulin-like fold (Ig-fold). The W520 is a unique hydrophobic residue, which is involved in the structuring and the positioning of the largest loop of Ig-fold. Residue T528 is composed of the β-sandwich core and stabilizes its conformation (21). Substitutions in these crucial positions will change the balance of weak and strong interactions interfere with the stability, folding and biochemical properties of Ig-domain.

In summary, this article presents the LMNA mutations identified in unrelated patients suffering from DCM with conduction defects, autosomal-dominant EDMD and LGMD type 1B. We consider the missense variants p.R190P, p.T528R and p.W520R as pathogenic, leading to dilatation cardiomyopathy. They were not detected in the ethnically matched control group and publicly available population databases. Their de novo occurrence led to the disease development that was not previously detected in the extended family. There are well-characterised mutations at the same codons associated with laminopathies. Previously reported cases with LGMD and EDMD demonstrated positive family segregation, supporting pathogenicity for p.W520R and p.T528R. Patients with LMNA mutations have a poor prognosis, a higher risk of sudden cardiac death. There is no specific treatment for laminopathies because their mechanism in humans is still unclear. The experience one can get while matching the definite laminopathy symptoms with the mutations re-

vealed in the patients LMNA gene, in any case, bring us closer to understanding the genetic basis of disease. Linkage analyses in affected families allow for prognosis and medication steps for mutation carriers.

Acknowledgments

The authors are grateful to the families involved in this study.

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CASE REPORTS

Is the epicardial left ventricular lead implantation an alternative approach to percutaneous attempt in patients with Steinert disease? A case report

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Steinert's disease or Myotonic Dystrophy type 1 (DM1) is an autosomal dominant multisystemic disorder characterized by myotonia, muscle and facial weakness, cataracts, cognitive, endocrine and gastrointestinal involvement, and cardiac conduction abnormalities. Although mild myocardial dysfunction may be detected in this syndrome with age, overt myocardial dysfunction with heart failure is not frequent. Cardiac resynchronization therapy is an effective treatment to improve morbidity and reduce mortality in patients with DM1 showing intra-ventricular conduction delay and/or congestive heart failure. We report the case of a patient with Steinert disease showing an early onset ventricular dysfunction due to chronic right ventricular apical pacing, in which an epicardial left ventricular lead implantation was performed following the failure of the percutaneous attempt. As no relief in symptoms of heart failure, nor an improvement of left ventricular ejection fraction and reverse remodelling was observed six months later, the patient was addressed to the heart transplantation.

Key words: cardiac resynchronization, epicardial left ventricular implantation, Steinert disease

Introduction

Myotonic dystrophy type 1 (DM1) or Steinert's disease,is the most common muscular dystrophy in adult life with an estimated prevalence of 1/8000. Cardiac involvement, including conduction abnormalities with arrhythmias and conduction disorders, contributes significantly to the morbidity and mortality of the disease. It is recorded in about 80% of cases, and may precede the involvement of skeletal muscles (1-3). The characteristic impairment of His-Purkinje system is the most common cardiac abnormality. Mild ventricular dysfunction has also been reported associated with conduction disorders, but severe ventricular systolic dysfunction is not frequent and usually occurs late in the course of the disease as the final stage of cardiomyopathy (1). Cardiac resynchronization therapy (CRT) is able to restore physiological pattern of ventricular depolarization, resulting in reduction of mitral regurgitation and improvement of left ventricular (LV) systolic function (4-6). CRT has demonstrated reduction in morbidity and mortality in patients with severe refractory heart failure (HF) and intraventricular conduction delay (4-6). The technique of choice for left ventricular pacing in ventricular resynchronization is the insertion of a lead through the coronary sinus, into the postero-lateral vein. The epicardial placement of ventricular leads is considered at present, a salvage technique for patients in whom the percutaneous procedure fails (7).

We report the case of a patient with Steinert disease showing an early onset ventricular dysfunction caused by chronic right ventricular apical pacing, in which an epicardial left ventricular lead implantation was performed following the failure of the standard percutaneous attempt. As no relief in symptoms of heart failure, nor

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an improvement of left ventricular ejection fraction and reverse remodelling was observed six month later, the patient has been addressed to the heart transplantation.

Case report

A 43-year-old man – affected by Steinert disease and regularly followed at Cardiomyology and Medical Genetics Service since the time of his diagnosis (2003) – was hospitalised for an exacerbation of signs and symptoms of congestive heart failure [fatigue, muscle weakness, dyspnea, ortophnea, edema and palpitations, New York Heart Association (NYHA) class III]. His blood pressure (BP) was 100/60 mmHg and heart rate (HR) 60/bpm. Crackles at the basal field of lungs and pretibial edema were detected. Chest X-ray revealed cardiac dilation and pulmonary congestion.

The diagnosis of DM1, at first based on the family history (one affected brother) and clinical features (myotonic phenomenon, mild distal skeletal muscle dysfunction, cataract, gastrointestinal disturbances, endocrine deficiency), was subsequently confirmed by molecular testing, that showed a pathological expansion of CTG triplets (E1 class). In 2005, a bicameral pacemaker (PM) was implanted because evidence of first degree (PR interval ≥ 255ms) plus second-degree type2 atrio-ventricular block (8-13), and concomitant paroxysmal atrial flutter (AF) episodes. The implant was made according to the current guidelines (14) and was followed by an improvement of symptoms and quality of life. To be noted that atrial arrhythmias are not rare in this population (15-17).

In 2013, the PM – according to the current guidelines (18) – was uploaded to a cardioverter defibrillator (ICD) due to the finding of not sustained ventricular tachycardia (NSVT) in pacemaker stored electrograms to prevent the high risk of sudden cardiac death, frequently observed in these patients as in others muscular dystrophies (19). The ICD was placed in the right position, because of the occlusion of left subclavian vein (20-22).

In 2016 during a routine clinical and instrumental follow-up, signs of congestive heart failure (CHF) were detected. The ECG showed a sinus rhythm and a wide QRS interval (165 ms) due to constant right ventricular apical pacing (Fig. 1). Transthoracic echocardiography showed dilation of the heart (left ventricular end-diastolic diameter – LVEDD – was 7.4 cm), left systolic dysfunction and overt intra- and inter-ventricular asynchrony. The ejection fraction (EF), calculated by the Simpson and Teichholz method, was 25% (Fig. 2).

The interrogation of ICD revealed absence of intrinsic spontaneous ventricular rhythm, not sustained paroxysmal episodes of atrial flutter/fibrillation and ventricular tachycardias and no episodes of malignant sustained ventricular

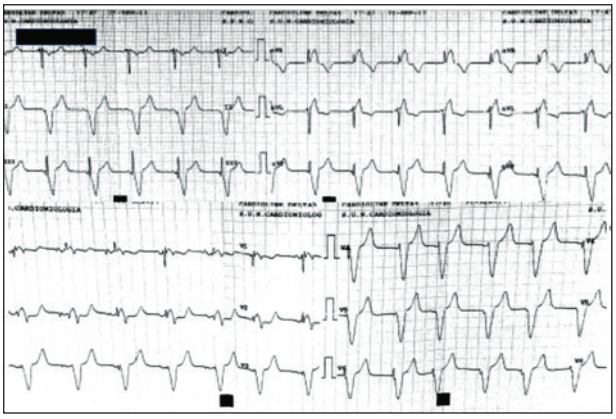

Figure 1. Pre-implant ECG showing sinus rhythm with wide QRS interval due to constant right ventricular apical pacing.

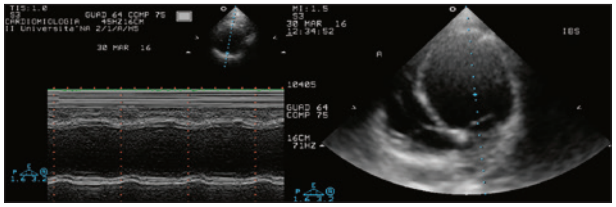

Figure 2. M-mode scan of the left ventricle derived from two-dimensional parasternal short axis view and apical view. A marked left ventricular dilation can be noted (end diastolic diameter = 74 mm). The interventricular septum is hypokinetic and motion of the left ventricular posterior wall is virtually absent.

arrhythmias requiring device intervention. According to the current guidelines (23), his medical therapy was adjusted and included aggressive loop diuretic therapy, β-blockers, spironolactone and ACE inhibitors. In order to rule out an ischemic aetiology of dilated cardiomyopathy and consequent heart failure, a diagnostic coronary angiography was performed showing normal coronary arteries. Despite the aggressive medical therapy the patient experienced two episodes of acute heart failure over one year period, posing the indication for cardiac resynchronization therapy by a biventricular ICD-CRT (24). Before the intervention, a right subclavian venogram was performed which revealed a long segment of occlusion; any attempt to recanalise the right subclavian vein percutaneously failed. Venous stenosis or occlusion due to thrombosis/fibrosis resulting from the presence of the lead is a frequent side-effect in patients implanted with devices. In these cases, an epicardial approach is planned.

Technical procedure

The procedure was performed after written informed consent. Left antero-lateral thoracotomy was performed

along the fourth intercostal space under general anesthesia. The patient was placed in a 45° rotation to the right side. A 3- to 4- cm long left minithoracotomy was performed through the fourth intercostal space between the anterior and mid- axillary line.

The pericardium was opened longitudinally anterior to the phrenic nerve and suspended with traction sutures to better expose the lateral wall. The epicardial lead (bipolar) was fixed at the anterolateral wall of LV. Electrical parameters were measured to verify the correct positioning of the new leads. Once a site with satisfactory pacing threshold was identified (impedance > 200 Ω and < 2000 Ω, sensing > 5 mV and pacing threshold measured at 0.5 ms < 2.0 V), the lead was sewn with 5/0 polypropylene sutures. The connector of the lead was tunelled to the ICD-CRT device pocket in the right pre-pectoral region. The previous endocardial right ventricular defibrillation lead was connected to the ICD-CRT generator (Fig. 3). The patient was extubated in the operating room and observed in the cardiac surgery recovery unit for 24 hours.

Patient's follow-up

The post-operative follow up included the assessment of NYHA functional class, ECG with determination of QRS duration and echocardiography. Left ventricular ejection fraction, left ventricular end-diastolic dimension and severity of mitral regurgitation (MR) values were collected to analyse the effect of CRT via epicardial LV lead placement on reverse ventricular remodelling. One month later an optimization of the atrio-ventricular and inter-ventricular intervals during cardiac resynchronization was performed by both ECG and echocardiogram.

At six-months follow-up, no relief of symptoms was reported by the patient. In that occasion ECG revealed paced biventricular rhythm with a still wide QRS interval (150 ms, Fig. 4), though of reduced size compared to the previous one, and no changes in the repolarization dispersion time. Despite an adequate biventricular pacing the patient remains in NYHA class-III and experienced a further episode of acute heart failure requiring hospitalization. The echocardiogram didn't show an improvement of EF and LV stroke volume (Fig. 5). The ICD analysis showed no significant modification of the electrical parameters, paroxysmal atipical atrial flutter/fibrillation and 98% biventricular pacing rhythm. The patient experienced one episode of slow sustained monomorphic ventricular tachycardia (140 bpm), recognized in monitoring zone of the device, which required external electrical cardioversion.

Discussion

Conduction abnormalities are the most frequent finding of cardiac involvement in patients with DM1 and minor conduction defects can be present in early stages of the disease (1, 2, 25-27). More severe conduction defects may be cause of shortness of breath, dizziness, faint-

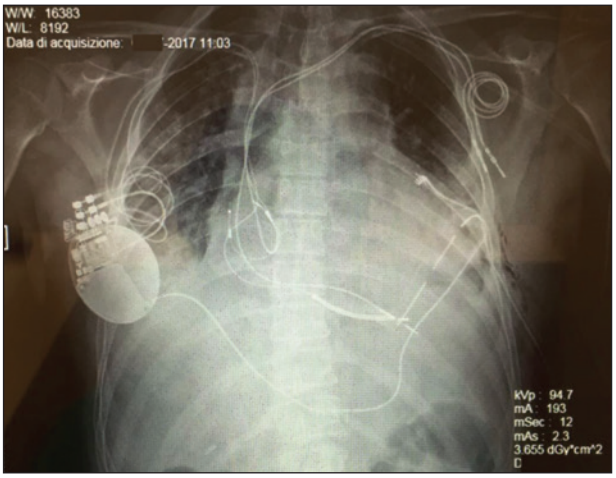

Figure 3. Chest X-ray postero-anterior (PA) view showing leads and ICD-CRT generator placement.

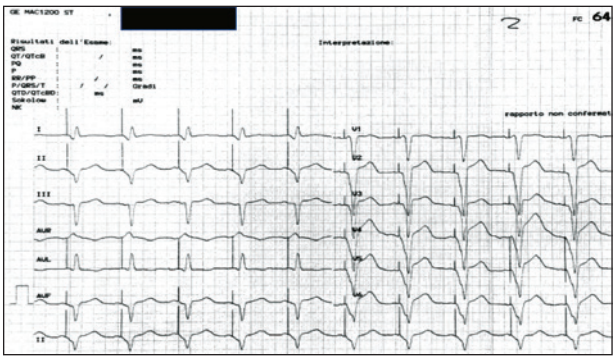

Figure 4. Post-implant ECG showing sinus rhythm with QRS different morphology (compared to the pre-implant one), due to constant biventricular pacing.

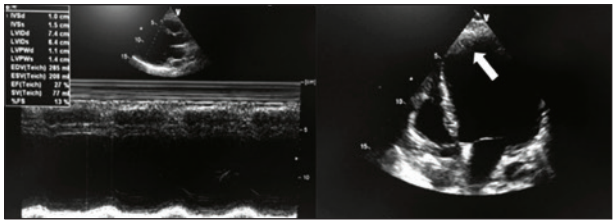

Figure 5. Echocardiographic findings after 6-month ICD-CRT implantation. Please note that both heart dimensions and ejection fraction are unchanged. A blood clot (thrombus) in the left apical ventricle can be observed (white arrow).

ing, syncope, and even of sudden death. Left ventricular dilatation with overt systolic dysfunction is not frequent; however when present they may be more prominent than the muscle complaint. Cardiac symptoms generally occur later compared to the skeletal muscle weakness, but sometimes they may be the initial manifestation of the disease (1, 2, 25). In patient here reported, the early onset of heart failure could be related to the electromechanical delay caused by both intra- and inter-ventricular asynchrony due to chronic right apical pacing; the latter leads to regional structural changes causing a uncoordinated heart contraction that in turn accelerates the progression of the heart failure (28). Beside advances in the optimal medical treatment, strategies for medically refractory symptomatic advanced heart failure have emerged, including cardiac resynchronization therapy. Patients with NYHA class III or IV, with EF 35% or less, sinus rhythm with a QRS duration ≥ 130 ms and left bundle branch block (LBBB) or a QRS duration ≥ 150 ms irrespective of the QRS morphology, are eligible to receive a cardiac resynchronization therapy, according to the current guidelines (23). Basing on the progression of LV dysfunction, AV conduction disturbances and the frequent occurrence of ventricular tachyarrhythmia, Said et al. (29) hypothesized a role for biventricular ICD in patients with DM1 who need a permanent pacemaker implantation. Two previous papers (7, 30) reported an improvement in symptoms of heart failure, LVEF and reverse remodelling in one patient with DM1 showing an early onset ventricular dysfunction secondary to a complete LBBB by this approach. However, a clear consensus about biventricular pacing or the usage of ICD does not exist for this kind of patients.

The "standard of care" of left lead implantation for CRT still remains the less invasive transvenous approach (31). However, several issues may result in failed transvenous implantation of the LV lead such as anatomical limitations due to occlusion of the subclavian vein or the superior vena cava, or an abnormal anatomy of the coronary sinus. Furthermore, lead-related issues such as lead instability with repeated dislodgement, phrenic nerve stimulation despite electrical or physical optimization, or systemic conditions such as endocarditis may contribute to failed transvenous LV lead implantation (31). In these cases, the surgical placement of an epicardial LV lead is required with satisfactory long-term results (32).

Conclusions

The case here reported is the first patient with DM1 in which an epicardial left ventricular lead implantation was used for cardiac resynchronization therapy after failure of percutaneous attempt. At six-months follow-up, based on this experience, the epicardial CRT did not induce either symptom relief, nor improvement of the ejection fraction or reduction of the arrhythmic risk. A possible explanation of the heart failure in this patient may be the prolonged apical pacing; further studies are in progress to determine the consequences of long-term constant apical pacing in patients affected by Myotonic Dystrophy type 1.

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Acta Myologica • 2017; XXXVI: p. 218-222

Complete resolution of left atrial appendage thrombosis with oral dabigatran etexilate in a patient with Myotonic Dystrophy type 1 and atrial fibrillation

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Myotonic Dystrophy type 1 (DM1) is the most common muscular dystrophy in adult life characterized by muscle dysfunction and cardiac conduction abnormalities. Atrial fibrillation frequently occurs in DM1 patients. It's related to the discontinuous and inhomogeneous propagation of sinus impulses and to the prolongation of atrial conduction time, caused by progressive fibrosis and fatty replacement of the myocardium. AF predisposes to a hyper-coagulable state and to an increased risk of thromboembolism. We report the first case of complete resolution of left atrial appendage thrombosis with oral dabigatran etexilate in a myotonic dystrophy type I patient with atrial fibrillation scheduled for transesophageal echocardiogram-guided direct current cardioversion.

Key-words: myotonic dystrophy, atrial fibrillation, dabigatran etexilate, atrial thrombus

Introduction

Myotonic dystrophy type 1 (DM1) is the most common muscular dystrophy in adult life with an incidence of 1:8000 births and a worldwide prevalence ranging from 2.1 to 14.3/100.000 inhabitants. Cardiac involvement is noticed in about 80% of cases, and it often precedes the skeletal muscle one (1). Paroxysmal atrial arrhythmias (atrial fibrillation, atrial flutter, atrial tachycardia) frequently occur in DM1 patients with a prevalence up to 25% (2) and seem to increase mortality in this population (3). Modern pacemakers (PMs) and implantable cardiac defibrillators (ICDs) include detailed algorithms and functions to facilitate the diagnosis and management of frequent paroxysmal atrial tachy-arrhythmias often undetected during conventional clinical follow-up (4-11). Atrial fibrillation (AF) predisposes to a hypercoagulable state and an increased risk of thromboembolism (TE) (12, 13). The incidence of left atrial appendage thrombosis before direct current cardioversion (DCC) has been widely studied in AF population, ranging from 6 to 18% (14, 15). Non-Vitamin K Antagonist oral anticoagulants (NOAC) are increasingly used for the prevention and treatment of thrombi formation owing to the inherent limitations of Vitamin K antagonist oral anticoagulants (VKAs) (16). We report the first case of a left atrial appendage thrombosis effectively treated by dabigatran etexilate, a direct inhibitor of thrombin, in a DM1 patient with AF scheduled for transesophageal echocardiogram (TEE)-guided direct current cardioversion (DCC).

Case report

A 45-year-old DM1 woman with arterial hypertension, previously implanted with a dual chambers pacemaker for advanced atrioventricular block, came to our observation for PM check and cardiologic therapy optimization before cataract surgery. She was taking perindopril (4 mg/die) and magnesium pidolate (2.25 g/die).

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She referred a recent onset of palpitations and dyspnea. Standard (12-lead electrocardiogram) ECG confirmed the diagnosis of atrial fibrillation with a mean ventricular rate of 160 bpm (Fig. 1). PM interrogation showed atrial high rate electrograms (AHRE) faster than 220 bpm, that lasted longer than 5 minutes with irregularity and incoherence of RR intervals (Fig. 2) and arose five days before the cardiologic evaluation. Transthoracic echocardiogram showed a slightly reduced left ventricular systolic function (Simpson's biplane ejection fraction: 48%) and a mild left atrial enlargement (left atrial volume index: 29 mL/m^2). Considering the patients' symptoms and the need to restore sinus rhythm before surgical procedure, a TEE-guided DCC was performed, which showed the presence of a thrombus in left atrial appendage (Fig. 3). The patient started a beta-blocker therapy for rate control (bisoprolol 2.5 mg/die) and oral anticoagulant therapy (warfarin 5 mg/die) to dissolve the thrombus and prevent the risk of systemic thrombo-embolic events. However, at one-month follow-up, due to a non-optimal response to warfarin therapy, evaluated by the International Normalized Ratio (INR) of the prothrombin time, a switch form VKA to NOAC therapy with dabigatran was performed at a dosage of 150 mg/bid. Eight weeks after, TEE revealed the complete resolution of the left atrial appendage thrombus (Fig. 4), allowing us to perform a safe and successful direct current cardioversion, that restored the sinus rhythm at 65 bpm. The therapy with dabigatran was prolonged for 4 weeks after cardioversion due to the high risk of thromboembolic events (CHA2DS2-Vasc Score: 2). At the date, twelve months after DCC procedure, no bleeding events or side-effects are reported.

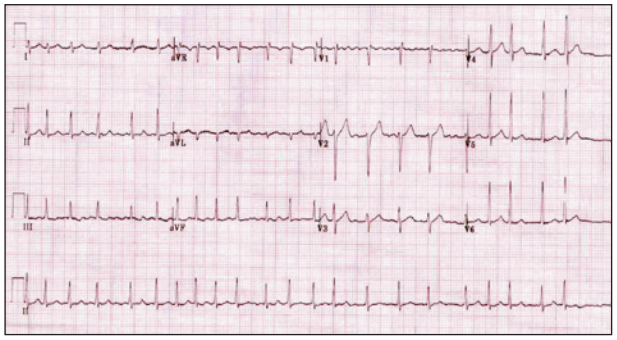

Figure 1. Atrial fibrillation detected by standard 12-lead electrocardiogram.

Discussion

Cardiac involvement in DM1 patients occurs as a degenerative process with progressive fibrosis and fatty replacement not only limited to the specialized conduction system, but also extended to initially unaffected areas of the atrial myocardium (17). This anatomo-pathological substrate, causing the discontinuous and inhomogeneous propagation of sinus impulses and the prolongation of atrial conduction time, may facilitate the onset and perpetuation of atrial arrhythmias in these patients (18-24), as usually happens in other clinical conditions (25-30). AF is one of the most common supraventricular arrhythmias observed in DM1 population, characterized by chaotic and uncoordinated atrial activity which predisposes to a hypercoagulable state and an increased risk of TE (12, 13). DCC quickly and effectively converts AF

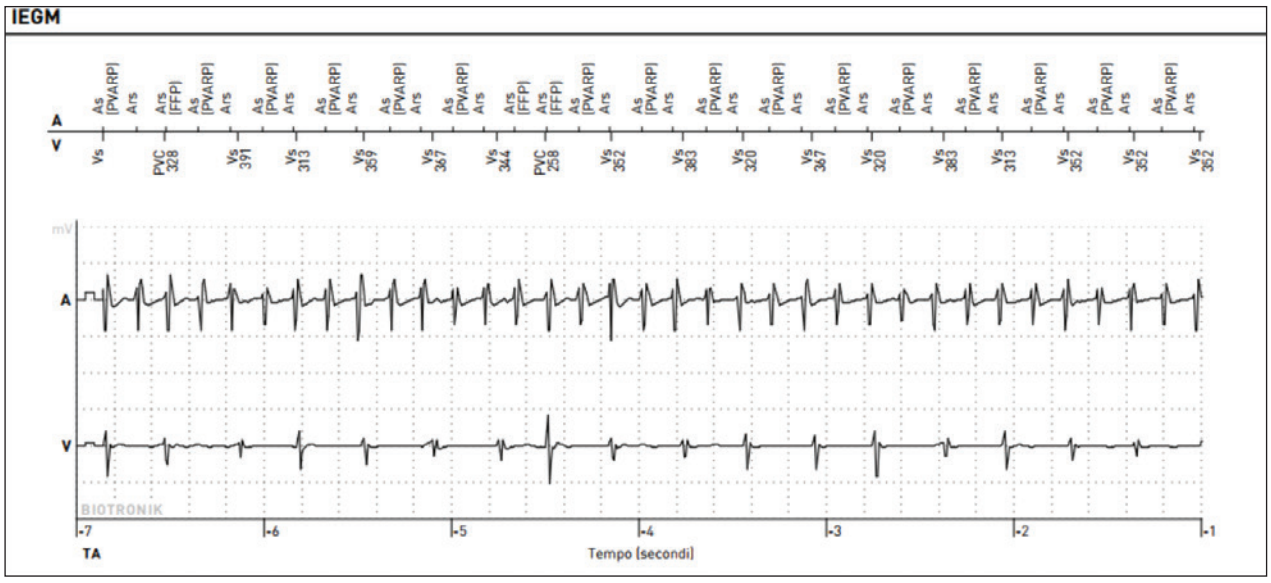

Figure 2. Atrial high rates electrograms detected by pacemaker diagnostics.

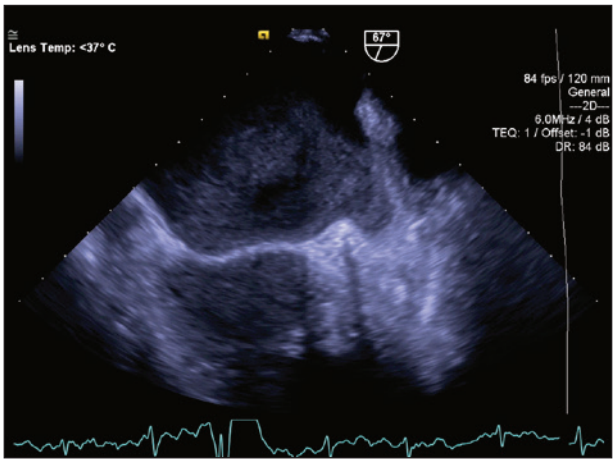

Figure 3. Transesophageal echocardiographic evaluation. See the presence of a thrombus in left atrial appendage.

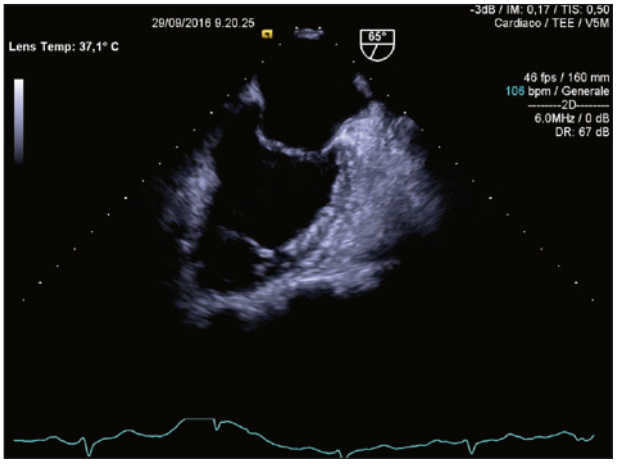

Figure 4. Transesophageal echocardiography evaluation eight weeks after treatment with dabigatran etexilate.

to sinus rhythm; however, it carries an inherent risk of stroke, which is substantially reduced by the administration of anticoagulation therapy. An early initiation of such therapy is important in all patients scheduled for cardioversion. Patients who have been in AF for periods longer than 48 h should start oral anticoagulation therapy at least 3 weeks before cardioversion and will continue it for at least 4 weeks afterwards (31). However, the difficulties in achieving an optimal anticoagulation with conventional warfarin therapy, likely related to several factors such as the slow onset of action, variable pharmacologic effects, numerous food and drug interactions and periodic closely target INR monitoring (32) make it difficult the therapeutic management in clinical practice and reduce the real-life patients' compliance. All these challenges have prompted an extensive research and developed NOAC, now available for stroke prevention in AF patients and used in various clinical settings (33-38). Dabigatran etexilate, a direct inhibitor of thrombin, emerged as the first new generation oral anticoagulants potentially able to replace warfarin in preventing arterial TE in patients with AF (39-41). A post-hoc analysis of the Randomized Evaluation of Long-Term Anticoagulation Therapy (RE-LY) study in patients who underwent cardioversion with or without TEE guidance, showed that dabigatran treatment has a low and comparable frequency of adverse events compared to warfarin (42). These results were confirmed by a long term propensity score matched study in real world setting (43). The potential thrombolytic effect of dabigatran has been previously described (43, 44) as it is able to create a easier and faster anticoagulation milieu while inhibiting thrombin binding to fibrin and fibrin degradation products. In contrast warfarin anticoagulation, in its loading phase, could also exert a transient thrombogenic action (45).

Conclusions

The present case is the first report of a complete left atrial appendage thrombosis resolution obtained by oral dabigatran etexilate in a DM1 patient with AF, scheduled for TEE guided direct electrical cardioversion. The use of NOAC therapy should be particularly useful in this population of patients, for their variable cognitive impairment and consequent poor compliance with periodic INR monitoring.

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OBITUARY

Professor Giovanni Nigro (1931-2017)

Giovanni Nigro (born 24 April 1931) died at his home in Naples on 13 October 2017. After graduating in Medicine and Surgery from the University of Naples "Federico II" in 1954 at the age of 23, he was invited to Sweden, where he had the privilege to work with Gunnar Biörck and Hugo Theorell, recipient in 1955 of the Nobel Prize in Medicine for his studies on myoglobin, cytochrome c and the respiratory chain.

At only 29 years of age he became the youngest Professor of Internal Medicine in Italy, and a few years later was also appointed Professor of Clinical Biochemistry. Following his return from Sweden until his retirement, he worked tirelessly at the University of Naples, first as an unpaid teaching assistant, then as Professor of Special Medical Pathology and later Professor of Therapy.

During his long academic career, he served in key roles on the Department Council, the Department Executive Board, and the University Council, contributing to the drafting of the Statute of the Second University of Naples – now the University of Campania "Luigi Vanvitelli" – with a true and increasingly rare spirit of public service.

His interest in muscle diseases stemmed from an encounter with a young girl suffering from a muscle disorder while working as a university teaching assistant.

In 1961, he attended a conference on muscular diseases in Trieste – organized by a patient – after reading about it in the newspaper. There, he learned of the Italian Union for the Fight against Muscular Dystrophy (UILDM) and returned to Naples determined to set up a local branch of the association to help families of affected children. Over the following years he served as National President of the UILDM from 1979 to 1986, and chaired its Medical-Scientific Committee from 1984 to 1990.

In 1971 he opened the first rehabilitation center in Campania for patients with muscle conditions, carrying out the last request of a dystrophic boy who had entrusted him with the entire contents of his piggy bank. Named in memory of the boy, the Gaetano Torre Center for Muscular Diseases is still running today, and over the years has supported about 70 families, helping unemployed parents of children with muscular dystrophy.

In 1976 he set up first Center of Cardiomyology and Myology at the Naples University Hospital, which went on to become a multi-specialty department in 1980.

In 1981 he organized the first international meeting on muscle diseases in Naples, attended by world-renowned researchers in the field.

In 1985 he invited Prof. Yves Rideau from the University of Poitiers to Naples University so that young Italian Duchenne patients might benefit from his innovative surgical-orthopedic therapy.

In 1989, as Chair of the UILDM's Medical-Scientific Commission, he imported the Téléthon event run by the French Association against Myopathies (AFM) to Italy, inaugurating the very first Italian Telethon Marathon, now in its 28th year.

He was one of the founder members of the European Neuromuscular Centre, set up in 1992, and served on both

its Executive and Research Committees, later becoming one of the organization's honorary members.

In 1993 he established the Mediterranean Society of Myology (MSM) in Ischia, uniting members from 22 different countries, with the aim of bringing together experts in striated muscle diseases and neuromuscular disorders from across the Mediterranean area, fostering collaborative medical and biological research, holding scientific meetings, sponsoring a journal for the publication of scientific papers, and creating a Mediterranean-wide medical network for muscle diseases.

In 1995 he established the "Gaetano Conte Prize" in honor of the Neapolitan physician who in 1836 – 32 years before Duchenne de Boulogne – first described two patients with muscle degeneration. The prize is awarded to distinguished scientists working in the field of muscular dystrophies. In the same year he set up the World Muscle Society (WMS) in Bologna together with Victor Dubowitz and Luciano Merlini, and was one of the founder members of the Italian Association of Myology (AIM), created in 2000.

His name is inextricably linked with ground-breaking research in the field of cardiomyology – a term he himself coined – having first underlined how the heart may be a primary site of the dystrophic process in Duchenne patients. This was in 1976, 10 years before the discovery of the dystrophin gene. He was also one of the first to report cardiac involvement in Duchenne and Becker carriers, and heart disease as a major cause of death in numerous muscle disorders, such as myotonic dystrophies and Emery Dreifuss muscular dystrophies, if not properly diagnosed and treated.

Prof. Nigro remained active for many years after his retirement, working as editor-in-chief of the journal *Acta Myologica* and organizing conferences – including the World Federation of Neurology Congress in Naples in 2010, and MSM meetings in 2011, 2013 and 2015. The upcoming 13th MSM conference will be held in Cappadocia, Turkey, on 27-29 June 2018, and is dedicated to his memory.

He also continued to be actively involved in running the rehabilitation center he himself created, working on a voluntary basis as Medical Director until April 2016, as well as the G. Torre Association, where he served as an honorary member up until the day before his death.

On a more personal level, Prof. Nigro will be remembered for his quick wit, his ability to make light of difficult situations, his optimism, his capacity to come back stronger after each setback, his immediate empathy with patients and their families, and his pioneering approach. He was in no way jealous of his ideas or insights, and would always stress the importance of disseminating ideas, not their authorship.

Prof. Nigro leaves behind a great legacy, and his memory will live on among the tens of thousands of people – students, colleagues, patients and friends – who had the privilege of knowing him. We can only thank him for everything he taught us.

Luisa Politano and Vincenzo Nigro

The Pacini family and the editorial staff of Pacini Editore wish to remember Professor Giovanni Nigro for his precious collaboration in the realization of the Journal *Acta Myologica*.
His profound devotion as Editor-in-Chief affirmed the Journal as a high-profile scientific periodical in the national and international field.
His professionalism and correctness have allowed us to work in full harmony enriching our editorial experience.
It was always a pleasure to welcome him to our Company in Pisa.

Patrizia Pacini
Managing Director
Pacini Editore srl

NEWS FROM AROUND THE WORLD

AIM

Below the letter of the end year of the AIM President, Gabriele Siciliano, outlining the activities of the Association.

" Dear AIM Members,

It's with a special feeling I'm sending to you the usual end of year message and my wish for a sparkle end of it and a very happy new year.

The first six months of the new year are the last of my triennial mandate, I am going to face them with a bit of melancholy for the usual exit phase but also with so much determination in bringing our Association towards always more ambitious and rewarding goals, cherishing the awareness of representing an united and enthusiastic working group. Therefore, this is not the time to make final summaries, rather I would like to stress the viability of our Association in its primary activities of disseminating knowledge, stimulating research and acting in favor of the patients.

The number of AIM members, especially young people, is further increased compared to the previous year, almost 20%.

We were involved in several educational events. We were regarded as advisory consultants at various institutional levels and authoritatively participated in research groups and national and international scientific events. Something more certainly is awaiting us from today to the next Genoa AIM Congress in June 2018.

After concluding to draft the new statute on December 12 (we updated and necessarily regularized some organizational and administrative aspects), we have been called, even as an affiliated association to the Italian Neurology Society, to work on drafting guidelines and clinical recommendations in our field of study, in compliance with the recently introduced legislation (the Gelli's Law). It is my purpose to request your collaboration and your participation in working groups in this regard.

Another current topic is the increasing advisory role that institutions ask us to carry out in evaluating appropriateness of the new therapies for diseases of our interest, which is a fundamental and challenging task for all of us. This is part of the recognition of the role of our Association also in wider initiatives such as the one related to the ERN. In this regard, we were invited to a recent meeting in Rome, on 20 December, by authoritative representatives of the State-Regions conference on health issues.

The on-going project of the Coordination of Neuro-Muscular Patient Associations, in which we are involved as a technical partner, will continue albeit some difficulties (also economic for the smaller Associations) have slowed the legal recognition of this Coordination board. However, I firmly believe that we can not miss the opportunity to create synergies between doctors and the different patients' associations.

In this view, we have been present at the audition with the Minister of Public Administration in September 27, and will participate at the Rare Disease Day on February 28 and, especially, at the next edition of the Neuromuscular Day, GMN2018, on March 10.

Our commitment will continue in divulging knowledge on muscle diseases and more generally in the matter of myology, in this organizing further scientific-and educational events, collaborating with other twin and related Associations, supporting research grants for young scientists, contributing to growth of our journal Acta Myologica.

With these brief notes I finish my message, I embrace all of you and I wish you a happy 2018."

MSM

The 13th Congress of the Mediterranean Society of Myology will be held in Turkey on 27-29 June 2018, organised by Prof. Haluk Topaloglu. The symposium was in the traditional two-days MSM format with selected topics (see brochure).

WMS

The 23rd International WMS Congress will be held in Mendoza, Argentina from 2 to 6 October 2018. The symposium will follow the traditional format with 3 selected topics:

- New developments in genetic and acquired disorders of the neuromuscular junction.
- Mitochondrial function and dysfunction in neuromuscular disorders: pathogenesis and therapies.
- Advances in the treatment of neuromuscular disorders.

One day of the symposium will be dedicated to each of the selected topics. Invited keynote speakers will summarize the state of the art on the selected topics, covering clinical, molecular and other aspects. The sessions will comprise selected oral papers and poster presentations with guided discussions. Contributions will also be welcome on new advances across the neuromuscular field. The 16th WMS Pre-Congress Teaching Course will be held on 1-2 October 2018. Please note only 45 places are available. Early booking is advised.

FORTHCOMING MEETINGS

2018

February 20-24

Biospecimen Research Symposium: Quality Matters. Luxembourg. Information: website: *http://meetings.isber.org*

May 20-24

ISBER 2018 Annual Meeting & Exhibits. Dallas, Texas, USA. Information: website: *http://meetings.isber.org*

June 6-9

18th National Congress of Italian Association of Myology. Genua, Italy. Information: website: *www.miologia.org*

June 16-19

European Human Genetics Conference 2018. Milan, Italy. Information: website: *conference@eshg.org*

June 16-19

4th Congress of the European Academy of Neurology. Lisboa, Portugal. Information: website: *www.ean.org*

June 27-29

XIII Congress of Mediterranean Society of Myology. Avanos, Cappadocia, Turkey. Information: *msm2018@flaptour.com.tr; htopalog@hacettepe.edu.tr*

July 6-10

15th International Congress on Neuromuscular Diseases (ICNMD2018), Wien, Austria. Information: *www.icnmd2018.org*

August 25-29

European Society of Cardiology (ESC). Munich, Germany. Information: website: *https://www.escardio.org/*

October 2-6

23rd Congress of World Muscle Society. Mendoza, Argentina. Information: website: *www.worldmusclesociety.org*

October 16-20

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

October 17-21

Asia Pacific Heart Rhythm Society (APHRS). Taipei, Taiwan. Information: website: *http://www.aphrs.org/*

October 31- November 2

World Congress on Human Genetics. Valencia, Spain. Information: website: *http://humangenetics.conferenceseries.com/*

November 9-10

9th International Conference & Exhibition on Tissue Preservation and Biobanking at Atlanta, USA during, 2018. Information: website: *http://biobanking.conferenceseries.com/*

2019

May 2019

Heart Rhythm 40th Annual Scientific Sessions (HRS). Chicago, IL. Information: website: *http://www.hrssessions.org/*

June 15-18

The European Human Genetics Conference 2019. Gothenburg, Sweden. Information: *conference@eshg.org*

September 24-28

24th Congress of World Muscle Society. Copenhagen, Denmark. Information: website: *www.worldmusclesociety.org*

October 22-26

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

To be announced

Asia Pacific Heart Rhythm Society (APHRS). Bangkok, Thailand. Information: website: *http://www.aphrs.org/*

2020

June 6-9

The European Human Genetics Conference 2020, Berlin, Germany. Information: *conference@eshg.org*

October 27-31

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

To be announced

25th Congress of World Muscle Society. Toronto, Canada. Information: website: *www.worldmusclesociety.org*

13th Meeting of the Mediterranean Society of Myology

This congress is memory of our late Professor Giovanni Nigro, one of the first pioneers for neuromuscular research in the Mediterranean area

in connection with the

2nd Congress of the Turkish Neuromuscular Society

27-29 June 2018
Avanos, Cappadocia, Turkey

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Topics of the congress: Limb-girdle muscular dystrophies, Advances in the field

Extra activity 1: 26-27 June 2018, Clinical neuromuscular course for physicians

Extra activity 2: 27 June 2018, Outcome measures course for physiotherapists

CONTENTS

INVITATION

Dear Colleagues,

Thirty-six years ago, a group of researchers with interest in the field of muscular dystrophies felt the need to promote a mutual cooperation among the people of the Mediterranean area, and created the Mediterranean Society of Myology in 1993, in Ischia.

The initiative had a rapid success with the accession of the representatives of 22 Mediterranean Countries and was a model to establish other International Societies of Myology, such as the European NeuroMuscular Center–ENMC (established in 1992 by Ysbrandt Portman, Reinhardt Rudel and myself) and the Word Muscle Society (established in 1995 by Victor Dubowitz, Luciano Merlini and myself)

The presence of the Turkish delegates has always enriched the value of the Society, and the organization of the 13th Congress attests their contribution.

Therefore I am very pleased and grateful to Prof. Haluk Topaloglu for accepting the task (and load) of the Congress organization, and I'm convinced it will be a successful event.

I hope that many of you will be present next year in Cappadocia.

Giovanni Nigro
30 April 2017

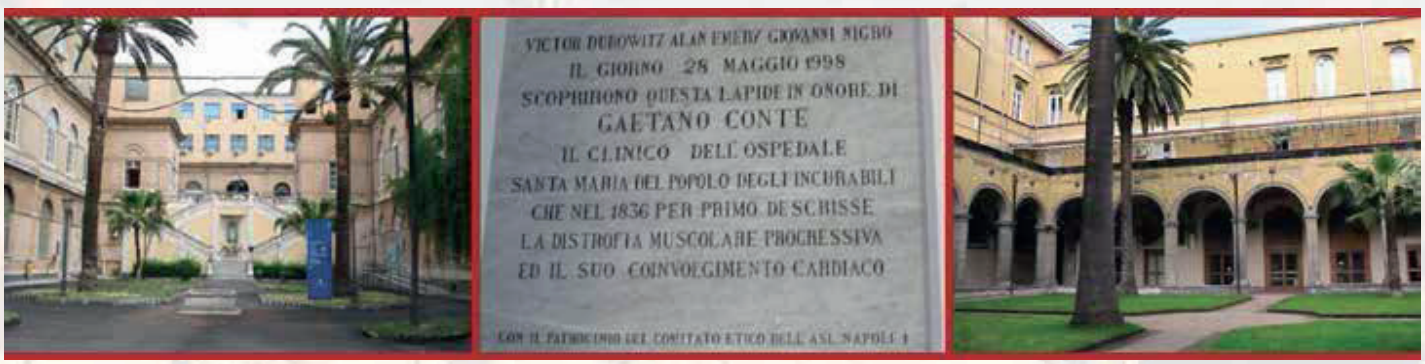
Dear Colleagues,

We invite you to attend the 13th Meeting of the Mediterrranean Society of Myology (MSM) in Cappadocia, Turkey, June 27-29 2018. MSM has been originated in Italy, rapidly escalated, and within a decade has become an internationally renown group of enthusiasts. Bi-annual meetings have been traditional. With the spirit we have received from the past congresses of the Society, it will be our aim to bring researchers together with interest in basic and clinical science. The special topic for this congress has been chosen as ''limb-girdle muscular dystrophies''. We shall try our best to create an exciting programme. This congress will jointly be done with the 2nd Turkish Myology meeting.

Cappadocia which was the population zone of the Assyrian civilization later has hosted the Hittite, Frig, Pers, Byzantine, Seljuk and Ottoman civilizations. Cappadocia is an important tourism site in Turkey.

We think that your visit to Cappadocia in the summer of 2018 will be rewarding academically and educationally, and also from the social aspects.

Prof. Haluk Topaloglu
President of the Mediterranean Society of Myology



COMMITTEES & KEY FIGURES

Congress Presidents

Giovanni Nigro, Haluk Topaloğlu

Local Organizing Committee

İpek Alemdaroğlu (secretary)
Hayat Erdem
Göknur Haliloğlu
Ayşe Karaduman
Müjgan Sönmez
Beril Talim
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G. Kale

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Treasurer
Ö. Yılmaz

Members
A. Karaduman
B. Talim
H. Topaloğlu

SCIENTIFIC PROGRAMME

27 June 2018, Wednesday

19.00 – 19.30 Welcoming Lecture Followed by Reception

28 June 2018, Thursday

Limb-Girdle Dystrophies

Session 1. Genetics and Classification of Limb-Girdle Dystrophies

08.30 – 09.00 Classification and Pathophysiology
Marco Savarese, IT

09.00 – 09.30 Solve the Unsolved LGMDs: the Next Approach
Vincenzo Nigro, IT

09.30 – 10.00 The Gene Therapy Field in LGMD
Isabelle Richard, FR

10.00 – 10.30 Oculopharyngeal Muscular Dystrophy: From Bench to Bedside And Back Again
G Butler-Browne, FR

10.30 – 11.00 Break

Session 2. Clinical features of limb-girdle dystrophies

11.00 – 11.30 Clinical Features of Limb Girdle Dystrophies: an Overview
Jordi Diaz-Manera, SP

11.30 – 12.00 Metabolic Myopathies Mimicking Limb Girdle Dystrophy
Corrado Angelini, IT

12.00 – 12.30 Myofibrillar Myopathies
Duygu Selcen, USA and TR

12.30 – 13.00 Muscular Dystrophies with Mental Retardation
Haluk Topaloglu, TR

13.00 – 14.00 Lunch, Poster Viewing

14.00 – 15.30 Oral Presentations

15.30 – 16.00 Break

16.00 - 17.30 Posters

17.30 - 19.00 MSM General Assembly

20.00 – 24.00 Gala Dinner

SCIENTIFIC PROGRAMME

29 June, Friday

Session 3. Advances and Therapies I

09.00 – 09.30 **Genetic Diagnosis**
Roula Cristodoulou, Cyprus

09.30 – 10.00 **Laminopathies**
Giselle Bonne, FR

10.00 – 10.30 **Dysferlinopathy, Calpainopathy and Imaging**
Giorgio Tasca, IT

10.30 – 11.00 **Contribution of Muscle Biopsy in Diagnosis of LGMD in the Third Millennium**
Rita Baresi, UK

11.00 – 11.30 **Break**

Session 4. Advances and therapies II

11.30 – 12.00 **Future of genetics**
Judith Melki, FR

12.00 – 12.30 **Clinical and Molecular Heterogeneity in Limb-Girdle Muscular Dystrophies**
Giacomo Comi, IT

12.30 – 13.00 **Cardiac Involvement in Muscular Dystrophies: Contribution of the Naples's School**
Luisa Politano, IT

13.00 – 13.30 **Treatment of Pompe Disease**
Antonio Toscano, IT

13.30 – 14.30 **Lunch, Poster Viewing**

Session 5. Advances and therapies III

14.30 – 15.00 **Update in spinal muscular atrophy treatment**
Eugenio Mercuri, IT

15.00 – 15.30 **Therapy of GNE Myopathy**
Zohar Argov, IL

15.30 – 16.00 **Duchenne Muscular Dystrophy: Future Perspectives**
Yoram Nevo, IL

16.00 – 16.30 **Sarcoglycanopathies, Therapeutic Approaches**
Dorianna Sandona, IT

Closure of the meeting

TOPICS & PARTICIPANTS & IMPORTANT DATES

Topics of the congress:
Limb-girdle muscular dystrophies
Advances in the field

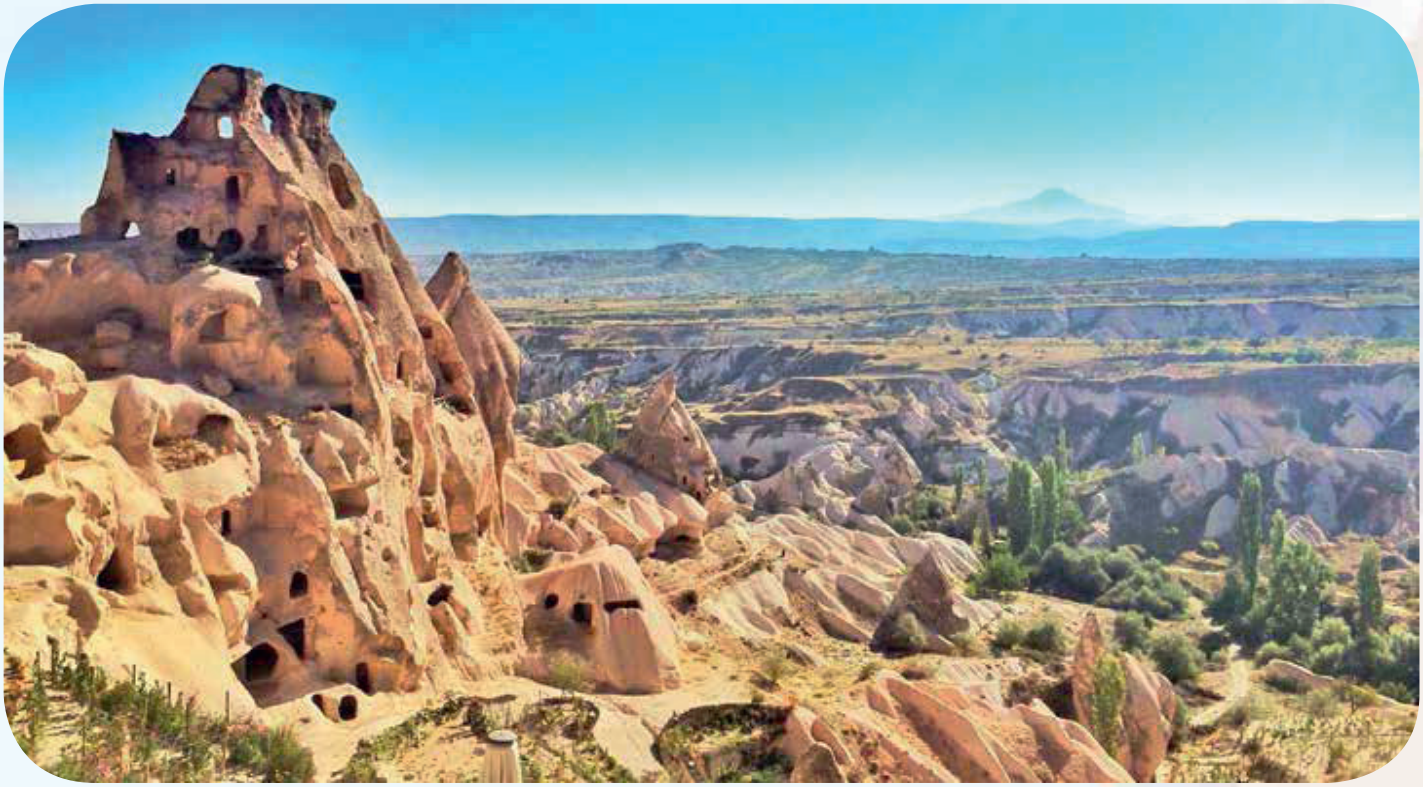
Extra activity 1: 26-27 June 2018
Clinical neuromuscular course for physicians

Extra activity 2: 27 June 2018
Outcome measures course for physiotherapists

World Muscle Society members are highly specialised professionals active in the neuromuscular field, are on research for neuromuscular disorders or involved in the management of patients with these disorders.

IMPORTANT DATES	
Beginning of Abstract Submission & Registration	1 January 2018
Abstract Submission Deadline	30 April 2018
Early Bird Registration Deadline	30 April 2018
Pre-Congress Course Registration Deadline	15 May 2018

CAPPADOCIA



Cappadocian Region displays a beautiful combination of nature and history.

Some three million years ago, violent volcanic eruptions covered the plateau in this area with tufa, a soft stone comprised of lava, ash and mud. Subsequently, the wind and rain have eroded the brittle rock to form a spectacular surrealistic landscape of rock cones and capped pinnacles, called “fairy chimneys” that are painted in colors ranging from warm reds and gold to cool greens and grays. Fairy chimneys and carved houses and churches inside these formations and adorned these settlements with frescos, carrying the traces of the thousands of years of their civilizations. During Byzantine times, Christian chapels and monasteries were hollowed out of the rock, and later these dwellings served as refuge for Christians, persecuted by the Romans.

Göreme National Park and Cappadocia were placed on the UNESCO World Heritage List in 1985 as 7 parts: Göreme National Park, Derinkuyu Underground City, Kaymaklı Underground City, Karlı Church, Theodore Church, Karain Güvercinlikleri (Karain Columbaries) and Soğanlı Archaeological Site.

Hot-air ballooning is very popular in Cappadocia and is available in Goreme. Daily hot-air balloon tours are organized in various concepts (from an hourly trip to lunch&dinner trips) and you can enjoy the fascinating view from the wonderful sky.

BENEFITS OF SPONSORS

- Sponsor companies will be show-casing in front of a elite, highly specialised professional group
- With its geographical outreach, Cappadocia is accesible for participants from all around Europe and Middle - Near East
- Participating companies will book their place in setting that will shape the future trends in development of drugs and equipment in the field
- Brand awareness for participating companies will be raised during the all event and will be set for a higher level for it will be a unique and vivid meeting
- Direct contact with trend-setters
- Direct exposure to possible clients

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Todisco, Vincenzo

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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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Manuscript submission must be effected on line: **www.actamyologica.it** 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.
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